

RESOLUTION 6.22
CETACEAN LIVE STRANDING

The Meeting of the Parties to the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area:

Recalling Resolutions 1.10 on “Cooperation between national networks on cetacean strandings and the creation of a data base”, 3.25 on “Cetacean live stranding” and 4.16 on “Guidelines for a coordinated stranding response”,

Taking into consideration Recommendation 10.10 of the ACCOBAMS Scientific Committee,

Recognizing that in recent years the ACCOBAMS Area has been the scene of cetacean live stranding events, involving mass strandings over wide geographical areas, which have evoked great concern and have attracted considerable attention from the scientific community,

Aware that cetacean live strandings can present national governments with specific challenges that are exacerbated when they become a transboundary event,

Recalling that in emergency situations, one possible major barrier could be due to general difficulty of administrative authorities to produce immediate responses,

Conscious of the related work underway under the Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas (ASCOBANS), and *noting* ASCOBANS Resolution 8.10 on Small Cetacean Stranding Response,

Considering that the joint ACCOBAMS/Pelagos workshop on cetacean live stranding held in Monaco on 29th and 30th October 2014 proposed harmonized procedures in case of cetacean live stranding, stressing that, in case of transboundary emergencies involving cetaceans, rapid intervention, participation and cooperation from different experts, stakeholders and within scientific organizations are required to ensure an effective response and an adequate coordination,

Also considering that the International Whaling Commission (IWC) held an Expert Workshop in September 2013, which, in particular, stressed the need for human safety, developed a decision tree related to rescue versus euthanasia, provided an authoritative and comprehensive review of various euthanasia methods and provided advice on data collection protocols and event management,

1. *Takes note*, as guidelines, of :
 - the common definitions of terms related to stranding events as presented in [Annex 1](#);
 - the common best practices for a basic post-mortem examination of stranded cetaceans as presented in [Annex 2](#);
 - the common data collection protocol for live strandings as presented in [Annex 3](#);
2. *Requests* the Scientific Committee, to approach the ECS, IWC and ASCOBANS in order to:
 - review during the triennium, if necessary, the common definitions, common data collection and common necropsy protocol;
 - develop principles and guidelines for handling live strandings events, including prevention, recognizing the cultural, political and socio-economic differences between countries;
3. *Requests* the Permanent Secretariat to:

- encourage training and exchange programmes for national stranding networks aimed at creating a common framework for rescue teams, in particular with respect to rehabilitation, intervention on live strandings and euthanasia procedures and dealing with the public;
- undertake trainings on necropsies, live strandings and response to emergency situation in the ACCOBAMS Area;
- maintain / establish (sub)regional mailing lists of participants in the stranding networks to facilitate exchange of information, in particularly in the South Mediterranean region;
- encourage data / tissue exchanges through collaboration with relevant databases and tissue banks. In this context, list of tissue banks registered with the CITES Secretariat should be made available.

ANNEX 1 COMMON DEFINITIONS OF TERMS RELATED TO STRANDING EVENTS

Sandro Mazzariol
DVM, PhD

In order to come up with a unified approach on how to manage strandings in general and live stranding in particular within the ACCOBAMS area as well as to facilitate data and information exchanges, it is fundamental to consider the presence of different approaches currently existing in different member states as potential barriers. The starting point towards the establishment of common procedures is a shared definition of all stranding events that can be identified and the possible stakeholders involved in these events, as stated during the joint workshop ACCOBAMS/PELAGOS on cetaceans live strandings organized in Monaco (October 29th-30th, 2014) in order to define common procedures in case of transboundary emergencies involving live stranded animals

This document summarize all proposed common definitions of term related to stranding events.

1. **Stranding**

Literally, a **stranded cetacean is one cetacean which body lies entirely on land**. The term is used to include both dead and live animals, the latter found in a helpless state after faltering ashore ill, wounded, weak, or simply lost. The term is sometimes expanded to include animals, dead or alive found floating or swimming, respectively, in shallow waters, in the latter case, showing clear signs of physiological dysfunction. One should keep in mind that many, if not most, of the dead stranded animals stranded while still alive and therefore the distinction between live and dead strandings relates to the timing of human attendance. The distinction is however crucial, as human intervention in a live stranding may prevent death, or hasten it to prevent suffering. On the basis of the number of animals involved, it is possible to distinguish between single and mass strandings.

1.1 **Single stranding**

This term refers in general to a **single animal involved, including a female and her calf**. Such events are the most common ones occurring in the Mediterranean Sea. Further definitions involve characteristics and features of the animal found stranded and general conditions of the findings. Accordingly, it is possible to distinguish:

- A. **Dead stranded cetacean: an animal lacking vital signs, which means without brain, respiratory and circulatory function.** This type of event requests specific procedures involving public actors (i.e. coast guard, local governments, sanitation authorities, public veterinarians, research institutions, NGOs, news media, etc.) in order to ensure public health and safety (delimitation of the carcass, rapid removal of the corpse, disposal of the carcass according to existing laws), research (biological information, postmortem investigations, recovery and storage of tissue samples and skeleton) and on-site public education; some countries consider dead stranded large whales as unusual events due to the compound logistics and procedures required.
- B. **Beached cetacean: this is another term sometimes used to define an animal found dead completely ashore.**
- C. **Live stranded cetacean: this term refers to a cetacean found alive, ashore or free-swimming in shallow waters.** Live-stranded animals are usually in need of medical attention and are unable to return to their natural habitat without assistance. In these cases, specific approaches should be considered in order to react to different situations. All interventions should be coordinated by a rescue team, including one or more expert veterinarians, able to assess the situation and apply its best knowledge and past experience through a well-established triage procedure. The latter should be used to decide whether the animal is immediately releasable, releasable after a period of rehabilitation or if euthanasia is the only option. In general, medical condition and the stranding characteristics (i.e., epidemic on going, mass stranding, etc.) are the basic criteria to decide the possible release into the wild but behavioural responses, ecological and ethological parameters and ethical statement may also be used in assessing the situation and in the decision process.

- D. Stranded cetacean: referring to an animal still in the water that is trapped, cannot cope or is outside of its natural environment;** these conditions suggest a perilous situation with a possible risk of stranding that may demand preventive measures and highlight the quandary of whether and when to act. More in detail in the ACCOBAMS area, this term referred to specific situations, often involving pelagic cetacean species, observed in unusual proximity to the coastline. Distance from the coasts depends on geography and bathymetry of the area. This term could refer also to coastal species when they are observed inside ports, estuaries, basins or in highly congested areas which could represent a risk for the animal's survival.
- E. Entangled cetaceans: cetaceans are included in this term when found entangled in fishing gear** and this condition impairs their swimming and diving abilities thereby compromising their feeding activities. Animals could be completely or partially entangled by nets. If human safety and animal welfare are ensured by available trained personnel and equipment, a procedure to release the animal could be attempted.

1.2 Multiple strandings

- A. Unusual Mortality Event (UME): this term refers to unexpected mortality of cetaceans at an abnormally large scale compared to average stranding reports for the species involved in the event and the area and period considered.** An immediate response is required and special investigation teams may be assembled to investigate the causes of these events. Main recognized causes are a rapid diffusion of a disease, biotoxins, human interactions (including environmental accidents) and malnutrition. Features of these mass mortalities (i.e. temporal and spatial distribution) do not correspond to mass strandings, as defined below.
- B. Disease outbreak: specific UME involving infectious agents.** A disease outbreak is the occurrence of cases of disease in stranded individuals in excess of what would normally be expected in a defined population, geographical area and/or season. An outbreak may occur in a restricted geographical area, or may extend over an entire basin involving several countries. It may last for a few days or weeks, or for several years. A single case of a zoonotic or a communicable disease absent from a given cetacean population, or caused by an agent (e.g. bacterium or virus) not previously recognized in that species or area, or the emergence of a previously unknown disease, may also constitute an outbreak and should be reported and investigated.
- C. Mass stranding: these events involve two or more cetaceans (excluding cow/calf pairs) stranded at the same time and place.** Several causes may be responsible for this event, including, but not limited to, extreme weather conditions, tidal changes, disease of one or several group members, or human-related events. It is noteworthy that some individuals involved in a mass stranding may be completely healthy.
- D. Atypical mass stranding: this definition refers to those mass stranding related to sonar exposure** in which animals do not strand all together as a single cluster but over a very short and defined time lap and within a confined space, both in association to the SONAR event. .

1.3 Usual vs Unusual stranding events

In order to implement a stranding network, it is often useful, depending on the internal organization, to define usual and unusual strandings. This definition is based on resources, knowledge and organization necessary to face these kinds of events.

- A. Usual strandings: this term refers to those stranding events occurring more frequently in a routine fashion.** In the Mediterranean Sea, small odontocetes found dead on the shore or close to the beach are included in this category. In these events, small teams are involved to recover the carcass, collect data, perform necropsy, store tissues, preserve skeleton and dispose of the corpse. Due to the limited scope, no immediate response is often necessary.

- B. Unusual strandings: occur rarely** and, due to the amount of animals, the size of the cetaceans involved and/or the presence of live animals, request an immediate and coordinated response that faces several problems such as animal welfare, administration of euthanasia and associated socio-ethical considerations, decisional processes and emergency. These kinds of events are in need of equipment and a well-trained and coordinated, often multinational, emergency team.

2. Terms related to dead stranded cetaceans

Postmortem investigations on cetaceans found stranded dead ashore are fundamental diagnostic procedures aimed to reveal and report any threats for cetaceans' conservation, by using an evidence-based approach. In the last years, an increasing number of skilled and expert veterinarian have been involved and forensic protocols and techniques have been developed and used, thus increasing the quality of the data collected. In addition, PM investigations are an essential source of biological data, including dietary, morphometric, genetic, etc. Dead cetaceans could have stranded alone or have been a part of a multiple stranding.

- 2.1. **Necropsy/autopsy:** synonyms of postmortem examination, a specialized procedure that consists of a thorough examination of a carcass by dissection to determine the cause, the mechanism and manner of death and to evaluate any disease or injury that may be evident. It is usually performed by a specialized veterinarian with specific training in animal pathology. If trained personnel are not available, veterinarians and/or biologist with an adequate training in cetaceans' anatomy could perform part of the gross and sampling procedures, as well as some of the main ancillary analyses.
- 2.2. **Cause of death/stranding,** could be defined as: the disease, injury or abnormality that alone or in combination with other factors (environmental, other concurrent diseases, age, etc.) is responsible for initiating the sequence of functional disturbances that ended in death. In the case of an animal stranded on the shore, the necropsy is aimed to determine the cause of stranding. During necropsy the following may be further defined:
- a) Immediate cause of death: final disease or condition resulting in death;
 - b) Underlying cause of death: the disease or injury that initiated the chain of morbid events that led directly and inevitably to death;
 - c) Contributing factors: other significant diseases, conditions, or injuries that may have contributed to death but which did not constitute an underlying cause of death.
- 2.3. **Mechanism of death:** the immediate physiologic derangement resulting in death. A particular mechanism of death can be produced by a variety of different causes of death. In an animal stranded alive that later died on shore, the mechanism is often asphyxiation due to mechanical compression of the chest by the animal's own weight.
- 2.4. **Manner of death:** how death came about; in the case of wildlife and, specifically, in cetaceans, we could distinguish: natural (due mainly to natural disease or toxic processes); related to anthropic activity (accidental - ship strikes, by-catch - and non-accidental or due to a volitional act - direct killing); undetermined (inadequate information regarding the circumstances of death in order to determine manner).

3. Terms related to live-stranded cetaceans

May strand singly or be a part of a mass stranding; may be found completely ashore or in shallow waters. Stranded cetaceans sighted swimming close to the shore, in ports or lagoons with clear avoidance behaviour and entangled cetaceans should not be considered stranded and a different approach with specific protocols should be used in handling such cases.

- 3.1. **Triage:** a process of determining the priority of treatments, based on the severity of patient's condition. The process rations patient treatment efficiently when resources are insufficient for all to be treated immediately (i.e. mass strandings). This approach has been developed and is used in emergency medical centers. In its application to cetaceans stranded alive, specific decisional matrices have been developed by several rescue

teams and stranding networks, in order to define the final destination of an animal, given that technical, economical and personnel resources are limited.

- 3.2. **Releasable cetaceans:** animals stranded alive, the ecological, ethological and health conditions of which, as evaluated by skilled veterinarians, are considered appropriate for an independent life and do not pose any risk to wildlife populations and public safety.
- 3.3. **Conditionally releasable cetaceans:** animals stranded alive, the ecological, ethological and health conditions of which, as evaluated by skilled veterinarians, are considered appropriate for an independent life and that do not pose any risk to wildlife populations and public safety, after further examinations or after a period of rehabilitation/quarantine, when national laws allows such procedures.
- 3.4. **Non releasable cetaceans:** animals stranded alive, the ecological, ethological and health conditions of which, as evaluated by skilled veterinarians, are considered NOT appropriate for an independent life and/or pose a risk to wildlife populations and public safety, even after a period of rehabilitation/quarantine. Euthanasia or permanent captivity, when national laws allow such procedure, are the most suitable options.
- 3.5. **Euthanasia:** Has been defined by the IWC and by the American Veterinary Medical Association in 2013 as “the use of humane techniques to induce the most rapid, painless and distress-free death possible”. It could be chemical (use of drug) or physical (firearms). A specific IWC report is available (Report of the IWC Workshop on Euthanasia Protocols to Optimize Welfare Concerns for Stranded Cetaceans).

4. Common code system for strandings

As already proposed during the aforementioned workshop on transboundary procedure, an alert system is proposed including coded definitions of stranding events herein presented.

CODE A: live cetacean/s at risk (close to the coastline or stranded)

In this category are included animal/s that are still alive in the water but with obvious signs of trouble in swimming, abnormal behavior for the species or unusual location, potentially threatening their safety. No rehabilitation efforts are attempted because it is difficult to approach the animal in the water.

CODE B: single live animal refloated after stranding or stranding and rehabilitated or following disentanglement (cetaceans stranded alive and entangled).

Single animal rehabilitated and released after being stranded alive in shallow waters, or lying on the beach, or entangled and released after its health assessment.

CODE C: mass strandings involving dead animals including atypical events

Simultaneous stranding of two non-dependent (not recognized as mother and offspring) or more dead cetaceans of the same species. Atypical mass strandings that may comprise of more than one species, are also considered.

CODE D: mass strandings involving live animals, including atypical events

Simultaneous stranding of two non-dependent (not recognized as mother and offspring) or more live cetaceans of the same species. Atypical mass strandings that may comprise of more than one species, are also considered.

CODE E: unusual mortality events

Increase in seasonal and/or regional stranding rates related to diseases or environmental factors (i.e. oil spills, biotoxins, peak of by-catch phenomenon), involving both live and dead animals.

CODE F: presence of anthropic activity using sound

The use of anthropic sound sources have been often related to mass strandings or unusual mortalities.

5. References

GERACI, J.R., and V.L. LOUNSBURY. 2005. Marine mammals ashore: a field guide for strandings, Second Edition. National Aquarium in Baltimore, Baltimore, MD, USA

General document on transboundary emergencies involving cetaceans in the PELAGOS Sanctuary, Monaco, October 29th-30th 2014.

ANNEX 2

COMMON BEST PRACTICES FOR A BASIC POST-MORTEM EXAMINATION OF STRANDED CETACEANS

Sandro Mazzariol
DVM, PhD

Conservation of cetaceans in the Mediterranean Sea and riparian waters is menaced by several threats. Often these are estimated on the basis of simple observation, but they are not associated to marine mammals' mortality by using an evidence based approach.

In order to quantify and explain the real impact of diseases, human activities and other causes of stranding, it is necessary to perform systematically postmortem examination of cetaceans found stranded on the coast. These procedures should be carried out through a shared approach in order to compare and exchange data collected during necropsies.

These approaches should be maintained not only within the ACCOBAMS Area but worldwide since the need of comparison and sharing is a common feeling. For these reasons, the present document has been prepared after consulting several colleagues (i.e. pathologists, stranding responders) working in the ACCOBAMS and ASCOBAMS Areas and also within the International Whaling Commission (IWC). This document should be considered as the starting point for a joint effort to build up a common procedure in order to study the causes of cetaceans' strandings and, in particular, the real impact of human activities on marine mammals' conservation.

In preparing this document it has been considered that in the ACCOBAMS area there are evident differences in the approach to cetaceans' strandings; procedures can be really informal or very well structured, services and equipment can be completely unsuitable or adequately organized, education and competences on the field can be at the forefront or totally insufficient. In some countries, National Stranding Networks are official or well-functioning and could have already adopted a national procedure for examining stranded marine mammals. For countries where National Stranding Networks are not existing or operate on the base of the volunteers' involvement there, a procedure based on the standards of more advanced countries could be too difficult to achieve.

The present document should be considered as a postmortem examination guideline supporting the development of national postmortem best practices in the Mediterranean Sea, Black Sea and riparian waters in order to standardize data collection and support those stranding networks without specialists working in these fields.

For those countries without a structured network including veterinarians and laboratories, these procedures could offer a simple tool to collect data in the proper way also by untrained personnel; furthermore, the document give also indication and suggestion to develop a more detail postmortem examination. On the other hand, for countries where a more developed procedure has been established, the present guidelines could give the minimum standard to be achieved.

These guidelines should be considered as the first step of a multi-level approach considering:

BASIC: basic gross examination and data collection

- collection of data on stranding event (date and location coordinates)
- data on animal involved (species, sex, age class, physiological status)
- measuring the animal
- gross examination with general description of main findings
- possible external signs of human interaction
- stomach content examination

INTERMEDIATE: sampling for general ancillary analyses

- sampling and performing microscopic examination and tissue bank
- sampling and performing microbiology
- sampling and performing toxicology
- sampling and performing and life history

ADVANCED: specific postmortem examinations and analyses with specific data and samples collection

- Dolphin morbillivirus
- Human interaction (bycatch and ship strikes)
- Sound related mortality
- Mass strandings

In order to diagnose specific causes of death, more detailed analyses and diagnostic procedures should be implemented: for these reasons, the creation of a list of internationally recognized experts and diagnostic laboratories is proposed and it is recommended to give whoever needs a proper support for more detailed examinations and/or in case of specific causes of strandings and diseases. In particular, this “expert panel” could develop dedicated diagnostic protocols in case of specific problems, as dolphin morbillivirus mortalities, ship strikes and interaction with fisheries, sound related unusual mortalities or be considered as advisory consultant. They could also support ACCOBAMS directly in the case of specific problems related to cetaceans’ mortality or intervene in case of unusual mortality events.

Finally, the expert panel could be appointed to revise and implement the present document with those indication and recommendation coming from the dialogue with ACCOBAMS and IWC in order to compare and share data as well as implement the guidelines with new information and diagnostic approaches. These could be foreseen periodically during international meeting as European Cetacean Society which could also support a common protocol for postmortem investigation to be used around Europe.

PROPOSAL FOR POSTMORTEM BEST PRACTICES IN CASE OF CETACEANS STRANDINGS

An autopsy, also known as a postmortem examination or necropsy, is a specialized procedure that consists of a thorough examination of a carcass by dissection to determine the cause and manner of death and to evaluate any disease or injury that may be evident. It is usually performed by a specialized veterinarian with a specific training in animal pathology. If trained personnel is not available, veterinarians and/or biologist with an adequate training in cetaceans' anatomy could perform part of the gross and sampling procedures, as well as some of the main ancillary analyses (for instance life history, genetics, gastric content analyses, toxicological studies).

1) **Main goals of a postmortem examination**

As already stated, through a standardize procedure, necropsies are aimed to determine:

- a) cause of death/stranding: it could be defined as the disease, injury or abnormality that alone or in combination with other factors (environmental, other concurrent diseases, age, etc.) is responsible for initiating the sequence of functional disturbances that ends in death. In the case of animal stranded on the shore, the necropsy is aimed to determine the cause of stranding. During necropsy it could be defined a:
 - b) - immediate cause of death: final disease or condition resulting in death
 - underlying cause of death: the disease or injury that initiated the chain of morbid events that led directly and inevitably to death;
 - contributing factors: other significant diseases, conditions, or injuries that contributed to death but which did not result in the underlying cause of death;
 - Cause of death does not always could be determined due to limiting factors (i.e. knowledge, lack of equipment, carcass preservation, etc.).
 - c) Mechanism of death: it is defined as the immediate physiologic derangement resulting in death (for example, haemorrhage, cardiac arrhythmia, cerebral hypoxia, sepsis, etc.). A particular mechanism of death can be produced by a variety of different causes of death. In animal stranded alive and dead on the shore, the mechanism is always mechanic compression of the chest acting on breathing;
 - d) manner of death: how the death came about; in the case of wildlife and, more in detail, in cetaceans, we could distinguish: natural (due mainly to natural disease processes; related to anthropic activity (accidental - ship strikes, by-catch - and non-accidental or due to a volitional act - direct killing); undetermined: inadequate information regarding the circumstances of death to determine manner.

In order to achieve these goals, it is necessary a very strict and well define procedure to collect data, in order to ensure a good quality of the information. This information obtained from stranded animals depends on a number of factors including:

condition, location and numbers of the carcasses

- quality of human resources: size, skills, organization, interests of the teams involved;
- existence of clear and detailed protocols;
- availability of equipment and supplies;
- time available;
- care in managing samples (packaging, labeling, shipping and storing).

2) **Documenting Data**

Information has scientific value only when carefully documented data are collected systematically using appropriate terminology. Depending on conditions listed in paragraph 1, data collection, as well as the postmortem procedure, may be basic (Level A), intermediate (Level B), or detailed (Level C) ([Appendix I](#)). The use of standardized data sheets and forms is recommended working on the field. Examples are reported herein ([Appendices III-V](#)).

Beyond written observations, photographic and video records may bring to life main details as color pattern, distinctive markings, scars or injuries, and the pattern of a mass stranding. Photographic documentation should include pictures of main distinctive pictures as well as a general view: at minimum, a full lateral view of the stranded

animals and of the head with exposed teeth or baleen should be attempted. For those species included in photo-ID catalogues, additional pictures of identifying characteristics should be taken. Photographs should include a reference scale of known standard size and, possibly, a label with date and location.

Rare specimens are especially valuable and require an extra measure to ensure a complete body of data. The entire carcass removal to a suitable laboratory or museum for study or preservation should be attempted.

3) Public Health

Dead and decaying marine mammal tissues harbor a variety of potentially harmful organisms, some of which can infect humans (i.e. Brucella, Salmonella, etc.). Dangerous consequences from exposure can be reduced by wearing appropriate clothing (protective overalls and rubber gloves), eye and mouth protection (safety glasses, sun glasses, disposable masks), and by a careful handling of tissues. Persons should protect open wounds with dressings and avoid contact with fluids or airborne droplets. Keep disinfectant solutions at hand.

In implementing the postmortem protocol, a list of equipment and disposal wearing should be prepared. In [Appendix VI](#) a list of these tools is presented considering the minimal kit that should be always available in case of emergencies.

4) Evaluation of the carcass

Before beginning postmortem examination, the quality of the carcass must be evaluated to determine its suitability for collateral examinations and further studies. The condition of the carcass should be evaluated by observation of external and internal features.

a. External Features

The condition of a marine mammal carcass cannot be evaluated solely by its outward appearance or estimated by knowing the time since death. The rate of decomposition is influenced more by body temperature which is influenced by blubber layer (higher in more robust animals) and by environmental temperature. Larger, rotund carcasses retain heat longer than smaller, thin ones.

Cetaceans (except mysticetes) sink initially at death, then float days or weeks later when buoyed by decomposition gases (putrefaction gas is produced in 36 hours after death in large whales), and arrive ashore outwardly slightly changed but internally decomposed. At the other extreme, seagulls may begin gouging the eyes and penetrating the skin and blubber of the jaw and body openings of a living dolphin, perhaps already mutilated by shells and rocks during stranding. By the time the animal dies, the carcass may already appear to be spoiled.

Rigor mortis (stiffening of the body after death) is not a valuable indicator of the time of death in cetacean species as it is in terrestrial ones. Also skin, eyes, and exposed mucous membranes dehydration cannot be considered a reliable indicator, since it occurs rapidly after death during air exposure, while these tissues retain their vital appearance longer in water or with humidity or precipitation and then, too, may be unreliable indicators. During buoyancy, sides of the carcass in the water are better preserved than those exposed to sun and air.

Bloating is generally a sign that a carcass is not fresh, though some diseases may cause gas production in tissues even in live animals. Tell-tale signs of decomposition include a protruding tongue and penis. At some point the gases escape, and it may not be obvious whether the process has just begun or ended. The only reliable approach is to examine the carcass internally.

b. Internal Features

The blubber of a fresh carcass is firm, mostly white, and only moderately oily, depending on the species. With time, it may become tinged with blood (imbibition) from underlying tissues. Eventually, the oil begins to separate (delipidation) and pool, leaving behind a lacework of greasy connective tissue fibers.

Fresh muscle is dark (except in fetuses and manatees) and firm, and the bundles are distinguishable and easily separated. As a carcass decomposes, the muscles become soft, pale, translucent, and pasty; fiber bundles become almost indistinguishable.

The rate of decomposition may be increased by the animal's terminal condition, such as a generalized infection with increased body temperature (fever) or wounds that expose the body to rapid bacterial invasion. Because blood tends to promote the process, decomposition is retarded in animals that bleed to death.

The rate of decomposition of an internal organ is related to temperature, the amount and arrangement of connective tissue, and proteolytic enzyme content. Skin, blubber and muscle can remain intact and may even show gross lesions for as long as seven to nine days after death. The heart and lungs maintain their integrity for perhaps two or three days, while adrenal glands, liver, spleen, brain, kidney, and mucosa of the digestive tract decompose with frustrating rapidity.

c. Carcass Classification

Despite uncertainties inherent in determining the stage of decomposition, any study on carcasses requires a system to define the quality of the material. Animals or carcasses are assigned to one of five basic categories, determined by specific characteristics, as specify here below and in [Appendix II](#).

CODE 1: Alive or just died (< 2 hours post mortem).

Uses: morphometrics; limited life history, external gross pathology, parasitology and microbiology; biopsies; blood studies, including DNA analysis and clinical chemistry. If died in two hours same Uses of Code 2.

CODE 2: Fresh carcass (< 24 hours post mortem).

Uses: morphometrics; DNA analysis; life history; parasitology; histopathology; toxicology; microbiology; limited blood studies; gas bubble analysis.

Characteristics: Normal appearance, usually with little scavenger damage, fresh smell, minimal drying and wrinkling of skin, eyes and mucous membranes, eyes clear, carcass not bloated, tongue and penis not protruded. Blubber firm and white; muscles firm, dark red, well-defined; blood cells intact, able to settle in a sample tube; serum unhemolyzed; viscera intact and well-defined; gut contains little or no gas; brain firm with no discoloration, surface features distinct, easily removed intact.

CODE 3: Moderate decomposition. Carcass intact, bloating evident (tongue and penis protruded) and skin cracked and sloughing, possible scavenger damage, characteristic mild odor, mucous membranes dry, eyes sunken or missing. Organs are basically intact.

Uses: morphometrics; DNA analysis; limited life history; parasitology; gross pathology; stomach contents; marginal for microbiology (virology, mycology, molecular analyses for bacteria while is limited for bacterial agents by direct methods) toxicology (useful for metal and organochlorines, poor for biotoxins); histopathology of skin, blubber, muscle (skeletal and heart), lung, and possibly firm lesions. Brain, lymphoid organs, liver and genital tract should be examined in any case since partial information could be collected; GI tract and related glands (i.e. pancreas) can provide limited information.

Characteristics: carcass intact, bloating evident (tongue and penis protruded) and skin cracked and sloughing; possible scavenger damage; characteristic mild odor; mucous membranes dry, eyes sunken or missing; blubber blood-tinged and oily; muscles soft and poorly defined; blood hemolysis, uniformly dark red; viscera soft, friable, mottled, but still intact; gut dilated by gas; brain soft, surface features distinct, dark reddish cast, fragile but can usually be moved intact.

CODE 4: Advanced decomposition

Uses: morphometrics; limited life history (teeth, baleen, bone, claws, some stomach contents, possibly reproductive condition); DNA analysis parasitology, microbiology (virology with sensitive technique) gross pathology and toxicology.

Characteristics: carcass may be intact, but collapsed; skin sloughing; epidermis of cetaceans may be entirely missing; often severe scavenger damage; strong odor; blubber soft, often with pockets of gas and pooled oil; muscles nearly liquefied and easily torn, falling easily off bones; blood thin and black; viscera often identifiable but friable, easily torn, and difficult to dissect; gut gas-filled; brain soft, dark red, containing gas pockets, pudding-like consistency.

CODE 5: Mummified or Skeletal Remains

Uses: morphometrics; limited life history (teeth, baleen, claws, bone), DNA analysis, toxicology; paleopathology.

Characteristics: skin may be draped over skeletal remains; any remaining tissues are desiccated.

5) General Considerations on Necropsy Protocol

The effectiveness of a postmortem examination is increased by following clear and concise protocols. The procedure should be prepared implementing a basic protocol considering main anatomical and physiological feature of the species, main diseases and pathological findings, logistics, number and available economical resources, personnel and equipment. In case of insufficient experience, knowledge and/or means to dedicate at this activity, it is important to standardize a very basic procedure in order to collect useful and comparable information, concentrating on fresh specimens and avoiding losing of resources.

In order to obtain best samples, a careful dissection should be planned, avoiding contamination of tissues by contact with dirty instruments, other organs, or body fluids and ensuring before the type and quality of equipment and packaging materials. With thoughtful planning, it should be possible to obtain morphometric data first, followed by external samples for microbiology.

Once the carcass is opened, tissue samples for microbiology and toxicology take precedence, followed by sampling for histopathology, parasitology, and life history. This order follows the sequence of a routinely performed gross examination as reported in the example in [Appendix II](#).

6) Examining the Carcass

Procedures for dissecting and examining carcasses depend on the size and species of the subject and personal preference of the investigator. The outlines reported in [Appendix II](#) is one approach to carrying out systematic examination of a carcass and it is based on specific protocols and personal experience.

This protocol could be varied on the basis of the experience, knowledge and researches of specific diseases or pathological condition, such as Morbillivirus, damages related to sound, mortalities related to by-catch and ship strikes, etc., and it could be implemented on the basis of available diagnostic technique and resources. Here below main steps of the procedures are resume.

- IDENTIFICATION of the species and DETERMINATION of the sex.
- DESCRIPTION and PHOTOGRAPH form, colour pattern, scars, other distinguishing features (e.g., number and position of teeth or characteristics of baleen), injuries, external lesions, etc.; for populations included in photo catalogues, photograph pertinent characteristics in order to identify the individual
- TAKE MEASUREMENTS (at least total length), including blubber thickness; obtain body WEIGHT if possible.
- EXTERNAL AND INTERNAL GROSS EXAMINATION. Note, describe and illustrate any changes, lesions, parasites and discharges considering their:
 - distribution: focal, multifocal, disseminate, diffuse, segmental, etc.;
 - location: the region, apparatus, organ and/or tissue involved, mono-lateral or bilateral;
 - volume: increased, decreased, maintained;
 - shape: bi-dimensional or tridimensional description of the lesion (round, spherical, target, irregular, etc.)
 - edges: definition (well defined, not defined, infiltrating), shape and profile;
 - surface: smooth, rough, depressed, raised, wet, dry
 - dimension: measure the lesion
 - texture and consistency: note any changes compared to normal features of the interested tissue and organ;
 - smell: if any

These feature allow an objective description of the change observed compared to normal anatomical features. In case of inexperienced personnel, these approach is quite simple and it could allow advices of skilled experts, along with pictures taken during examination.

- TAKE PICTURES of any features, changes considered anomalous for the experience of the person carrying out the necropsy

- At each stage of the examination, SAMPLE tissues as soon as they are exposed, starting from virology and microbiology, histopathology and toxicology.

7) Sampling

a) Blood and urine samples.

They provide an opportunity to evaluate the functional capacity of organs, as one approach to determining what processes might have been responsible for or associated with the stranding event. A broad spectrum of analyses can be performed, including plasma chemistry, hematology, antibody titers, and toxicology, as a means of investigating a range of pathologic conditions. Blood samples only have value for clinical pathology when taken from live animals, or within minutes after death. Organs deteriorate rapidly causing progressive changes in concentrations of blood gases, enzymes and electrolytes, among other parameters. Samples collected from animals dead for more than a few minutes are useful only for serological studies.

b) Morphometrics

Morphometric and descriptive data provide basic biological information and have added value when correlated with factors such as age, stage of maturity, reproductive status. The accumulation of such data results in a better understanding of general population health, demographic trends, and identification of discrete stocks. Every carcass provides some morphometric data, even skeletal remains. The amount available depends on the state of the carcass. Measurements are taken according to the appropriate protocol for the species. All measurements can be valuable, but standard length is consistently useful. It is the straight line distance from the tip of the snout (or the melon, if more anterior) to the tip of the tail or notch of the flukes. Blubber thickness (does not include skin) is measured from a perfectly perpendicular cut.

c) Life History

This analysis is aimed to obtain information on age, genetics, reproductive status, and feeding habits to understanding the general biology of the species. Certain life history information makes interpretation of pathologic and toxicological data more meaningful.

In general, biological data are additive; the more we can obtain on a given specimen, the more meaningful each element becomes.

d) Gross and Histopathology

Carcasses are a biological record of illnesses endemic in populations, diseases and disorders underlying natural mortality, and conditions that might have led the animal to strand. The information is tapped by careful selection of tissue samples for pathology studies. Injuries such as fractures and lacerations remain evident for long periods of time, as do certain firm lesions (e.g., tumors). Carcasses too decomposed for histopathology may still be useful for describing gross pathologic conditions. Brain, spleen, liver, and other enzyme-rich organs are the first to deteriorate.

e) Microbiology

This sampling procedure is aimed to evaluate factors underlying occurring in mortality. Studies reveal that marine mammals harbor a variety of microorganisms, some of which are known to have pathogenic potential. We now recognize that certain endemic diseases can periodically erupt into epidemics causing large-scale mortalities that have significant influence on the status of populations or stocks.

Even under ideal conditions, it is often difficult to associate bacteria isolated from a carcass with specific lesions. Bacteria associated with active infectious processes tend to endure longer in viable concentrations, and certain species may be isolated from more deteriorated carcasses, even frozen stored specimens.

Most viruses are fragile and have a short life span in decomposing tissue. Viruses that persist long enough to be harvested and identified, however, are generally responsible for some infectious process.

f) Parasitology

Virtually every marine mammal carcass has parasites. Most of these are innocuous and have value as ecological markers. Others, however, may cause serious illness to individuals and, perhaps, ultimately affect populations

g) Contaminants and Biotoxins

Marine mammals are the potential ultimate repository for oceanic contaminants passed through the food chain. Stranded inshore residents provide information on regional conditions and trends. Offshore species signal the extent to which the seas are being spoiled. Both groups reveal the influence of contaminants and toxins on health.

A commitment to collection and long-term storage of marine mammal tissues will enable us to follow patterns of biological toxins, organochlorines, heavy metals and other contaminants, and to recognize the need for change and help guide future policy. To be effective, the collection and preparation of specimens that form this resource must be impeccable, and the samples matched with reliable life history information.

h) Samples for Skeletal Preparations

While photographs and measurements can document the specific identification of some animals, skulls and skeletons can do it much better. In addition, osteological material provides a means of determining physical maturity of a specimen and may document skeletal abnormalities or injuries.

8) Necropsy forms

During postmortem examinations, it is necessary to collect data, observations and samples using a standardized approach. For these reasons, it is useful to prepare specific forms containing all information to be collected during necropsies. These forms are useful tools during the postmortem procedure which could be used both on the fields and in the laboratories. In [Appendix III-V](#), examples of these forms are attached to the present document. In particular, [Appendix III](#) is a necropsy form to be filled during gross examination noting any pathological change, peculiar feature or finding; in [Appendix IV](#), are listed all the information necessary to support the hypothesis of an human interaction; [Appendix V](#) is a simple checklist to remember all the samples to be collected during necropsy.

9) Specific analyses

These guidelines give information to implement a general and basic necropsy protocol, that could be carry out also by unexperienced and trained personnel with some basic knowledge of animal anatomy. In case of unusual mortality events, specific causes of death and/or threats related to cetaceans' strandings more detailed or different protocols should be applied. In particular:

- dolphin morbillivirus: this is one of the most relevant biological threats for cetacean in the Mediterranean Sea, since it caused several mortality outbreaks. Specific sampling protocols and molecular techniques has been implemented;
- by-catch: interaction with fishing activity is one of the most frequent cause of death of human origin. In order to determine if the animals died entangled in fishing gears, a detailed forensic protocol completed by microscopic analyses has been implemented;
- ship strikes: in order to understand if collision with vessels occurred with an alive animal or the interaction is postmortem, specific techniques has been developed for microscopic observation;
- gas and fat embolic syndrome and other sound related mortalities: mortality related to sound sources became famous after atypical mass strandings occurred spatially and temporally associated to military exercises using mid frequency sonar. Animals exposed to this sound source developed an embolic syndrome that could be diagnosed by gross, microscopic and chemical examination which require a specific sampling protocol. Further sound related damages could be found analyzing inner ear through electron microscopy examination: also this investigation require specific sampling and preservation protocol.

A list of scientists and/or institutions with specific expertise in the ACCOBAMS area should be provided along with their contacts for advisory service, creating an expert panel to support Countries of the Mediterranean Sea, Black Sea and Riparian Waters in case of necessity. If necessary, these reference laboratories are able to perform investigations and studies and could give specific information on sampling, preservation, packaging and delivery of samples collected during necropsy.

10) Tissue Banks

During postmortem examination tissue samples should be collected, properly preserved and forwarded to reference Tissue Banks as specified in the corresponding Guidelines.

If no national or neighboring tissue bank is available, the Mediterranean Marine Mammals Tissue Bank (www.marinemammals.eu) located in Padua is available for support, storage, and/or distribution of cetacean samples free of charge.

Appendix I DATA COLLECTION

1. Level A Data: Basic Minimum Data collected on the field

- a. Investigator: name and address (institution)
- b. Reporting source
- c. Species
 - preliminary identification (by qualified personnel)
 - supporting material (photographs; specimens, including tooth counts from odontocetes, or 2 pieces of mid-row baleen from mysticetes)
- d. Field number
- e. Number of animals, including total and sub-groups (if applicable)
- f. Location
 - preliminary description (local designation)
 - latitude and longitude GPS
- g. Date (mm\dd\yy), time of first discovery AND of data and specimen recovery
- h. Length (girth and weight when possible)
- i. Condition (recorded for both discovery and recovery times)
Codes as follows:
 - 1) alive
 - 2) freshly dead
 - 3) decomposed, but organs basically intact
 - 4) advanced decomposition (i.e., organs not recognizable, carcass intact)
 - 5) mummified or skeletal remains only
- j. Sex

2. Level B Data: Supplementary On-Site Information collected by direct observation or reported

- a. Weather and tide conditions
- b. Offshore human/predator activity
- c. Behavior
 - pre-stranding (e.g., milling, directional swimming)
 - stranding (e.g., determined effort to strand, passive, thrashing)
 - after return to sea (e.g., disoriented swimming, listing); note also ID number given after release and color
 - location of sighting
- d. Samples collected for life history studies: if these could not be collected during necropsy, they could be collected on the field
 - teeth, ear plugs or bone for age determination
 - reproductive tracts
 - stomach contents
- e. Samples collected for blood studies
- f. Disposition of carcass

3. Level C Data: Necropsy Examination and Sample Collection

- a. Gross pathological changes noted during necropsy
- b. Sampling of tissues for ancillary examination
 - microscopic examination (i.e. histopathology, fat emboli, electron microscopy)
 - microbiology
 - parasitology
 - toxicology
 - genetics
 - gas emboli
 - research of biotoxins

Appendix II

BASIC NECROPSY PROTOCOL

Before beginning postmortem examination, some biometrical data and life history information concerning the stranded animal should be collected in order to collect as many information as possible about the species and to gain further insight into the cause/s of death. In particular, data and information concerning any interaction with humans and with anthropic activities must be collected. Before handling the carcass, it is important to prepare all opportune protective equipment to prevent any transmission of infectious diseases to humans (zoonoses) and to prevent possible accidents with cutting tools.

1 Preliminary Information

Harmful zoonotic organisms can dwell within the carcasses of marine mammals, and personal and public safety precautions should be taken when handling dead marine mammals and tissues. Protective gear, such as disposable gloves, goggles, face masks, or splash shields should be worn to reduce the risk of contamination. All existing wounds should be well bandaged prior to beginning the necropsy and any injuries sustained during postmortem procedures should be thoroughly cleaned, bandaged and documented. Well stocked first aid kits must be on site at all times. Proper disposal receptacles for blades, knives, and needles as well as chemical spill treatment kits should be easily accessible. All chemicals should be handled in a well ventilated area. Exposed skin should be thoroughly scrubbed before leaving the lab or site. Equipment should be cleaned and disinfected. Disposal of the carcass should be well thought out in order to avoid exposing the general public to potential hazards. Prior to commencement of the necropsy, all necessary equipment should be set up and accessible.

1.1 Life History

Strandings offer a unique opportunity to study marine mammals. It is thus important to know the history of the stranded animal in order to evaluate any evidence of human interaction and to determine the cause and mechanism of death. It should likewise be remembered that a thorough necropsy begins with the stranding itself. Information that should be collected before the necropsy begins includes:

- The time and date of the stranding;
- Environmental conditions prior to and at the time of the stranding
- Location of stranding, including Global Positioning System (GPS) coordinates and topographic features
- Behavior prior to and during the stranding;
- Single or mass stranding (if the stranding was mass, it should be specified if it was a single or multi-species);
- Time and date of death;
- Euthanized or natural death;
- If there is a current Unusual Mortality Event (UME) under investigation;
- Mode of storage prior to necropsy;
- Details of any ropes, nets, or fragments attached to the carcass during recovery, including gear no longer on the animal at the time it was collected or of the necropsy;
- Record of any trauma known to be inflicted (ante- or post-mortem).

If storage prior to necropsy is necessary, such as overnight, refrigerate the carcass as soon as possible. The carcass must be examined for evidence of human interaction and morphometric data collected before storage. It is best to avoid freezing prior to necropsy as it interferes with microscopic examinations.

Other information that may be useful is the time lapse between the first sighting and the first response as well as any treatment or therapies carried out if the animal was alive. Any photos that taken by the first person on the site should be requested as these may have been taken when the carcass was in better condition.

An age estimate is initially made on the basis of weight and total length (adult, juvenile, adult, and neonate) and then confirmed by more other data such as microscopic teeth examination, ossification of the shoulder, gonadic features and the fatty acids in the crystalline.

1.2 Human Interaction Evaluation

Post-mortem investigations should be carried out scrupulously and carefully, following an established necroscopic protocol. Using this protocol will yield two relevant information: the first is an objective evaluation of an animal or carcass to determine if any evident sign of human interaction, could be ante- or post-mortem, healed or recently inflicted. The second is a subjective analysis by the examiner who will use all available information to evaluate if human interaction could have contributed to the stranding event. Objective findings proving anthropic activities affecting the conservation and management of cetaceans' population, should be promptly communicated to authorities. Documenting this types of interaction and identifying the spatial and temporal patterns associated may shed light on measures that can help to prevent future events. Nonetheless, it is important to avoid misinterpreting strandings and data relative to human interaction and all findings should be recorded as contributory causes.

In cases in which it is opportune or necessary to take legal action, physical evidence must be conserved. This evidence can include nets or fragments that have been removed from the animal, photos, and samples of tissues.

1.3 Relevant issues for a post-mortem examination

Post-mortem investigations need be carried out scrupulously and carefully following an established necropsy protocol. The diagnoses that are formulated may be utilized to review management and political strategies. Then, it is important to be cautious in formulating any hypothesis which need be proven and irrefutable for every animal. If there are any factor that could compromise the possibility of evaluating the carcass in a thorough and appropriate manner, the final report should reflect this uncertainty and the diagnosis could consider that it "could not be determined." The factors that can affect possibility of emitting a certain diagnosis, also for human interaction include but are not limited to: decomposition, damage caused by scavengers, inexperience in conducting these examinations, logistics (large animals that are difficult to manage and to evaluate from all points of view). All individuals/organizations utilizing and implementing this protocol must collect data in the same manner to permit the data to be analyzed on a broader scale.

1.4 Images and video

In addition to describing the physical observed evidences, it is very important to document any observations with images (photographs and videos). Digital pictures and videotaping can be extremely important when human interaction is being evaluated. Iconographic documentation can support any evaluations and the final diagnosis. With regard to documenting physical data, it is important to:

- Photograph or film everything even if there are no evident marks;
- A label and a ruler should be used in all images; the label should include the identification number, the date of the stranding, the species and the organization, close-up views should indicate the lesion/body part;
- Images should be taken from a wide angle to allow a viewer to place close ups in context;
- Care should be taken with regard to shadows, glare and fingers;
- All marks should be drawn and/or described.

Pictures are the virtual support of descriptions of the pathological report. They will also aid the pathologist in identifying the sampling area and to put together microscopic observations with macroscopic evidence. During a necropsy, labels should be used and must contain the following data:

- An identification number;
- The species;
- Date of death and/or necropsy;
- Where the stranding took place;
- Tissue/lesion.

A measurement scale (cm) should always appear in all images to have an idea of dimensions. Both the scale and the identification number must be clearly visible in all images. When photographing/filming wounds caused by propellers

images should be shot with the objective placed perpendicularly with respect to the axis of the surface of the lesions. It is important to photograph the organ or the entire tissue whenever there are lesions; other pictures can then be taken at a closer distance to provide more detailed information. If the tissue or organ have been removed from the carcass it is good practice to rinse and dry it to avoid blood excess or abnormal reflexes.

2 State of Conservation of the Carcass

It is possible to classify the state of conservation of a carcass found along the coastline using the criteria outlined by the most important manuals on the management of cetacean strandings. The following table delineates the criteria, which is based on physical parameters easily identified even by persons without any veterinarian experience, used to classify the state of conservation of a carcass and the code number assigned to each category; it also lists other investigations, depending on its status, that should be carried out.

Code	State of conservation	Description	Possible investigations
1	Alive/just died	Animal found alive or died at most 2 hrs earlier	Clinical examination, blood and urine exams, Microbiology/histology swabs, cytology, virology (from the tissue/PCR), serology, microbiology (cultures from tissues or PCR), parasitology, contaminants, biotoxins, genetics, biology (life history)
2	Carcass in good condition	Death took place within 24 hrs of the finding; minimal scavenger damage; normal smell; minimal drying or wrinkling of skin or eyes; eyes clear; no bloating; tongue and penis not protruded	Histology, cytology, virology, (from the tissue/PCR), serology, microbiology (cultures from tissues or PCR), parasitology, contaminants, biotoxins, genetics, biology (life history)
3	Moderate decomposition	Integral carcass with evident bloating (tongue and penis protruding) skin not integral with some sloughing, some damage by scavengers possible, mild odor, mucous membranes dry, eyes shrunken or missing	Histology (limited) virology (PCR) parasitology, contaminants, biotoxins, genetics, biology (life history)
4	Advanced decomposition	The carcass may be integral but collapsed; ample areas of sloughing skin, serious scavenger damage, strong odor, muscles and blubber easily detached from the bone, liquefaction of internal organs	Histology, (limited) virology (PCR), parasitology(PCR), contaminants (limited) biology, paleopathology (on the skeleton) (life history), genetics
5	Mummified or skeletal remains	Dehydrated, dry skin draped over desiccated bones	Biology (life history), genetics, paleopathology (on the skeleton)

Once the classification code has been made authorization has been given by the pertinent health authorities, one of three avenues are possible.

2.1 Category 1

1.a A living animal. A live stranding response unit should be contacted immediately and the animal should be transported to an appropriate facility if there is any hope that it can be recuperated and returned to the sea. The other possibility is euthanasia if the animal's state of health is seriously compromised.

1.b An animal found dead or one that has been euthanized. In this case the closest appropriate reference center should be contacted immediately. The center should in any case dispose of a veterinarian with some pathology training and experience with marine mammals and a biologist who can collect the necessary samples that will need to be conserved.

The necropsy should be carried out in an accredited facility or by personnel working for an accredited facility which disposes of appropriate equipment and logistics to carry out a thorough necropsy and to prepare for all the analyses listed above or are connected to appropriate organizations which do. In view of the rarity of the event and the perishability of the samples, all actions need be timely and coordinated. Efforts must be made to collect all the samples, possibly multiple ones, to guarantee that material is recuperated for scientific as well as diagnostic research. Again, in view of the rarity and importance the event and maintaining in all cases the role of coordinating the activities involved, the veterinarian/s in charge must carry out the necropsy taking into consideration, if this does not interfere with the protocol, the requests of various research groups to participate directly. When animals of large dimensions/weight are concerned, the extraordinary intervention of the Fire Department and Civil Protection Authorities or the assistance of the City administration may be necessary. Transportation may need to be organized to tow the animal to an appropriate site where the necropsy can be carried out and the skeleton can be recuperated. According to most ordinances, the city where the stranding took place is responsible for covering the cost of disposing the skeleton.

2.2 Categories 2-3

In these cases the carcass can still furnish useful information about the cause of death for both health and conservation purposes. An expert veterinarian as described in the point above is necessary. The value of the carcass is, however, inferior and as a result all activities can be carried out with greater tranquility and fewer samples will need to be collected. The standard protocol should be followed with the principal objective being that of diagnosing the cause of death, of establishing if any human interaction has taken place, and to furnish tissue samples for further investigations.

2.3 Categories 4-5

In view of the poor state of conservation, the qualified veterinarian of the Local Health Authorities who in any case is responsible for carrying out the samples requested and forwarding them together with photographic documentation to the appropriate centers can delegate personnel to collect the samples.

3 Life history and physiological parameters estimation

3.1 Age estimation

It is useful to estimate the age of beached cetaceans as this can modify the prognosis and all of the operations that need to be carried out.

Age estimation of cetaceans can be based on microscopic evaluation of the exemplar's teeth, but the procedure cannot be carried out on live animals. Age estimates can also be based on the dimensions and on other properties of the layer of dentin (calf, juvenile, young adult, old). The specimen's total length is the physical parameter that help to define both physiologic parameters that is age class and estimated weight. The mean lengths ascertained in particular make it possible to differentiate between neonates (dimensions similar to the mean ones outlined in the literature for the species) and adults. Neonates a few days old can be identified by the presence of lingual papillae and a patent umbilical cord. Other factors of importance are obviously length and in some species the season.

Animals which are suspected to be dependent maternally should not be liberated unless there is clear evidence of members of that same species in the vicinity.

Intermediate length conditions falling between adult and neonates make it possible to classify the subject as young. That estimate can be confirmed by the color of the livery in some species of odontocetes (Risso's Dolphin, Beaked whale, etc.) and the limited number of signs attributable to intra-specific interaction.

Older specimens are characterized by dimensions comparable to those of an adult cetacean with perhaps some signs of muscular atrophy along the trunk or absent or worn out teeth. The table below outlines typical correlations between approximate lengths and age classes in species that are frequently beached on Mediterranean coastlines.

Species	Total length at birth (cm)	Total length calf (cm)	Total length 1 year (cm)	Total length 2 years (cm)	Approx age weaning (years)	Total length Weaning (cm)	Total length Adult (m)
Striped Dolphin Stenella coeruleoalba	93-100	100	166	180		170	2.2-2.6

Bottlenose Dolphin Tursiops Truncatus	117	100-130	170-200	170-225	1.5-2	225	2.2-3 cost. 2.5-6 pel.
Risso's Dolphin Grampus Griseous	110-150	120-160					3-4
Common Dolphin Delphinus Delphis	80-90	80-100				110-120	2.3-2.5
Rough Toothed Dolphin Steno Bredanensis	100						2.4-2.7
Long-finned pilot whale Globicephalea melas	177	160-200			2-3	240	4.5-5 F 4.5-6 M
Beaked Whale Ziphius cavirostris	270	200-300					6.7-7
Sperm Whale Physeter macrocephalus	300	350-500		670	>2	670	11-13 F 15-18 M

3.2 Weight estimation

It is important to estimate the weight of stranded animals for therapeutic purposes (to calculate drug dosages and other support therapies) or for logistics. The total length is once again used to hypothesize the subject's weight. The table below outlines some estimates underlining the relationship between the two parameters in five species of small cetaceans well represented in the Mediterranean Sea.

Total Length (m)	Maximum estimated weight (kg)		
	Striped Dolphin Common Dolphin	Bottlenose Dolphin Risso's Dolphin	Long-finned pilot whale
1	20		
1.5	60	65	
1.75		150	75
2	125		150
2.5	150	260	
3		370	

3.5		480	
4		600	2000
6			3500

To have more precise estimate it is possible to resort to a linear regression according to the $\log_e M_{\text{media}} = a + b \log_e L_{\text{max}}$ where M is the mass expressed in kg and L is length in centimeters. For a and b coefficients there is a variation linked to the species (there are differences between odontocetes and mysticetes) and sex. The sperm whale has a linear regression similar to that of mysticetes perhaps confirming its phylogenetic relationship to whales. A different formula to calculate weight is outlined for this species given its anatomic peculiarities ($M = 0.218 \times L^{2.74}$). The table below indicates the coefficients for the various typologies.

Family	Sex	a	b
Mysticetes	M	-7.347	2.329
	F	-7.503	2.347
Odontocetes	M	-8.702	2.382
	F	-9.003	2.432

3.3 Sex determination

The sex of a small cetacean can be determined by examining the ventral midline of the animal. Both male and female cetaceans possess a genital slit between the umbilicus and anus. The distance between the centers of the anal and genital openings are generally less than 10 cm for female cetaceans. The distance is generally greater in the male. A single short mammary slit can be seen on either side of the genital slit in most female cetaceans and occasionally males also possess this feature. One of the simplest ways to determine the sex in a cetacean is by blunt-probing the genital slit. If the probe angles forward, it has entered the vagina and it is, thus, a female. If the probe angles backward it has entered the penile opening of a male. Confirmation of gender is of course exposing the penis (in animals in moderate or poor state of conservation) or by internal examination.

4 Nutritional status

The nutritional status of a cetacean can be evaluated by examining the dorsal axis from a slightly inclined perspective in order to verify the profile of the body at the sides of the dorsal fin revealing the dorsal fin muscles formed by the epiaxial muscles. In a healthy, well-fed animal, the profile will be rounded and convex. A thin animal will show some loss of muscle mass and may show bilateral retraction of the dorsal-lateral profile. An emaciated animal will show a greater loss of epiaxial muscle girth and may be concave along the dorsal-lateral body. Cachectic animals will show even greater concavity at the nape.

5 External examination: examining the integumentary system

The external examination should include the investigation and description of the eyes, mouth, blowhole, umbilicus, genital opening, anus and skin. Take note of the dimensions (height x width, height x depth, diameter) shape, color, consistence, localization and distribution of any abnormalities noted.

- When examining the eyes, operators should look for discoloration, injuries and/or discharge;
- All lesions, signs of parasites, the color of the mucus membranes as well as worn, broken or missing teeth should be documented;

- The color and amount of discharge from the blowhole as well as the presence of parasites and/or obstructions must be noted. Culture swabs should be taken (in the case of code 1 or 2 conservation);
- The umbilicus should be examined in neonates for signs of infection and degree of healing;
- Lesions, discharge, or growth around the genital opening and anus should be noted and samples should be taken for histology, microbiology, molecular and ancillary investigations;
- If the animal has mammary glands, operators can attempt to express milk and note its color, consistency and estimate quantities (cc or ml). Milk can be expressed by pressing on the body about 10 cm dorsal and cranial to the mammary slit and massaging downward toward the nipple;
- Any scars, abscesses, ulcerations, erosions, wounds, and parasites on the skin should be thoroughly examined and documented;
- Photograph the dorsal fin in order to permit comparison of individual signs with ID photo records.

Take samples of all tissues mentioned and all lesions following the modality outlined in section 2. In particular, the following samples should be taken:

- Skin: make a sample of the skin of the apex of the dorsal fin (skin without blubber) for genetic analysis, take double samples (frozen and placed under a DMSO solution) and for histology. Select the skin, cleaning it from other tissues.
- Teeth: at least 4-6 teeth should be removed from the center of the lower left mandible to investigate the age and to carry out toxicological investigations (heavy metals). Teeth can be extracted by inserting a tooth extractor or a flat head screwdriver between the tooth and the alveolar wall. In some older animals a knife can be used instead of a scalpel to avoid breaking the blade. It is important to avoid breaking or crushing the tooth as this damage can render it useless for analysis purposes.

6 Removal of the external layers: skin, blubber, muscle

The procedures to evaluate the integumentary system and the muscles of the axial skeleton are outlined below.

6.1 The skin and blubber

The blubber must be removed before the examiners proceed to evaluate the body cavity. In the case of a small cetacean, the animal should be positioned left side up. Using a scalpel or a knife, a longitudinal incision starting just left of the dorsal midline posterior to the blowhole should be made and continued down the entire length of the animal ending at the dorsal tail stock. The incision must not penetrate or damage the skeleton but should cut through only the skin and blubber layers. A dorso-ventral incision perpendicular to the previous body length incision just cranial to the anterior insertion of the left pectoral flipper should then be made. Parallel incisions should be made down the length of the animal every 20-25 cm thus creating a series of flaps along the lateral body. The blubber should be separated from the muscle by cutting through the fascia or connective tissue at the top of each flap. By remaining between the blubber/muscle interfaces and reflecting the panel of skin down and away from the body in a dorsal to ventral direction, the blubber should easily separate from the muscle.

At this point it is possible to evaluate the thickness, color and texture of the blubber. The thickness of the blubber should be measured at three points (dorsal, midline and ventral) cranially to the cranial insertion of the dorsal fin. Parasites and abnormalities within the blubber layer should be noted. Samples of the blubber and of the subcutaneous tissue should be collected for histology and for analysis of contaminants. In the latter case, it is necessary to collect blubber without skin or muscle being careful to collect samples always from the same area, generally from the mid-thoracic region. Once the blubber has been examined the flaps can be separated from the carcass along the median sagittal line.

6.2 Skeletal muscle

Before removing it, the quality of the fascia and muscle on the body should be examined and all color, texture, thickness and abnormalities should be noted. Signs of hemorrhage, post mortem pooling of blood in vessels (hypostasis or post-mortem lividity) and bruising (hematoma) should all be noted. It is to be remembered that bruising generally result in a deep maroon to purple colour and gelatinous texture.

The large dorso-lateral muscle mass or epiaxial muscle spanning from the occipital ridge down to the tail stock can now be removed using the dorsal and lateral spinal processes as landmark boundaries for this muscle. It is opportune to trim away as much muscle as possible from the backbone and ribs. Samples of muscles for histology and contaminant analysis should be collected.

7 Internal examination

Once the external layers have been examined and removed the next step is the internal examination.

7.1. Removal of the scapula and pre-scapular lymph nodes

The pre-scapular lymph node must be located prior to the complete removal of the scapula, the oval to triangular shaped, beige to peach tissue located just underneath the cranial corner of the scapula proximal to the external ear. Normal lymph nodes throughout the body usually share the same characteristics: a well-defined oval shape, slightly firm texture, color is diffusely beige to peach with slight differentiation between the cortex and the medulla. If the tissue begins to vary from the homogenous peach to tan, it is indicative of a reaction. The size, shape, color and texture of the prescapular lymph nodes should be noted. Samples for histology, microbiology, molecular and accessory investigations should be collected.

The left scapula and appendage should now be removed by cutting through the connective tissue and muscles just underneath the bone. If the scapula is pulled ventro-laterally, reflecting it down, it should detach easily and a crackling sound as the connective tissues and muscles are being pulled and cut confirms that the incision is in the correct spot between the muscle groups.

Before cutting into the body cavity it is important to obtain uncontaminated bacterial and viral samples from the thoracic and abdominal cavities.

7.2 Opening the body cavity

In order to open the body cavity an incision should be made along the costal arch with the flat side of a knife or a scalpel keeping the tissue raised with tongs and leaving the muscle exposed the muscle. Once the peritoneal cavity has been penetrated, the incision should continue in a dorso-caudal direction first and in a caudo-ventral direction later following the muscular axis and moving towards the anus.

A sample of transudates, exudate or liquids, can now be collected with a sterile disposable syringe and can be described and weighed. The abdominal wall can then be folded over ventrally in order to complete the cranial and caudal incisions reaching the median sagittal line, arriving respectively at the xiphoid process and caudally at the anus. Once reaching the ano-genital region the pelvic rudiments can be recuperated dorsally and laterally to the anus in the abdominal wall and easily available in the male whose penis is anchored to the pelvic elements by two crura which are fused in the body of the penis to form a single corpus cavernous.

The organs in the abdominal cavity can now be examined and all its abnormalities (for example, ectopic spleens) can be verified. The intestine encumbers all of the peritoneal cavity and it is best to remove it before examining the other organs after collecting microbiological specimens and evaluating topographic variations of the organs. After having extracted the intestinal bundle using a scissors or the blade of knife, the mesentery should be cut at the point where it is inserted into the intestine in order to liberate the bowel loops. This operation will make it possible to observe the color of the mesentery and to reduce the pressure of the abdominal organs on the diaphragm making it possible to view it by lowering with a hand the stomach chambers and the liver.

The diaphragm is an elastic, expandable, thin, smooth textured dark brown muscular membrane inserted into the caudal ribs separating the thoracic cavity with the abdominal one. Note all variations in consistency and appearance. White streaks are frequent. Samples should be collected for histology.

7.3. Opening and examination of the thoracic cavity

The diaphragm should be punctured with a scalpel or scissors to evaluate the presence of negative intra-thoracic pressure (its absence is a sign of a pneumothorax, thoracic trauma, effusion or pneumonia) which can be verified by the presence of a sucking sound of air. The diaphragm can thus be separated from its insertion into the thoracic wall by resting the blade of the knife on the costal pleural surface and proceeding in a dorso-ventral direction from the spinal column to the xiphoid process following the costal profile.

To open the thoracic cavity, the cutter should start at the caudal end of the left rib cage and feel for the articulation between each individual rib and vertebrae. It is easy to separate the ribs from the costal cartilages without breaking any bones with the blade of the scalpel or a knife. While cutting, virology and microbiology samples and all liquids should be collected using a sterile syringe. Even chondro-sternal articulations can be cut to widen the window facilitating the operations pushing the sternum down. Beginning at the caudal ribs, the cutter can proceed to disarticulate the costo-vertebral articulations without breaking the bones and making the ribs rotate to favor the retrieval of the joints and the separation of the rib from the corresponding vertebra. The cutter should proceed from rib to rib from the diaphragm towards the head maintaining a constant angle of the scalpel on the articulation and cutting the intercostal muscles in order to move and work on the single bones. Both pathologic states and old age can affect the way the joints disarticulate. Since the more cranial ribs present twin costo-vertebral articulation, the cutter must cut the first articulation and then proceed with the scalpel going down along the body of the bone until the second one is found and cut turning the blade in the direction of the animal's longitudinal axis.

The articular surfaces should be smooth and not granular. The cutter can feel with his hand if there are any fractures or bone alterations of the thoracic cage. No matter how labored and long this procedure may seem, it is the only way a skeleton be preserved for use in pathological bone investigations or for a museum collection or other educational uses.

Once the thoracic cage has been completely opened, the topography of the thoracic organs and any possible lesions, color alterations, adherences, fluids or particular odors can be appreciated. At this point the examiners can go on to evaluate the internal organs using a systematic approach. The organs can first be examined in situ and then extracted for further examination. The collection methodology is based on sampling requirements, the state of conservation of the exemplar, and personal preferences. Internal fluids such as those from the gastrointestinal tract must not be contaminated by other tissues.

7.4 The tongue, larynx and trachea

To extract the tongue connected to the pharynx, larynx and trachea, the cutter cuts the floor of the oral cavity with the blade of a knife following the medial side of the mandible extracting the tongue with his hand. Once the cutter has reached the pharynx and the hyoid bone which sustains the tongue, he must search for the chondral articulations severing them with a scalpel or knife keeping the bones integral for future donation to museums. It is possible to penetrate the pharynx with a hand and dislocate the larynx with a slight amount of traction. As already mentioned, the larynx is elongated in a dorso-cranial direction and is situated in the choanae permitting the separation between the airway and the food passages. The structures of the soft tissues of the short visceral space of the neck together with the esophagus should be separated using a firm traction and helping oneself with a cutting instrument. Once these are dislocated and extracted from their natural location, they appear as elongated, hard, short, whitish, flexible, tubular, slightly dorso-ventrally compressed organs formed by continuous rings.

The pharyngeal mucosa should be examined and possible color and appearance alterations of every lesion, foreign body or exudate should be noted. One penetrates with a scissors the epiglottis lumen continuing the cut on the dorsal side between the two arytenoids highlighting the pharyngeal tonsil and continuing to cut the tracheal wall until reaching the bronchial bifurcation. Luminal contents (foam, fluid, blood, puss), the appearance of the mucous and of the folds of the laryngeal tonsil (hyperemia, edema, hemorrhage, petechiae, erosions) must be examined. Samples should be collected for histology.

7.5 The thyroid and parathyroids

The thyroids, sitting ventrally and the cranial branches of trachea are rather difficult to locate and identify as their aspect and consistency are similar to that of smooth muscle (Fig. 3.34). The parathyroids are small, light colored tissue attached to the thyroid along the cranial margin of the thyroid and can aid in identifying the tissue correctly if found. The tissue must be examined externally and internally using serial cuts, and evaluating the form, dimensions, color and consistency. A sample in formalin for histology, microbiology, molecular and ancillary (toxicologic and molecular profiles of enzyme induction) investigations should be collected.

7.6 The thymus

The thymus is a large, lymphoid organ that is primarily found in neonates and some juveniles. It is situated at the base of the thoracic inlet, cranial to the anterior margin of the heart. The primary function of this organ is to generate T-cells. The thymus is absorbed with time after weaning, thus it is not usually visible in adult marine mammals. The tissue should be examined externally and internally. Its size, shape, color and texture should be noted. A sample in formalin for histology, microbiology, molecular and ancillary investigations should be collected.

7.7 The tracheobronchial (TB) lymph node

The TB lymph node is located along the distal cranial ventral surface of the lung proximal to the bifurcation of the trachea. It can easily be located by reflecting the cranial lung tissue away from the cavity and palpating the connective tissue between the lung and anterior to the trachea bifurcation. This tissue should be identified and removed prior to removing the lung or trachea as it can easily be lost if there are no anatomical landmarks. The lymph node should be examined externally and internally by cutting it into a sandwich and describing the differences between the cortex and the medulla as well as any other variations in size, shape, color and texture. A sample in formalin for histology, microbiology, molecular and ancillary investigations should be collected.

7.8 Lungs

The lungs occupy the greater part of the thoracic cavity and are generally bright pink with a consistent sponge-like texture. Depending on its dimensions, it can be examined attached or detached from the trachea. The plural surface must be examined and the color pattern and texture noted and possible alterations in consistency can be found by palpation. Normal air-filled lung tissue bounces back immediately after being pressed with a finger (like a sponge) and float when placed in water or formalin. The internal organs should be examined using scissors to trace the trachea from the bifurcation along the bronchi and into the bronchioles of each lung. Note if there are any signs of fluid, froth, and/or parasites and describe the quantities and appearance.

Serial, parallel cuts perpendicular to the long axis of the body into the tissue should be made using a long knife and single sweeping movements to examine the parenchyma. The parenchyma should be examined and its color pattern and texture noted. A sample in formalin for histology, microbiology, molecular and ancillary investigations should be collected from the cranial lobes of both lungs (four sampling sites).

7.9 Heart and vessels

It is best to examine the heart with the organ still in situ if the dimensions of the animal permit. If this is not possible the heart can be separated maintaining the roots of the vessels cutting the lung arteries and the aorta at least 6-10 cm from their starting points. The pericardium is to be observed and described first and any thickening, increase in liquid, exudate or the presence of gas bubbles within the pericardium vessels (important in freshly stranded animals) should be noted.

Once the pericardium has been removed the external surface of the heart can be observed. Abnormalities in dimension, appearance, color and consistency of every heart structure must be noted. Once the right ventricle has been identified scissors should be used to make a small opening in the cranial right atrium and cut down along the

medial edge of the right ventricle down to the apex. The operator should continue cutting along the right ventricle side of the septum until this chamber joins the pulmonary artery and cut up through the vessel.

The left side of the heart can be examined using a knife or scissors and making a cut on the ventricular wall perpendicular to the septum from the apex to the base of the heart, cutting also the atrial wall. In this way the flaps of the mitral, the atrial valve, the atrial cavity, and the venous sinuses and the descending branch of the ventricle can be viewed. By cutting the atrial flap of the bicuspid inserting the point of the cutting instrument under it, one reaches the bulb of the aorta, exposing the origin of the coronary arteries above the semilunar and the aorta whose wall can be cut following the first bifurcations. Operators must look for signs of thrombi, endothelial plaques, whitish mineralization, aneurysms, or breaks and the consistency of the ductus arteriosus should be evaluated. The other alternative is to proceed as in the right part of the heart, by penetrating the atrium and following the coronary sulcus and the interventricular septum.

It is thus possible to evaluate the endocardium and to examine both chambers of the heart for the presence of nematodes or other abnormal material. The width of the ventricular chambers should be measured to verify their ratio (the normal ratio between left and right is 3-4:1 in adults and 2:1 in neonates or fetuses). Variations in width, thickness, appearance and consistency of the atrioventricular valves, which are normally homogeneously thin and slightly opaque, should be noted and described. Once the endocardium has been examined the muscle part can be evaluated by making bread-slice cuts, in particular in the subvalvular apparatus, in order to detect any variations in color, consistency, and to verify if there are any abscesses or granulomas. The right and left ventricles and the atria, septum, apex, atria and aorta should be sampled for histology.

7.10 The spleen

The shape and size of the spleen vary among cetacean species. The spleens of most dolphins are palm-sized, spherical and mottled dark purple to white with a smooth external texture. In other species it can be similar or smaller and elongated. Normally the spleen is located close to the main stomach chamber on the left side. The organ can be removed by detaching it from the omentum (thin, web-like, connective tissue). The shape, dimensions and appearance both externally and internally should be described. Verify and note the presence of smaller, accessory spleens on the visceral side. The organ should be sampled for histology, microbiology, and molecular investigations.

7.11 The adrenal glands

The right and left adrenal glands are located just anterior to the cranial pole of each kidney and are attached to the dorsal abdominal wall. The adrenal glands are small, oblong, light maroon tissues. Locating and extracting the adrenals prior to removing the kidneys is highly recommended as they can be difficult to locate without an anatomical landmark. The adrenals can be removed by gasping and pulling the tissue away from the body wall and cutting the surrounding connective tissue. Before sectioning, each adrenal should be measured and weighed (length x width x depth). Each adrenal should be cut with parallel cuts perpendicular to the longest axis. When cut, a normal adrenal will present a distinct darkened center (medulla) with a lighter perimeter (cortex). All alterations in shape, dimensions, color and appearance of the external and internal tissue as well as in ratios regarding the cutting surfaces (cortex:medulla equal to 1:1) should be noted and described. The presence of cavities, cysts and hemorrhages should be noted and the organs should be sampled for histology and secondary investigations.

7.12 The kidneys and the ureters

The kidneys are maroon, ovoid tissues immediately evident when the abdominal cavity is opened and made up of numerous, clustered reniculi (miniature kidneys) attached to the caudal dorsal abdominal wall. The kidneys can be detached using traction against their connective tissue after having identified and isolated the adrenal glands endeavoring to maintain the links with the bladder and the entire urinary system examining them after having removed them from the carcass.

The external capsule should be examined for the presence of fluid, hemorrhage or gas bubbles and their color, thickness, and opacity should be described and noted. The capsule should be cut and using tongs the cutter should attempt to separate the capsule while evaluating the degree of adhesion and the presence of sub-capsular alterations. The dimension, size, external color and appearance of the kidneys should be examined and then these should be cut longitudinally like a sandwich to examine the internal structure. The presence of stones and the differentiation between the cortex and medulla as well as the medulla:cortex ratio within each reniculus should be evaluated (the normal ratio is equal to 1:2). Each reniculus should be well demarcated but clustered together within the kidney itself. Samples for contaminants, histology, and microbiology, molecular and ancillary investigations should be collected.

7.13 The liver

Normally dark red, the liver is large and occupies a large part of the abdominal cavity adhering for the most part to the cupola of the diaphragm and covering the stomach. Once it has been separated from the abdominal organs and from the diaphragm together or after the gastrointestinal package, it is possible to examine the diaphragmatic and visceral surfaces of the organ and to note alterations in color, consistency and the sizes of the hepatic lobes. The organ should be weighed and the ratio with the weight of the rest of the carcass calculated: normally it is approximately 2-2.5%. Parallel cuts should be made of the parenchyma to detect any alterations in color and consistency in particular corresponding to lesions found externally. At the same time, the bile ducts should be examined for the presence of parasites. Samples for contaminants, histology, and microbiology, molecular and ancillary investigations should be collected. Note that all cetaceans lack a gall bladder.

7.14 The pancreas

The pancreas is a peach colored, irregularly shaped, pyramidal, softer tissue that is attached to the mesentery and sits in the curve of the duodenum. It can be removed from the cavity by detaching it from the connective tissue and duodenum. Its size, shape, color and texture of the surface should be noted and described. The parenchyma should be cut with two or three parallel cuts so that changes in color or texture can be noted. The ducts should be examined for parasites. Samples for histology, microbiology, molecular and ancillary investigations should be collected.

7.15 The stomach chambers

The stomach of most odontocetes are composed of three chambers: the fore stomach, main stomach and the pyloric stomach. The omentum is the thin, net-like connective tissue that is attached to the visceral side of the stomach. To avoid contaminating the remaining tissues in the body cavity or losing contents, it is necessary to tie off both ends of the stomach prior to extracting it. A tight, secure knot should be made at the location of the attachment of the esophagus to the fore stomach. A second one should be made just below the base of the pyloric stomach where the small intestines begin. The stomach can be extracted from the carcass by cutting beyond both knots. The serosal (external) surface of the stomach should be examined for discoloration and lesions. A gastric pathology can generally be suspected when the peri-gastric lymph nodes attached to the stomach are noticeably enlarged. Samples for histology, microbiology, molecular and ancillary investigations should be collected and a note about this should be made on the inventory list. Otherwise all excess attached tissue should be removed from the exterior of the stomach and it should be weighed.

Using a scalpel an incision should be made through the wall along the greater curvature of each stomach large enough to allow examination of the contents and the entire mucosal surface. Each compartment should be described as well as the composition of the stomach contents (fluid; whole or partially digested fish; fish bones; parasites; foreign objects) and their quantities, color and appearance. Before going on to further investigations, a sample of contents must be collected for biotoxins. The remaining contents can be emptied and rinsed into a sieve to ensure solid material is not lost and is thoroughly examined. All foreign objects must be saved for human interaction evaluation.

Once empty, the mucosa of the stomach should be examined and the color and texture of the mucosa of each compartment must be noted and described. The mucosa of the fore stomach is composed of squamous tissue and is usually white. The wall of the main stomach is stratified and usually thicker than that of the fore stomach and the mucosa is usually dark red. The pyloric stomach tends to be thin walled, glandular, and the mucosa is pink or stained

(yellow) with bile. The presence of ulcers, areas of discoloration and other abnormalities should be noted and described. The stomach should be weighed empty and samples of each compartment should be taken for histology.

7.16 The intestines

Examination of the intestines is preferably left until the end of the necropsy, even if it has already been extracted, in order not to contaminate the other organs. There is not a clear demarcation of the small and large intestines and as such the two can be examined together.

The transition from the colon to the rectum is indicated by the presence of a rectal lymph node near to the intestinal wall. It is to be remembered that cetaceans have anal tonsils near to the mucous-epithelial tissue junction near the anus.

The serosal surfaces of all the pieces should be examined for the presence of signs of hemorrhage, discoloration or parasites. The intestinal lumen can be inspected by making five to ten longitudinal cuts about 20-30 cm long. The colour, consistency, and appearance of the contents, the diameter of the lumen, the color and the appearance of the enteric mucosa and the wall thickness should be noted and described. Samples should be taken for histology. Feces should be collected for biotoxin analysis.

7.17 Mesenteric lymph nodes

Once called the pseudo-pancreas, the mesenteric lymph nodes are gray to cream colored finger-like connective tissue bands that are centrally attached to the mesentery. The lymph nodes should be removed from the mesentery and their form, dimensions, color and consistency should be noted and described. As these lymph nodes tend to have a more defined cortex and medulla, all of their parts and structures should be described. Samples for histology, microbiology, molecular and ancillary investigations should be collected.

7.18 The bladder

The bladder is a small, light pink organ that is found along the central body wall. It may appear as a thick walled, muscular organ, but if distended with urine, the walls may be thinned and semi-translucent. Before removing the bladder from the body, the contents should be extracted using a sterile syringe and a medium gauge needle. If none are available, the attempt should be made to clamp the bladder before removing it and to recuperate its contents without dissipating or contaminating them. The color, consistency and amount of urine must be described. Any stones detected must be described. Once the bladder is removed it should be examined internally by cutting along its length to expose the mucosal surface whose color and texture must be described. A sample of the cranial tip of the bladder should be taken for histology.

7.19 The reproductive tract

Female: Ovaries and uterus

The uterus and ovaries can most easily be identified by following the reproductive tract from the vagina to the uterus where it bifurcates to a right and left horn, each ending at the attachment of the ovaries. The uterus is a tan to pink tissue that varies in size and thickness depending on the maturity of the animal and its reproductive history. The size, shape, color and texture of the external and internal surfaces of the organ should be noted and described. The vagina and the lumen of the vagina should be examined and alterations in the mucous and/or the presence of lesions, foreign bodies or exudate should be noted.

If a fetus is present but is too small for a sufficient individual necropsy, the abdomen should be incised and microbiology and molecular samples should be taken and the fetus should be preserved whole in formalin. If the lung tissue floats in formalin or water this signifies that bronchiole expansion of the fetal lungs has taken place.

Off-while spindle-shaped ovaries are attached to the end of each uterine horn and their dimension, shape, color and appearance should be described. A mature ovary possesses random darkened notches or scars (corpora albicans)

which signify previous ovulations. The ovary of a pregnant female possesses a corpus luteum or a large yellow mass attached to the ovary. Before examining the organs internally the ovaries should be measured and weighed (length x depth x height), the scars should be counted, and the presence or absence of a corpus luteus should be recorded. The tissue should be examined internally and its color and texture should be recorded. Both the uterus and ovaries should be sampled for life history, histology, microbiology molecular and ancillary investigations.

Male: The testis and penis

The elongated off-white paired testes are located within the caudal abdominal cavity along the ventral wall, posterior to the kidneys and near to the midline. The testes (with the epididymis attached) should be removed from the body and measurements (length x depth x height) should be taken and the organs should be weighed. The size, shape, color and texture should be examined internally and externally. The epididymis should be sectioned to evaluate the presence/absence of sperm. Samples of each testis should be obtained for life history, histology, microbiology, molecular and ancillary investigations. The penis should be examined externally and evaluated for the presence/absence of discharge, papillomas or other lesions.

7.20 The central nervous system

As the brain is the most fragile and easily disrupted tissue in the entire body, extreme care should be taken when it is being removed from the skull. Before removing it, a sample of the cerebrospinal fluid should be taken for cytology and culture. To do so the overlying soft tissue at the back of the head and neck must be removed to gain access to the atlanto-occipital joint. Then a sterile needle and syringe should be used to collect the clear, viscous fluid.

The head should first be detached from the body to safely remove the brain. This can be done by cutting behind the blowhole down to the joint between the skull and cervical vertebrae, and then completing the cut ventrally. Then the articular capsule of the atlanto-occipital joint can be cut severing transversally the spinal cord, the meninges, and the ligaments in the vertebral canal. It is then possible to remove all excess skin, blubber, muscle and connective tissue from around the dorsal and caudal skull. Using a stryker saw or a hacksaw, transversal cuts can be made both to the left and to the right on the occipital condyles, then going up laterally to the cranium and crossing dorsally the cranial vault just posterior to the marked transverse ridge at the apex of the skull. It is important to be extremely careful and to fully penetrate the bone while avoiding contact with the brain. A chisel should be carefully placed in the incision between the cut bone and then turning the instrument in more than one place until the last bone fragments become detached and the skull comes away in one piece. Once again, the operation must be carried out cautiously and being careful not to penetrate the encephalic tissue and not to use edges or borders as levers so that the bony shelf (the tentorium cerebelli) does not damage the underlying tissue. Using their fingers, the cutters should try to separate the meninges from the cranium and to work under the brain to sever the cranial nerves. At times inversion of the head allows the brain to gently descend into the palm of the cutter's hand.

The brain should not be handled excessively. The external surface and any asymmetries of any of the structures (right and left cerebral hemispheres, cerebellum and brain stem) should be observed. The color, texture and presence of parasites or lesions should be noted and described. Samples should be taken for microbiology, molecular and ancillary investigations. The brain in toto should be placed in formalin for histology. It should be kept immersed in the fixative solution for an hour at -20° to achieve consolidation of the encephalic mass and cutting it in transversal parallel sections 1 cm thick permits a rapid and correct fixation of the nervous tissue.

Once the brain has been removed, the pituitary which is situated in a recessed bone at the base of the brain next to the optic chiasm, is exposed. It can be recuperated by lifting it out with tongs and/ utilizing a scalpel.

8 Samples management

The necropsy of a stranded cetacean is carried out to gain further insight into the species and into the cause of death. As a necropsy produces a series of gross observations, these can be utilized to establish not only the cause of death but, at times, also the cause of the stranding. Subsequent investigations such as histopathology are part of this process and can help to formulate the final diagnosis. Laboratories can also screen specific tissues for a wide array of potential

pathogenic agents. It is important in any case that while meeting the objectives of ordinary screening regimens, samples are taken to ensure that a full differential diagnosis can be attained. The entire process requires a precise sampling protocol. A necropsy sample inventory list is necessary to ensure that all the samples needed for the planned analyses have been taken and that the quantity of tissue/material needed and the opportune modality of taking and storing samples have been provided for/organized. It is thus of utmost importance that all involved understand the priority that should be giving to collecting samples. As a general rule, when in doubt, it is better to take unnecessary samples which can be disposed of at a later time. The table at the end resume sampling and preservation for each investigation it is possible to carry out on stranded cetaceans.

8.1 Sampling for Histopathology

Histopathology is the microscopic examination of tissue samples which leads to the diagnosis of disease. Histopathology is most effective when collected from the freshest (code 2) carcasses. Decomposition significantly alters the structures of tissue cells and diminishes the value of histopathological investigations. Only a limited reading can thus be expected from carcasses of later codes.

Two sets of samples should be collected for histological analysis: one for analysis and the other to archive. As a rule, the tissues should be fixed using a ratio of 10:1 of 10% neutral-buffered formalin to tissue. A lower ratio will prevent adequate fixation causing the tissues to decompose. It is helpful to rinse excessively bloody samples with a light stream of water to allow for more efficient fixation.

When sampling tissue for histological analysis, only a small 1 to 2 cubic cm sized section of the tissue is required in view of the fact that formalin penetrates at a velocity of 0.8-1 cm/24 hours, a parameter that varies depending on the tissue and the quantity of blood that are present. If the tissue is larger, it is helpful to make one or two parallel incisions to allow the formalin to adequately penetrate and to fix the tissue.

It is important to avoid altering the surface layers or mucosa of tissues intended for histology as these could cause artifacts that will be evident under the microscope. The best way to ensure that the highest tissue quality is submitted for histology is to trim tissues on a cutting board with a sharp knife or scalpel and to avoid using scissors.

Plastic, wide-mouth, screw-top jars are preferred for storing histology samples. Ideally the fixative should be changed after the first hour of exposure.

The list of histological samples includes the greater part of all of the tissues. Unless an abnormality is observed in lymph nodes in other locations throughout the body, only the tracheo-bronchial, prescapular, and mesenteric lymph nodes are suggested for histology. If tissues appear abnormal, it is important to obtain a single section that includes both normal and abnormal tissue. All samples should be clearly labeled. Representative samples from all sections (caudal, cranial, medial and distal) of larger, major tissues (i.e. Lung and liver) should be collected. Any additional tissues collected for histology should be listed at the bottom of the inventory list.

8.2 Sampling for cytology

Simple impression smears can furnish real time feedback to help formulate possible hypotheses. Impression smears are collected by pressing a clean microscope slide on a cut surface of interest, leaving it to dry, and staining it with one of the common staining protocols. It can then be examined under a microscope, if available.

8.3 Sampling for virology

For most virology screening protocols, the basic reference samples are: serum, lung, liver, spleen, lymph nodes and brain. Additional samples can include skin, muco-cutaneous junctions or the oral cavity, rectum, and urogenital tract. If a fetus is present, the same samples outlined above should be collected, as well as the adrenal glands and placenta. Tissues to collect and suggested storage media with regard to Morbillivirus screening tests are itemized on the sample inventory list provided in the appendix. For other specific tests, the reference laboratory should be contacted for the tissues they require and the proper storage protocols.

The most accurate virology results are derived from code 2 carcasses. Code 3 carcasses can, however, be successfully screened for virology by Polymerase Chain Reaction (PCR) analysis. Fresh tissue should be stored in sealed, sterile whirl-pack bags and transported on ice to the receiving laboratory as soon as possible. If fresh tissues will not be sent for immediate analysis, these should be stored at -80° . Virus isolation from frozen samples can be detected through PCR. Samples should be transported to the receiving laboratory on dry ice.

In some cases, fixed tissue can also be utilized for specific antigen detection by means of immunohistochemistry (IHC). Viruses can also be detected morphologically using electron microscopy.

8.4 Sampling for microbiology

- *Culture Swabs*: it is of utmost importance that the necropsy unit be in agreement with the microbiology laboratory about the nature of the swabs and storage and transportation media to use to ensure the best results and the greatest diagnostic capacity for aerobic and anaerobic bacteria. Modalities guaranteeing sterility while samples are being taken are essential to prevent contamination of tissues for microbiology culture swabs. Samples of internal organs should be carried out in situ. A new sterile stainless steel scalpel blade can be sterilized using a butane torch and the intended incision site can be flamed for one to two seconds. Then a single straight incision can be made to the tissue or cavity. The culture swab can then be inserted into the incision and rotated to facilitate imbibition. Fluids can be aspirated into a sterile syringe and microbiology, cytology and PCR cultures can be undertaken. Swabs should then be placed in appropriate transportation containers to decrease the chances of contamination and if possible sent for analysis to the laboratory on the same day. If the analysis must wait until the next day, the swabs should be stored at room temperature.

Results from culture swabs should be interpreted with caution as bacteria tend to multiply and travel through multiple organs soon after death. For this reason, culture swabs are preferably taken from fresh carcasses (codes 1-3) unless an unusual lesions is observed in a carcass of a later code.

- *Tissue samples and PCR*: PCR analysis can be utilized to identify the pathogenic agents found in the tissue samples of carcasses of varying conditions. Target tissues for these analyses can vary but generally include: liver, kidney, lung, spleen, pancreas, gonads, brain, lymph nodes, conjunctiva, and muco-cutaneous junctions of the oral and urogenital tracts. It is of utmost importance to consult with laboratory technicians in advance to come to an agreement about the tissues to sample. Only a small amount of tissue which can be collected in centrifuge tubes is needed. Sterile dry swabs can also be used to collect DNA for analysis. The swabs should then be placed in collections tubes. Swabs and tissues should be stored at -80° C.

8.5 Sampling for parasitology

The collection of parasites is important not only for species identification and documentation of specific parasites in marine animals, but they may also harbor pathogens and could be useful in viral isolation, such as morbillivirus. After fully rinsing the dead parasites with saline, these can be stored in ethanol at room temperature. If an in-house parasitologist is available and able to examine the parasites while they are still alive within a short time, samples should be stored in saline. The parasitologist can, in any case, furnish further information.

8.6 Sampling for toxicology

Toxins and other chemicals that exist in the marine environment, be they naturally occurring or human produced, can be ingested by marine life and incorporated into their tissues. Contaminants can bio-accumulate in the tissues of marine life during the lifetime of the animal and, as they are the top of the food chain, marine mammals have the potential to retain high levels of toxins in their tissues. High contaminant levels can have numerous, negative impacts on the health of marine mammals, including compromising their immune system and affecting their behavior and/or

development through hormonal disruption. Sampling tissues for the presence of contaminants can, therefore, lead to a better understanding of the factors involved in the deterioration of the general health conditions of these animals. The tissues collected for the analysis of contaminant levels are blubber, muscle, liver and kidneys. The laboratory may require that the skin and muscle attached to the blubber be removed. Each tissue section should weigh at least 100 grams and be wrapped completely in acetone washed aluminum foil and placed in ziplock bag and stored in a freezer at -20°C.

8.7 Sampling for biotoxins

Biotoxins are naturally occurring toxins produced by dinoflagellates and other marine algae that accumulate in animals and which are transmitted by the food chain. Fish and invertebrates contain biotoxins which, when ingested in large quantities, prove to be harmful in larger predators such as marine mammals. The most frequent algal biotoxins include domoic acid, brevetoxin, and saxitoxin, which are all neurotoxins. Biotoxin samples should be collected when an algal bloom is suspected in the surrounding area and/or the live animal exhibited neurological symptoms.

Biotoxin samples include tissues and fluids such as: liver, kidney, serum, aqueous humor, stomach contents, intestinal contents, feces, urine. Tissue samples can be stored in plastic, zip-lock bags. Stomach and intestinal contents, feces and urine can be collected in appropriate sized vials, usually 10-20 ml. Five to ten ml of urine and one to two ml of aqueous humor – the thick, watery substance that is located in front of the lens of the eye – should be collected using sterile syringes and needles and stored in appropriate sized vials. These samples should be stored at -80°C unless being shipped immediately on dry ice.

8.8 Life history and genetics

On the basis of data that is collected and information that is registered it is possible to evaluate the biologic parameters of the exemplar being investigated. Age, genetics, trophic position, habitat, and the reproductive status of a stranded animal can be assessed by collecting teeth, skin, stomach contents, gonads and skeleton. This information not only helps us to understand the dynamics of the specific exemplar and its species but it can also aid us to interpret other findings such as those concerning histopathology and contaminants. More can also be learned with regard to the impact and vectors of potential threats to the marine environment at large.

- Life history data

- Four to six teeth from the mid-lower left mandible of an odontocete should be collected and placed in a ziplock bag; half of these should be frozen and the other half should place in formalin.
- Any discharge from the mammary glands should be collected in a tube and frozen at -20°C.
- Sections of both gonads of both sexes and the uterus of the female should be fixed separately from all other tissues intended for histology clearly labeling the right and left sections.
- If a fetus is present and not large enough for a separate necropsy, the entire body should be placed in formalin.
- Collect the stomach contents and freeze it at -20°C for analysis. Diet scientists generally request an unopened stomach but this may compromise microbiology analyses.
- The entire skeleton should be conserved for osteological analysis, cleaning and museum archiving. It should be stored at -20° C until it can be cleaned.

- Genetics

Two, full thickness skin samples should be taken from each animal for genetic analysis. One sample should be conserved entire in a ziplock bag at -20° while the other can be diced into 1 mm cubic pieces and placed in 20% dimethylsulfoxide (DMSO) solution.

8.9 Labelling and grouping

It is wisest to use a double labeling system so that there is a legible, complete label available both within the container and another outside of it. The one on the inside should be written on water proof material in indelible ink. Each label should indicate the animal's field number, genus, and species ID, its sex, the date of death and/or stranding, its conservation code, how it died (use E for euthanasia and D for natural death), the place it was stranded and the tissue

type. For histology samples it is possible to attach the label directly to the container or to write the information with an indelible pen on a dry surface.

Once the samples have been collected and placed in appropriately labeled containers, these should be grouped together and placed in larger containers according to the type of storage they require; frozen samples taken for life history or genetics can, for instance, be placed in larger containers and labeled as life history and genetics. All samples for contaminants can be grouped together in larger containers, etc.

8.10 Tracking Samples

It is extremely important that all samples archived or sent for analysis are well documented in view of the fact that these animals are to be considered property of the state and are protected by the Convention of Washington.

DIAGNOSTIC INVESTIGATION	ORGAN OR TISSUE	COLLECTION MODE	CONSERVATION MODE
Virology	Lung Liver Spleen Brain Intestine Kidney Muscle Placenta and fetal tissue	2 cm ³ of aseptic sample	Freeze, -20°C
Microbiology	Lung Liver Heart Blowhole Spleen Kidney Brain Other pathological tissue	Aseptic sample or swab	Refrigerated, +4°C
Brucella spp.	Spleen Lymph node Blubber lesions Prostate Testicles Epididymus Uterus Placenta	Aseptic sample	Refrigerated, +4°C

Hystopathology	All organs and lesions	1 cm ³ of tissue	10% Formalin
Parasitology	Parasites		70% Ethanol
	Intestine	5 cm ³ of aseptic sample	Freeze, -20°C
	Liver		
	Lung Organs with parasites		
Age estimate	Gonads	At least one	10% Formalin
Diet and life history	stomach content	Plastic box	Freeze, -20°C
Serology	Blood	From right ventricle with a sterile syringe	Spin-dry the blood at 3000 rpm and freeze the serum at -20°C
Contaminants	Muscle	15x20 cm of aseptic sample	Freeze, -20°C
	Fat tissue		
	Liver		
	Spleen		
Algal biotoxins	Stomach content Urine Faeces	Plastic box	Freeze, -20°C
Life history and morphometric studies	Skeleton, skull		Freeze, -20°C
Genetic	Muscle	1 cm ³ of aseptic sample	Freeze, -20°C

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Appendix III - NECROPSY FORM

Event Info	Animal Info
Stranding Date: _____	Sex: M F CBD
Recovery Date: _____	Length: _____ cm / in / ft
Euthanized / Died	Weight: _____ lbs / Kg
Date : _____	Pup / Calf / Young / Sub-adult / Adult
Necro Date & Time: _____	Condition at Stranding: 1 2 3 4 5
Storage Prior to Necropsy: _____	Condition at Necropsy: 1 2 3 4 5

CARCASS DISPOSITION:

HISTORY:

COMMENTS:

Necropsy Observations: Please note general observations of color, condition, textures, etc. even when utilizing NA= not applicable, NE= not examined, NSF= no significant findings, NVL= no visible lesions. List weights (g) next to each organ examined.

EXTERNAL EXAM

Body Condition: Robust 5 - Normal 4 - Moderate 3 - Thin 2 - Emaciated 1
Skin / Hair Coat (color, condition):
Wounds / Scars:
Lesions:
Parasites:
Nostrils / Blowhole:
Mouth (tongue, teeth condition, ulcers) / Mucous membranes (color)
Eyes (discharge, color, ruptures):
Ears:
Genital slit / anus:
Umbilicus: Pink Open Healed:

INTERNAL EXAM

MUSCOLO/SKELETAL SYSTEM

Blubber:
Muscle:
Diaphragm:
Skeletal:

CIRCULATORY SYSTEM

Pericardium:
Heart:
Vessels:

PULMONARY SYSTEM

Trachea:
Bronchi:
Lungs (colour, condition, edema, congestion, consolidation, granulomas, emphysema, lesions):
(R)
(L)

GASTROINTESTINAL SYSTEM

Esophagus:
Stomach (contents, ulcers, mucosa, parasites):

Weight Full: _____ Weight Empty: _____

Small Intestine:

Large Intestine:

Colon:

Omentum, Mesentery, Peritoneum:

Liver (colour, congestion, lesions, size):

Gall Bladder / Bile Duct / Pancreaticoduodenal Duct (colour, amount):

Pancreas:

LYMPHATIC SYSTEM

Thymus:

Spleen:

Scapular Lymph Node:

Tracheobronchial Lymph Node:

Mesenteric Lymph Node:

Other Lymph (list location):

URINARY/REPRODUCTIVE SYSTEMS

ENDOCRINE SYSTEM

CNS

Thyroid:

Adrenals:

(R)

L x W x H cm:

(L)

L x W x H cm:

Other:

Kidneys (reniculi differentiation, colour, condition):

(R)

(L)

Bladder:

Testes / Ovaries: Immature / Mature

(R)

L x W x H cm:

(L)

L x W x H cm:

Mammary glands:

Uterus / Cervix / Vagina:

Pregnant? : Y N NA (male)

Spinal Cord:

Brain:

Pterygoid Sinuses:

OTHER FINDINGS

Thoracic Cavity:

Abdominal Cavity:

Head:

Internal Parasites (location, type, number):

Differential Diagnosis from Gross Exam:

**Appendix IV
NECROPSY FORM FOR HUMAN INTERACTION**

1. GENERAL INFORMATION					
N. ID			Species		
Sex	Length		Examiner		
Cause of death				Date of death	
Location of necropsy examination				Date of exam	
Video	YES	NO	Photo	YES	NO
Conservation Code			Fresh o frozen		
1	2	3	4	5	
Note					

ND: Not Determined – NE: Not Evaluable

2. EXTERNAL EXAM			
a. Body condition			
Emaciated	Not emaciated	ND	NE
b. Signs of fishing net or lines. (indicate if YES, NO, ND, NV for each area and in the positive case describe the lesion)			
Head	Dorsal fin		
Pectoral fin left	Pectoral fin right		
Caudal peduncle	Other		
c. Presence of fishing nets on the animal			
	YES	NO	
Fishing nets have been preserved?			
	YES	NO	
d. Penetrating wounds			
YES	NO	ND	NE
Describe gunshot wounds, puncture wounds, from harpoon, etc.			
e. Mutilations			
YES	NO	ND	NE

2. EXTERNAL EXAM			
Describe cuts, tears, cracks in the body wall, missing appendages, etc.			
f. Hemorrhages and hematomas			
YES	NO	ND	NE
Describe extension and area.			
g. Post-mortem damage from scavengers and opportunists			
YES	NO	ND	NE
Describe extension and area.			

3. INTERNAL EXAM				
a. Sub-epidermal haemorrhages				
YES	NO	ND	NE	
Describe extension and area.				
b. Fractures				
YES	NO	ND	NE	
Describe.				
c. Content of airway and lung				
AIR	FLUID	FOAM	ND	NE
Describe lungs' appearance (heavy, consolidated areas, colour variations, etc.) and airway's content.				
d. Stomach content				
Describe stomach content, amount, presence of parasites and foreign bodies.				
Stored in frozen		YES	NO	
e. Histopathology		YES	NO	
f. Presence of macroscopically visible lesions				
YES	NO	ND	NE	
Describe.				
g. DIAGNOSTIC HYPOTHESIS:				

**Appendix V
STANDARD SAMPLES**

	Standard Samples						
	Life History	Genetics	Contam.	Histo.	Morbilli	Brucella	Biotox
Tissue	(Frozen or fixed)	(Frozen &/or DMSO)	(Foil wrapped and frozen)	(10% Formalin)	(Frozen)	(Frozen)	(Frozen)
Adrenal (R)	///	///	///		///	///	///
Adrenal (L)	///	///	///		///	///	///
Aqueous humor	///	///	///		///	///	
Bladder	///	///	///		///	///	///
Blood/Serum	///	///	///		///	///	
Blubber	///	///			///	///	///
Brain	///	///	///				///
Colon	///	///	///		///	///	///
Diaphragm	///	///	///		///	///	///
Esophagus	///	///	///		///	///	///
Feces	///	///	///		///	///	
Heart	///	///	///		///	///	///
Intestine	///	///	///		///	///	///
Kidney (R)	///	///			///		
Kidney (L)	///	///			///		
Liver	///	///			///		
Lung (R)	///	///	///				///
Lung (L)	///	///	///				///
Mesenteric Lymph.	///	///	///				///
Milk/Mammary Discharge		///	///		///	///	
Muscle	///				///	///	///
Oral Mucosa	///	///	///		///	///	///
Ovary		///	///		///		///

Pancreas	///	///	///		///		///
Prescapular Lymph.	///	///	///				///
Skin	///		///		///	///	///
Spleen	///	///	///				///
Stomach	///	///	///		///	///	///
Stomach Contents		///	///		///	///	
Teeth		///	///		///	///	///
Testis		///	///		///		///
Thyroid	///	///	///		///	///	///
Trachea	///	///	///		///	///	///
Tracheobronchial Lymph.	///	///	///				///
Urine	///	///	///		///	///	
Uterus		///	///		///		///

Appendix VI

LIST OF EQUIPMENT FOR NECROPSY ON THE FIELD

Here below is a complete list of instruments and equipment, besides individual protection tools (overall, gloves, glasses and facemasks, possibly disposable). Those items considered indispensable are written in bold form.

- First aid kit with multiple small and large bandages and disinfectant;
- Kit for severe injuries including large compression bandages, tourniquets, and shock treatments; eyewash canisters containing sterile solution; thermal blankets;
- blade guards;
- necropsy jumpsuit (canvas and disposable kinds);
- a portable GPS;
- Digital camera (w/ disc space for at least 100 images);
- A video camera and video tape for 8 hours;
- Photo ID board to insert in all photo images;
- 2- metric tapes, 30 m long;
- A portable blackboard to write out communications/data;
- 30 m of 2 cm braided line;
- 30 m of 1 cm line;
- 1 very heavy (10cm wide) nylon towing strap;
- 4-6 high quality knives w/ 30 cm blades;
- 4-6 high quality knives w/ 20 cm blades;
- 4-6 high quality knives w/ 15 cm blades;
- 2 diamond "flat" steels;
- 2 normal "draw through" knife sharpeners;
- 2 ball shears or large boning shears;
- 4 30 cm metal meat hooks;
- 4 15 cm metal meat hooks;
- 4 n.4 scalpel handles and a box of blades;
- 4 large rat-tooth forceps;
- 4 small forceps;
- 2-4 15 cm plastic rulers;
- 2 30 cm plastic rulers;
- 2 plastic "turkey basters" for collecting urine and fecal samples;
- a meter long bow saw used for trimming tree branches;
- aerobic and anaerobic swabs;
- 100 tyvek labeling tags;
- Fine and large point indelible ink markers;
- Permanent ink pens;
- Pencils for recording data on datasheets and cassettes;
- Some 5 liter plastic containers to wash the jumpsuits;
- 2 rolls of scotch tape;
- Heavy garbage bags;
- 2 large plastic cutting boards to cut and photograph tissues;
- One box each of large, medium and small latex gloves;
- 4 pairs of fish cutting gloves in each of the above sizes;
- Boots, overalls and rain gear
- 2 torches;
- 5 medium to large coolers: 2 for dry equipment storage; 2 for tissue containment on site and during transport; one for food cooler for drinks and food
- A large plastic transport box for rain gear and boots;
- A large plastic transport box for plastic trash bags and ziplock bags;
- Soap and scrub brushes for cleaning;
- Safety glasses and facemasks;
- 20 litre container of 10% buffered formalin with pour spigot;

- 10 litre container of 95% alcohol;
- 2 bread box size waterproof plastic boxes for gross tissue collection;
- 2 packages of extra-large ziploc 5 liter bags;
- 4 packages of large ziplock 1 liter bags;
- 6 packages of medium ziplock .5 liter bags;
- 10 packages of small ziplock .1 liter bags;
- 2 packages of ziplock bags for macroscopic samples;
- Histo cassettes;
- 10 20 cc plastic syringes;
- 5 50 cc plastic syringes;
- Roll of aluminum foil;

ANNEX 3

COMMON DATA COLLECTION FOR ALIVE STRANDINGS

Sandro Mazzariol
DVM, PhD

One of the expectations arise during the joint ACCOBAMS/PELAGOS workshop on common transboundary procedures on alive animals organized in Monaco in 2014 (October 29th-30th) is a clear need of capacity building to create a common sense and common strategy through specific trainings and exchange of experiences and information.

Since the experience with alive animals are limited to few cases per year and, in most of the countries of the ACCOBAMS area, there are no established protocols or skilled personnel, sharing of procedures and guidelines built on the experience of rescue teams or experts has been considered fundamental in order to increase knowledge on this delicate topic. For this reason, the first step towards a common approach should be the circulation of information on strandings involving alive cetaceans. Data and information exchange could be done on the basis of a common way to collect them. These feelings has been discussed also with ASCOBAMS and IWC and further cooperation among these International Agreements have been recommended

The main aim of this document is a first standardization of data collection in case of cetaceans stranded alive within the ACCOBAMS area. These information should be compared and assess also with ASCOBAMS and IWC with the main goal of ameliorate and share internal procedures in case of live animals strandings and to create a common database where it should be possible to compare practices, approaches and results. When other international agreements will define their own procedure, the present standardize approach could be revise.

1. Preliminary information

In order to establish which are main data and samples to collect during a stranding involving alive cetaceans, we should think to the main steps in the management of this kind of events. Environmental and logistic factors (during stranding, rehabilitation and release efforts), features of the species involved, results of a physical examination on the stranded cetacean stranded and its clinical parameters should at least collected. More in detail, the previously mentioned items should be resume in a proper triage matrix in order to facilitate the decision process and define the final destiny of the stranded animal (release, rehabilitation or euthanasia) with the possible follow-up.

The triage procedure should be implemented for any country under veterinarian expert supervision and it should be applied only by trained personnel.

1. Logistic: several logistic factors including the availability of means of transportation, weather conditions, features of the stranding site and chances of rehabilitation and release must be taken into consideration. Human safety in the rescue operations must in any case be guaranteed. International guidelines and conventions recommend that all efforts should be directed to release the animal rather than attempting prolonged rehabilitation which could be a useless dispersion of energy and resources making later liberation impossible as the animal has become conditioned or no longer accustomed to life in nature. Lacking of trained veterinarians, volunteers and/or facilities impair any rehabilitation effort and possible choices could be limited to an immediate release or euthanasia. Also the absence of a post-release monitoring is a limiting factor.
2. Stranded animal information: it is important to know how long the specimen has been stranded, the species involved, and the subject's physiological features, as all these details may influence the outcome of rescue attempts. Knowing these parameters may help responders to select the animals with higher

chances of a successful release. Independent juvenile and young adults of small dimensions are good candidates since they are easy to move and to transport and respond to veterinary procedures. Coastal species certainly have more chances in respect to pelagic ones. Large size cetaceans could lay for shorter period on the shore due to circulatory impairment and subsequent hypoxic changes. In cases of mass strandings and mass mortalities, rescuers should use even greater caution in releasing single individuals to avoid further strandings of the same subject or to avoid transmitting infective agents to wild animals possibly responsible for the event.

3. **Physical examination:** the clinical examination for cetaceans does not differ greatly from the clinical evaluation carried out on terrestrial mammals; it should be performed by a veterinarian.

a. **General examination:** before carrying out the other parts of the examination, the veterinarian should observe the exemplar closely to evaluate its general physical condition and how it reacts to the environment, human exemplars, and other members of its species (if there should be any). Any external signs as well as the animal's attitude towards the external world should be evaluated. Nutritional status (i.e. malnutrition and cachexia), any skin lesions (i.e. wounds and traumas) and mucous membranes (possible inflammatory discharges and hemorrhages) changes should be reported.

b. **Buoyancy:** If the animal is in the water or has been observed while it was in the water, it is possible to note if there are problems with floating and/or swimming. In particular it is important to note if floating appears to be normal taking into consideration the surface during both the apneic and inspiration phases and during rest. An increase in the buoyancy is generally the consequence of an accumulation of gas (intestinal bloating, pneumothorax etc.). Impairment in swimming is generally associated to a reduction in lung capacity. Another parameter to evaluate is equilibrium and possible rotation respect the longitudinal axis.

c. **Behavior:** behavioral alterations may not be relevant at first glance unless the subject is in the water with others of its species or if it is compared with animals being rehabilitated. In the case of stranded animals, these should be evaluated in relation to their behavior towards humans and towards other members of their species and, above all, in relation to potential risks for operators. The animal's attitude toward the water and the beach should be evaluated; the exemplar, could, for example, appear lethargic or reactive. An ill animals may seem to be resting. It is important to note if the animal seems bright and alert or depressed and unresponsive.

d. **Clinical Evaluation:** once the exemplar's life history data has been collected and a general and behavior evaluation has been made, the physical part of the examination should be carried out and biological fluids for collateral examinations should be collected even if there are no signs indicating pathological states. These operations should be carried out as quickly as possible to avoid stressing the animal even further. The appearance of the mucous membranes, an assessment of main reflexes and muscular tone, associated the animal's breathing rate should be evaluated and reported. Temperature should be assessed in order to evaluate any relevant changes due to stranding or ongoing pathological condition. Respect to terrestrial mammals palpation of lymph nodes and heart's auscultation is limited due to their anatomy.

e. **Collected Samples:** blood samples can provide useful information about living, stranded exemplars and should be taken, whenever possible, and sent to the reference laboratory; the results may be useful when decisions about releasing the exemplar are being made. Even if there is little time to collect the samples and to have them analyzed in cases in which a healthy cetacean is released immediately, laboratory results can in any case be of retrospective value.

Samples from the blowhole are taken with the intention of carrying out culture tests and cytological examinations which can be conducted indirectly by positioning agar plates over the operculum or taking biological material with swabs. This kind of sampling makes it possible to evaluate the conditions of the upper airways although it does not provide extensive information about the entire respiratory system. Other samples that should be collected are those of urine, feces and milk.

Further information and data useful to be collected shared are those related to any diagnosis coming from the diagnostic procedure, results of any related therapy and the destiny of the animal after the triage and rehabilitation efforts. If any, the outcomes of a post-release monitoring should be collected in order to understand the success of different approaches. Specific protocols and procedures namely dedicated to

- . first aid and stabilization of the animal/s
- . diagnostic and laboratory analyses
- . therapeutic and euthanasic procedures
- . movement and transportation

should be implemented in any country according to national and/or EU legislation involving the supervision of expert veterinarians and biologists. International mentors and existing guidelines (i.e. British Divers Marine Life Rescue and NOAA protocols - listed in Annex I) could help in preparing these documents. Best practices and guidelines prepared by International Agreements (IWC, ACCOBAMS and ASCOBAMS) to support their implementation in each country could be useful.

2. Common data collection

Similarly to stranding events involving dead animals, data collection in case of cetaceans stranded alive may be basic (Level A), intermediate (Level B), or detailed (Level C) considering the capability of the stranding network to intervene in reasonable times and the involvement of trained personnel and/or veterinarians. The use of standardized data sheets and forms is recommended working on the field. Samples of these forms are suggested by already existing guidelines, as those proposed by the British Divers Marine Life Rescue (BDMLR) which already implemented in the UK well-structured protocols with the relative datasheets and forms to collect proper data.

2.1 Level A Data: Basic Minimum Data collected on the field

This level is aimed to report any stranding event to national and/or international Stranding Databases. Geographic information, as well as biological and logistics details concerning the stranding should be recorded and national datasheets concerning measures should be filled out. Once the event has been recorded, a unique identification number (ID), which should be used at all subsequent contacts, will be assigned to it. Information relative to the following data must be collected.

This level allow to know exactly how many stranding events involve alive cetaceans and how many animals strand alive; furthermore, main features of these events could be understand in order to focus properly any possible procedure and support for this relevant problem.

- a. Investigator: name and address (institution)
- b. Reporting source
- c. Responsible Veterinarian/Rescue Team
- d. Location
 - preliminary description (local designation)
 - latitude and longitude, GPS
- e. Date (mm\dd\yy), time of first discovery and of intervention of the rescue team
- f. Weather and tide conditions
- g. Offshore human/predator activity
- h. UME/Diseases outbreak ongoing
- i. Species
- j. Number of animals, including total and sub-groups (if applicable)
- k. Length
- l. Sex
- m. Refloating efforts attempted by person not being part of the stranding network/rescue team

2.2 Level B Data: Information collected by direct observation or reported and/or clinical examination by trained personnel.

This level of data collection allows to collect information in similar events: more in detail, data on physical parameters of the involved animals could help to assess and improve any procedure of clinical evaluation as well as features of cetaceans stranded alive. This level requests basic skills on animal physiological parameters and management. Veterinarian is preferred for physical examination but also trained biologists could carry out the examination.

- a. Veterinarian/biologist responsible for physical evaluation
- b. Behavior
 - pre-stranding (e.g., milling, directional swimming)
 - stranding (e.g., determined effort to strand, passive, thrashing)
 - after return to sea (e.g., disoriented swimming, listing); note also ID number given after release and color; location of sighting
- b. Reaction to environmental stressors
- c. Buoyancy
- d. Nutritional condition
- e. Skin conditions; evidences of wounds and traumas
- f. Orifices and Mucosal discharges and hemorrhages
- g. Reflexes and muscular tones
- h. Abnormality in breathing (i.e. rate and smell)
- i. Samples collected
- j. Diagnosis
- k. First aid and rehabilitation procedures attempted.
- l. Release/euthanasia/rehabilitation
- m. Time lapse between first reporting/first intervention/release or euthanasia

3. Level C Data: Veterinary Physical Examination, Samples Collection, Therapy and Follow-Up

This last step foresees the involvement of trained and skilled personnel able to perform advance diagnostic procedures, propose therapeutic approaches and follow the animal after the release into the wild. The collected data could be shared in order to increase knowledge, approaches and possible procedures in order to increase the knowledge on first aid and rehabilitation efforts for cetaceans stranded alive.

- a. Veterinarian/rescue team leader involved
- b. Results of any blood samples analysis
- c. Results of any urine analyses
- d. Results of any microbiological examination considering also DMV
- e. Results of any diagnostic imaging investigations (x-ray, TAC) and ultrasonography
- f. Diagnosis
- g. Final decision: release/euthanasia/rehabilitation
- h. Summary of any therapy and procedures adopted during rehabilitation
- i. Time of rehabilitation efforts.
- j. Logistics of rehabilitation efforts
- k. Procedure for release efforts
- l. Follow-up

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