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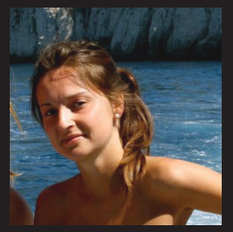

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This handbook should be considered as a useful field tool for people involved in cetaceans' stranding networks along the Mediterranean Sea and, more in detail, along the Adriatic coastlines. Recipients of this book are volunteers without any veterinary skills, biologists with no medical knowledge and veterinarians who have no informations about biology, anatomy and physiology of these very fascinating marine species.

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Handbook for Cetaceans' Strandings — Sandro Mazzariol, Bruno Cozzi, Cinzia Centelleghe

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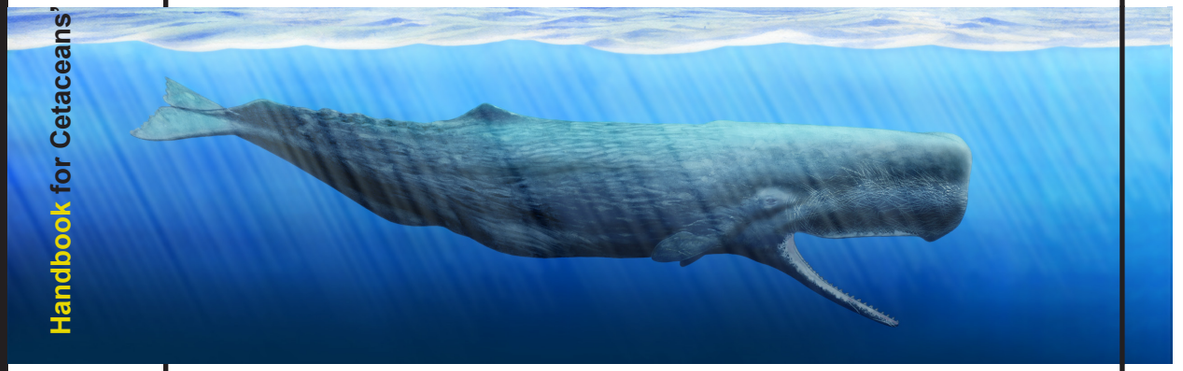
edited by
Sandro Mazzariol, Bruno Cozzi
Cinzia Centelleghe

netcet



Let's grow up together
Adriatic IPA
Cross Border Cooperation 2007-2013

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for
Cetaceans' Strandings

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the CoffeeHouse art&media

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Introduction

The aim of this handbook is giving to biologists, veterinarians and also volunteers involved in cetaceans strandings basic information on biology, anatomy, physiology and pathology of these species, in order to ameliorate the effectiveness of any intervention on any stranded animals along the Adriatic Sea.

The 7 chapters of this book try to give an historical and biological explanation of the stranding phenomena describing also which are the best practices and protocols to follow in these events; a complete necropsy protocol is also reported together with all the best pictures collected in these years describing main pathological changes observed during our experience and a brief description of all spontaneous diseases and evidences related to the interaction with anthropic activities that could be found during a post-mortem examination of a marine mammal; a specific protocol to certify an human interaction is also described. Finally, first steps to be done with an alive stranded cetaceans are reported. All protocols, procedures and pictures should be considered as a field guide for those person that want be involved in this events.

NETCET Project

Network for the Cetaceans and sea Turtle conservation in the Adriatic Sea

Our objectives

The Adriatic sea hosts several species of cetaceans and sea turtles and is considered a key foraging and development area for young sea turtles. Unfortunately cetaceans and sea turtles are vulnerable to interactions with human activities especially related to fisheries and coastal tourism (e.g. impacts with recreational boats).

The main objective of the NETCET project is to develop common strategies for the conservation of cetaceans and sea turtles in the Adriatic through a pan-Adriatic cooperation.

Cetaceans and sea turtles are a shared endangered natural heritage which cannot be managed by a single state in isolation. Due to the migratory nature of these species and the joint responsibility of Adriatic states, collaboration is essential to planning effective long-term conservation strategies.

Marine biodiversity conservation problems and more specifically cetaceans and sea turtles conservation are common to all countries across the Adriatic but experience across regions in this field varies and there is thus much to be gained by bringing together best practice and experience with the aim of defining a common conservation framework and tools/measures for the conservation of endangered marine species, making these tools readily available to other regions towards the end of the project. For these reasons the NETCET project, coordinated by the City of Venice, is managed by 13 partners situated in several Countries of the Adriatic Basin: Italy, Croatia, Albania, Montenegro and Slovenia.

What we want to achieve

- City network to enhance Adriatic cities role in the conservation of cetaceans and sea turtles. A Technical/Scientific network for the exchange of expertise,
- a common data collection framework based on a shared list of minimum mandatory data to be collected from all groups working along the Adriatic in a unique common database linked with main national and international existing databases on cetaceans and sea turtles,
- specific training courses and workshops for the exchange of expertise among partners and organisations (e.g. common clinical examination procedures and setting up of a rescue centre),
- creation/improvement of sea turtles rescue centres, in strategic locations. The rescue centres will also be permanent points of expertise for citizens and authorities and have proven to be powerful education tools,
- proposal for the development of an Adriatic emergency task force in order to respond to mass stranding and environmental emergencies,
- coordinated monitoring programme to increase availability of data and improve knowledge relevant for the conservation of cetaceans and sea turtles (e.g. 25 sea turtles will be equipped with GPS),
- a set of awareness raising activities targeting fishermen, boat drivers, school children and the general public to increase the awareness of the presence and the conservation needs of cetaceans and sea turtles (e.g. WWF Italy will design an educational kit for elementary school children),
- two distinct common strategies for cetaceans and sea turtles which foresee actions addressing the main threats challenging Adriatic populations (e.g. presentation of new National Action Plans for cetaceans and sea turtles, during the Final Conference).

Expected results

Shared standardized scientific knowledge and effective collaboration between the organisations involved in cetaceans and sea turtles conservation in the Adriatic,

greater institutional capacity for the conservation of cetaceans and sea turtles in partner states,

improved knowledge of Adriatic cetaceans and sea turtles populations, hot-spots and major threats,

increased technical capacity for sea turtle recovery in the Adriatic and effective treatment of stranded/injured sea turtles in high level structures and increased information available for citizens through the centres,

increased coastal communities' awareness of the presence and conservation needs of these species and widespread adoption by fishermen of correct handling procedures,

improved conservation and management of cetaceans and sea turtles conservation and their habitats in the Adriatic through effective regional cooperation,

a sense of ownership towards and pride in the natural heritage in the Adriatic.

Partners:

Comune di Venezia (IT) – coordinator

Università di Padova, Dipartimento di Biomedicina Comparata e Alimentazione (IT)

Comune di Pescara (IT)

Fondazione Cetacea (IT)

Istituto Superiore per la Protezione e la Ricerca Ambientale (IT)

Istituto Nazionale per la Protezione della Natura (HR)

Istituto Blue World per la Ricerca e la Conservazione Marina (HR)

Società Albanese di Erpetofauna (AL)

Associazione per la Protezione della Fauna Selvatica Marina dell'Albania (AL)

Istituto di Biologia Marina (ME)

Università di Primorska, Centro di Scienza e Ricerca (SI)

WWF Italia (IT)

Centro Educativo Marino di Pola (HR)

Associates:

Institute of the Republic of Slovenia for Nature Conservation (Associate)

Veneto Region (Associate)

Emilia Romagna Region (Associate)

Marche Region (Associate)

Introduction
NETCET Project
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Handbook for Cetaceans' Strandings

the art&media

1

CETACEAN STRANDINGS

Michela Podestà, Marco Affronte

Any marine mammals found dead or still alive stranded on the beach, floating near the shoreline, or being transported by sea currents is defined as stranded. Strandings are categorized as single events, involving an individual cetacean or a mother-child pair, or as mass strandings in those cases when two or more individuals that are not mother-child pairs are beached on the same stretch of coast over a narrow timespan; these are considered atypical when they involve mass strandings concerning different species over a lengthy stretch of coastline and over a long timeframe.

Taking place since ancient times, these events have always provoked strong public reaction and continue to represent an unresolved question concerning the animal kingdom. Generally speaking, mass strandings in which more than one individual is involved (especially gregarious species) attract more public attention. The causes underlying this phenomenon are unknown, but the hypotheses available define them as provoked by: social factors causing a group to follow a diseased element, spontaneous pathologies such as viruses or parasites which can apparently strike an entire group interfering with their orientation mechanisms, acoustic pollution or earthquakes which can modify the perception of space and cause confusion, variations in the planet's magnetic field linked to earthly factors or sun cycles, or geographic characteristics of the coast which impede the cetaceans from localizing themselves naturally and causing them to behave as if the area was acoustically dead.

There is usually less public clamor with regard to strandings of

single animals, in particular if the specimen found is already dead. Sometimes the causes can be easily identified. For the most part, in fact, the specimens are debilitated, infested with parasites, or present pathologies that have compromised the functioning of primary vital organs. In other cases, death was caused by old age and carcasses are transported to shore by sea currents. Other theories that cannot be considered scientifically valid include: scarce familiarity of pelagic species with coastal zones, regression to life on solid ground (primitive instinct), suicide, the necessity to recline against a surface in order to rest.

It is, nevertheless, important to underline that the greater part of live strandings presents numerous pathologies and concomitant lesions including the presence of parasites, viral and bacterial infections, algal toxins, tumors and organ abnormalities which can cause debilitation and in the end provoke a confusional state which may lead to the loss of orientation, coordination, equilibrium and/or hearing. Parasitic agents found in the large part of stranded organisms are in any case to be considered a sign of a markedly compromised health status probably due to numerous multifactorial causes that may be difficult to identify.

It is important to underline that stranded animals which have died of “natural” causes represent in any case an opportunity to conduct ecological, biological, pathological, genetic, microbiological, histological, immunohistochemical and ecotoxological analyses that can benefit the entire scientific community and widen our knowledge concerning marine mammals without directly impacting the natural populations and thus contributing to the safeguard of the population themselves. An efficient organization committed to quickly reporting sightings and to coordinate rescue activities is, without doubt, useful in rapidly and timely recuperating those organisms and collecting tissue samples which can be analyzed.

The first systematic, organized registration of data concerning strandings was begun approximately 70 years ago. In Italy, a network of researchers and collaborators of the Centro Studi Cetacei (Cetacean Study Center) has been involved in monitoring sightings and participating in rescue activities of live animals along the coasts of the Italian peninsula since 1986. In 2006 the Ministero dell’Ambiente e della Tutela del Territorio e del Mare (the Italian Ministry of the Environment, Land, and Sea) entrusted the task of data collection to the Centro Interdisciplinare di Bioacustica e Ricerca Ambientale (the Interdisciplinary Center for Bioacustics and Environmental Research) within the Dipartimento di Ecologia dell’Università di Pavia (Department of Ecology at the University of Pavia) and the Museo Civico di Storia Naturale di Milano Civic (Museum of Natural History in Milan). A Banca Dati Spiaggiamenti (Strandings Data Bank - BDS) which can be accessed online was created and is

continuously up-dated, although complete scientific data is reserved to authorized users (http://mammiferimarini.unipv.it). In 2002 the Ministry of the Environment, Land and Sea instituted the Mediterranean Marine Mammal Tissue Bank under the Department of Experimental Veterinary Sciences of the Faculty of Medicine of the University of Padua in response to a specific point in the ACCOBAMS agreement.

1.1 Strandings in Italy

Data relative to strandings along the Italian coastline inferred by the BDS database reflect the presence of species described in our seas. Nineteen out of the 80 known cetacean species have been observed in the Mediterranean Sea, 8 of which is regularly present. None of them is endemic. These are all cosmopolitan species plentifully present in all of the planet's oceans and which easily adapt to the varied conditions that the Mediterranean presents (noteworthy temperature range in the superficial waters and modest and often scarce primary productivity).

The cetaceans present in the Mediterranean sea and found stranded or floating offshore the Italian coastline are listed below. The morphological criteria for recognizing these species and the number of strandings of each species on the Italian coastline between 1986 and 2010 are specified. A more detailed treatment of biological, ecological and ethological aspects of the species described can be found in more specific texts. It is important to remember that the characteristic pigmentation of each species disappears quite rapidly after death. It is for that reason and in view of the difficulty of training operators, species recognition was not carried out for a large number of the cetaceans and in those cases exemplars were categorized as unspecified.

1.1.1 Regular Species

The distinctive morphological characteristics and the diet of the cetaceans present in the Mediterranean Sea are listed below, together with the number of strandings registered along the Italian coastline during the 1986-2010 timeframe.

Bottlenose Dolphin - *Tursiops truncatus* (Montagu, 1821)
Dimensions: adult 3.5 m, newborn 1.2 m, adult weight: 250-350 kg
Distinctive characteristics: the bottlenose dolphin has a more robust body with respect to other dolphins and has a stocky snout, short and broad, distinctly set off from its forehead by crease. The dolphin's lower jaw is shorter than its upper one. The upper parts of the body are dark gray, while its sides are lighter, and the ventral parts are almost entirely white. There are no demarcation lines between the colors. There is a dark V shaped line, on both sides of the melon. The shades of coloring are quite variable from one exemplar to another. The dorsal fin is sickle-shaped and located mid-back. It has approximately a hundred robust teeth.
Diet: Their diet prevalently consists of fish but they also feed on cephalopods, crustaceans and other invertebrates. They have been seen to hunt in groups.
Strandings in Italy (1986-2013): 1043.

Striped dolphin - *Stenella coeruleoalba* (Meyen, 1833)
Dimensions: adult approximately 2 m, newborn 80-100 cm.
Distinctive characteristics: Striped dolphins have a characteristic slender body shape combined with a relatively long beak, which is well separated from the forehead, and a distinct rostrum. The dark coloration of the back becomes lighter down the sides while the underbelly is almost white. A light gray spear-shaped band rises up from the thorax towards the base of the dorsal fin curving towards the darker region. Three black stripes begin at the eye area: one widens along the flanks reaching the ano-genital area, another reaches the base of the pectoral fins and the third, of the same length, is situated between the first two ones. A triangular dorsal fin is slightly sickle-shaped. The exemplars of this species usually have 160-200 small, sharp teeth.
Diet: Their diet is quite varied and adapts well to the availability of prey present in different zones at different seasons. In the Mediterranean, it feeds on cephalopods (among which species such as Histioteuthis, Ancistroteuthis, Todaroddes, etc.) and bony fishes (among which mictofids or lantern fish) and crustaceans
Strandings in Italy (1986-2013): 1940 along the entire coastline, found less frequently in the Northern Adriatic area

Risso's Dolphin - *Grampus griseus* (G. Cuvier, 1812)
Dimensions: adult as long as 4 m, weight 400 kg, newborn 1.5 m
Distinctive characteristics: Risso's dolphin has an extremely robust body, especially the anterior part. Without a distinct rostrum, they have a characteristic rounded, bulbous head with the anterior part presenting a slight crease at the center. The superior jaw is slightly prominent and the mouth rim is inclined upwards. Newborns are light grayish but they whitens with age, often presenting numerous whitish scars which in older exemplars are so numerous, especially at the head, as to make them seem almost white. There is a white anchor-shaped area between the pectoral fins similar to the one presented by the Long-Finned Pilot whale (Globicephala melas). Sickle-shaped and pointed, the dorsal fin is particularly high in adult exemplars. Most individuals have between 6 to 14 teeth, all in the lower jaw.
Diet: Risso's dolphins feed prevalently on cephalopods, a fact which can also be inferred from the number of teeth these mammals possesse. The species most commonly found in the stomach contents of exemplars stranded in Italy belong to the Histioteuthis and Ancistroteuthis genus. It also feeds on small fish.
Strandings in Italy (1986-2013): 156 animals stranded along the entire coastline, more rarely on the Adriatic coast

Fin Whale – *Balaenoptera physalus* (Linnaeus, 1758)
Dimensions: adult (in the Mediterranean) growing to 20 m, adult weight up to 60-80 tons; newborn 5.5-6 m.
Distinctive characteristics: The common rorqual or Fin whale is a long, slender marine mammal with a triangular head having a median longitudinal crest which extends to the blowhole made up of two orifices (as in all mysticetes). The back and sides of the body are black or dark brownish-gray and the ventral surface is white. The asymmetrical coloration pattern of the head which is dark on the left side of the lower jaw and white on the right side, is a distinctive characteristic of the species. They have a tall, hooked dorsal fin located about two-thirds of the way back on the body and between 70 to 100 accordion-like throat pleats. There are from 500 to 940 baleen plates (whale-bone) situated only in the upper jaw with asymmetric coloring reflecting that of the jaw (right white, left black).
Diet: quite varied. They feed on Euphausiacea but also on fish and small cephalopods. In the Mediterranean they feed primarily on Meganyctiphanes norvegica (horned krill).
Strandings in Italy: (1986-2013) 88 dolphins found dead along the entire coastline but rarely on the Adriatic side.

Sperm Whale - *Physeter macrocephalus* (Linnaeus, 1758)
Dimensions: There is a notable difference between the two sexes: males (in the Mediterranean) can grow up to 18 meters while the females up to approximately 12. The proportion of the head with respect to the total body length is likewise indicative of the sex: in adult males it is approximately 36% while in the females it is about 30%. Adult males can weigh as much as 60 tons and the female adult 18 tons. The newborn is 4-5 meters long and weighs approximately one ton.
Distinctive characteristics: The immense body is characterized by an enormous blunt head which makes up to ¼ to 1/3 of the entire body length. The blowhole is located toward the front and to the left of its head. The Sperm whale's lower jaw is long and thin. Exemplars of this species are usually a dark gray color. The skin around its mouth, particularly near the corners, is often whitish. The dorsal fin is very low and triangular followed by a series of smaller humps. It has a mouth full of approximately 50 conical teeth located only in the lower jaw that fit into sockets in the upper one.
Diet: fish and squid, including giant and colossal squid. In some areas, however, they feed on molluscs.
Strandings in Italy (1986-2013): 164 animals stranded along the entire coastline; more frequently in the Southern Tyrrhenian Sea and in Sicily and extremely rarely in the Adriatic Sea.

Short-beaked Common Dolphin - *Delphinus delphis* (Linnaeus, 1758)
Dimensions: adult exemplars typically reach 2.5 meters. A newborn is approximately 1 meter long.
Distinctive characteristics: The species has a fusiform, slender, hydrodynamic body shape. They have a relatively long beak, which is sharply separated from the forehead, and an elongated, thin rostrum. Their dorsal side is black to brownish black, while their underbelly is white. On each flank there is an hourglass pattern colored light gray-yellowish in front and light gray in back. They present a dark stripe which runs from their jaw to the base of their pectoral fin. The dorsal fin is curved and located half-way down the body. The species has approximately 200 small, sharp, conical teeth
Diet: The greater part of their diet consists of small fish and they prevalently prey on silver-skinned fish (sardine, anchovies, mackeral and bonita) and mictofids (lantern fish); they also feed on cephalopods (e.g. squid).
Strandings in Italy: (1986-2013) 43 reported more frequently in Sicily and Sardinia as well as in other areas with the exception of the Ligurian and Northern Adriatic sea coastline.

Long-finned Pilot Whale - *Globicephala melas* (Traill, 1809)
Dimensions: Adult males grow to up to 7 meters and weigh 3 tons, females reach approximately 5 meters, newborns 1.8 meters.
Distinctive characteristics: Exemplars of this species have a cylindrical body with a bulbous or squarish forehead and a slight rostrum. The pectoral fins are long and pointed. The adults are normally quite dark, almost black, while younger specimens are generally light brown. This species presents (just as some others) a whitish anchor-shaped patch that starts at the throat and extends to the uro-genital region on its ventral side. Their long, tapered, sickle-shaped pectoral flippers located quite forward on their bodies are the species' characteristic and they possess approximately 30-50 strong teeth.
Diet: They primarily feed on cephalopod molluscs but, if need be, also on pelagic fish.
Strandings in Italy: (1986-2013) 57 dolphins stranded especially in the Ligurian and Tuscan regions, in Sardinia and in a few cases in Calabria and Sicily.

Cuvier's whale - *Ziphius cavirostris* (G. Cuvier, 1823)
Dimensions: Adults are approximately 6 meters long, average weight is 3 tons, newborns are 2.5 meters long
Distinctive characteristics: the body of the Cuvier's whale is robust and cylindrical and their small head slopes down to a short beak and curved mouth; they have a small, poorly defined rostrum. Their lower jaw slightly protrudes with respect to the upper one. The color of these whales varies, especially as they age. Young exemplars are blackish-gray and their belly is usually lighter. In adults the color varies from slate gray to reddish-brown and males often have whitish heads. Exemplars commonly have numerous white scars, similar to scratches or stains. Small and triangular, the dorsal fin is located in a backward position. Adult males have two large, well developed teeth that protrude from the lower jaw and are visible even when its mouth is closed. Female teeth, instead, do not protrude from the gums.
Diet: These exemplars feed mostly on deep sea cephalopod molluscs as well as other kinds of deep sea fishes.
Strandings in Italy: (1986-2013) 82 exemplars along the entire coastline but not on the northern-central Adriatic coast.

1.1.2 Occasional species

Minke whale - *Balaenoptera acutorostrata* (Lacépède, 1804)
Dimensions: An adult (in the Mediterranean) grows up to 8 meters and weighs up to 3 tons, a newborn 2.5 meters
Distinctive characteristics: The Minke whale has a body that is similar to the common Fin whale but the extremity of the head is pointed and the blow hole is made up of two orifices (like all baleen whales). They have streamlined bodies and a distinctively triangular, narrow pointed snout and a flat rostrum. The upper parts of the body tend to be dark while the ventral ones are whitish. Minke whales have a characteristic white band on the dorsal side of each of their two, long flippers. The dorsal fin is hook-shaped and located in a slightly higher position with respect to the common Fin whale. They have 50 to 70 ventral throat grooves and approximately 600 baleen plates in the upper jaw.
Diet: this species feeds primarily on squid and small vertebrates such as cod, herring, and sardines
Strandings in Italy (1986-2013): 6 exemplars on the Ligurian, Tuscan, Sardinia and Campania coastlines

Rough-toothed dolphin - *Steno bredanensis* (Lesson, 1828)
Dimensions: Adults range between 2.2 and 2.4 meters; they are known to weigh approximately 150 kg; the length of a newborn is approximately 80 cm
Distinctive characteristics: These dolphins have long, sleek bodies and their most characteristic feature is their conical head and slender nose. They are generally dark gray with light spots on their flanks. Their ventral side, lower jaw and lips are white and they have a darker marking that extends over their eyes to the upper part of their flanks. Like other marine species, they may show scars resulting from encounters with other species/exemplars. The dorsal fin is high, curved and tapered to a point and is located in the middle of its back. Their teeth have a distinctive roughened surface formed by numerous narrow irregular ridges.
Diet: their diet consists of fish and pelagic cephalopods
Strandings in Italy (1986-2010): 7 exemplars, of which 6 contemporaneous sightings in Sicily and one in Sardinia

False Killer Whale - *Pseudorca crassidens* (Owen, 1846)
Dimensions: A male adult can measure up to 6 meters and weigh up to 2 tons, females can reach 5 meters, a newborn averages 1.6-2 meters
Distinctive characteristics: It has a slender body with a small, elongated, tapered head. They tend to be black or dark gray and have a white blaze between the pectoral flippers. The dorsal fin is sickle-shaped with a typically rounded tip which is found approximately in the middle of its back. Their flippers are somewhat unusual in that they feature an “elbow.” It has approximately 40 teeth.
Diet: They feed on cephalopods and fish, and with regard to the latter they are known to prey on large pelagic species which they are able to capture thanks to their agility and speed. They have also been reported to feed on smaller dolphins.
Strandings in Italy (1986-2010): 3 exemplars in Sicily and in Sardinia

Harbour Porpoise - *Phocoena phocoena* (Linnaeus, 1758)
Dimensions: The adult Harbour porpoise reaches 1.4 meters and a weight of approximately 50 kg; the newborn is approximately 70 cm long.
Distinctive characteristics: These porpoises have stocky bodies that taper toward the tailstock and they have a blunt, rounded heads; their mouths are short and they have inward-curving lips and spade-shaped teeth. They are dark gray or dark brown on their dorsal (upper) side, lighter gray on their flanks, and white on their ventral (under) sides. A thin, dark stripe runs from the back of the mouth to their pectoral fin. They have a small, triangular-shaped dorsal fin located half way down their body with a convex trailing edge and undercut rear margin. They have a total of 100 teeth.
Diet: They feed primarily on fish but also on molluscs and crustaceans
Strandings in Italy (1986-2010): No cases of strandings have been reported in Italy. The species present in the Black Sea is, according to some authors, a separate subspecies (P.p.relicta).

Killer Whale - *Orcinus orca* (Linnaeus, 1758)
Dimensions: Adult males average 3.7 tons and grow to 8-9 meters (females to a maximum of 5 meters), newborn between 2-2.5 meters
Distinctive characteristics: Killer whales have long, rounded bodies with indistinct rostrums and large dorsal fins at the middle of their backs. Their coloring is distinctive as they are strikingly solid black and white (their dorsal surface and pectoral flippers are black) and they have a gray patch called “saddle” on the back just behind the dorsal fin. The ventral surface, lower jaw and undersides of the tail flukes are mostly white and they have lobed white blazes which run down their sides to the genital area. They also have white patches near (slightly behind) each eye. Triangular, the male’s dorsal fin is quite characteristic as it can grow to 1.8 meters; in females it is typically smaller and slightly curved. Killer whales have a total of 40-50 strong teeth.
Diet: A typical predator, the killer whale has a wide diet and cooperates with others specimens of the same pod to locate prey. They feed on a variety of fishes, molluscs, birds and aquatic mammals and have been known to attack other whales.

1.1.3 Accidental Species

Rare sightings of the following species have been made in the Mediterranean Sea basin: the North Atlantic Right Whale (Eubalaena glacialis), the Sei Whale (Balaenoptera borealis), the Dwarf Sperm Male (Kogia sima), the Humpback Whale (Megaptera Novaeangliae), Blainville's beaked whale (Mesoplodon densirostris), the Indo-Pacific humpbacked Dolphin (Sousa chinensis).

1.2 Data Collection

As explained above, stranded cetaceans give us the opportunity to access a large amount of information concerning their specific biology and the population to which they belong. It is essential then to gather all scientific data possible each time that a stranding takes place on the Italian coastline. The persons in the position to report sightings of cetacean strandings are often poorly trained and are able to furnish only approximate estimates of the exemplar's dimensions and state of conservation; at times they are able to identify important, specific species characteristics. Trained operators normally require only a few pieces of specific information to deduce the exemplar's species (or at least its genus); as a result, important, at times unique, opportunities can be recognized. In fact, even if the carcass is in a poor state of conservation and has little value from a diagnostic point of view in identifying the cause of death, it is nonetheless valuable from biological and ecological perspectives especially those cases linked to species rarely noted in Italian waters, and skeletal material can be recuperated and conserved in museums of natural history.

The first response to sightings should then be that of attempting to recognize the species and to collect standard morphometric data. This should precede all other veterinarian investigations of the carcass. An extensive photographic documentation should complete the data gathered and be conserved together with it. Photos should be taken of the body from different positions, both laterally and ventrally and, if possible, together with a metric reference in view. Detailed photos of injuries, amputations and of any aspects of interest clarifying the description of the state in which the beached exemplar was found should be taken. Whenever possible, photos (usually perpendicular) of the pectoral and dorsal fins (see the pages concerning the ventral and dorsal fins), always with a metric reference, should be taken. The data that should be registered with regard to strandings is listed in the first page of sheet 1.1.

Biometric data is another important source of information; body

measurements should be taken carefully following standardized procedures so they can later be compared. The entire body should be measured using straight measurements. When this is not possible and the contours of the body are included in the measurement, this should be indicated in the report. It is also important to correctly identify the anatomic parts being described when measurements are being taken. The designs in the second page of the appendix illustrate numerous measurements that can be taken of the carcass of a cetacean and help to identify the points needing to be identified. The basic measurements referring to those marked by points 1, 2, 3, 6, 9, 11, 12, 21, 24, 25 should be included even in short reports.

Morphometric data is used to estimate the exemplar's age, its reproductive status, and the pathological processes of the populations of marine mammals. All measurements must be expressed in centimeters (cm) except for the blubber which is registered in millimeters. Measurements of blubber thickness help to define the subject's nutritional status. To measure blubber thickness, three single 2" – 3" long dorsal to mid-ventral incisions should be created through the blubber layer down to the blubber/muscle interface: one intersecting the dorsal midline, one intersecting the lateral midline, and one intersecting the ventral midline along the plane of the axillary insertion of the forelimbs. A ruler should be placed inside the incision and the thickness of the blubber from the blubber/muscle interface to the skin/blubber interface should be measured. Weight, either actual or estimated, should be registered in kilograms (kg).

Determining the sex of beached cetaceans is a rather simple and easy operation unless the carcass is resting ventrally or is too heavy to rotate. Few species present evident sexual dimorphism. The ventral area in which the genital and anal regions are located must be examined. In the female the the genital and anal openings are quite close to one another and are often contained in a single, externally visible slit. Two noticeable folds, where the nipples are situated, are located to the sides of the genital opening. In the male, instead, the genital and anal openings are further apart and they are generally clearly distanced from one another. Often the penis falls out of its slit in dead stranded males, in which case the exemplar's sex is clearly indicated. Greater detail is furnished in the chapter concerning the necropsy procedure.

It is important to furnish a description of the teeth of odontocetes indicating, whenever possible, the number contained in each jaw and, if the skeleton is not to be exhibited, to remove two teeth (taken from the central part of the row) which will make it possible to determine the exemplar's age.

The stomach contents of the exemplar are extremely important as they

indicate the cetacean's diet. These should be collected and frozen for future analysis. If this is not possible immediately, they can be placed in 70% ethanol. The material should be handed over to researchers as soon as possible so it can be subdivided as some parts can deteriorate if they remain in ethanol for too long.

In accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), in those cases in which a museum may be interested in recuperating the skeleton for exposition an authorization/permit must be obtained from the appropriate CITIES office.

Necropsies should be carried out in collaboration with the museum's technical personnel to facilitate the correct conservation of all parts of the carcass and in view of recuperating the skeletal one.

2

THE ANATOMY OF CETACEANS

Mattia Panin, Bruno Cozzi

Cetaceans are marine animals that are uniquely adapted to the particularly challenging habitat in which they live. The sea represents, in fact, a potentially hostile environment for warm-blooded mammals such as whales and dolphins: dispersion of heat is quicker in water than in air; food, which is difficult to find and to capture, is often available only at extreme depths; seawater is characterized by high concentrations of salt which, due to the osmotic gradient, may lead to dehydration of air breathing animals. Marine mammals have evolved over time adaptive mechanisms that permit them to survive even in these difficult conditions.

2.1 The Integumentary System

The skin of marine mammals must isolate their bodies to prevent thermal dispersion due to the cold ocean temperatures and must contemporaneously make them hydrodynamic. In effect, water conducts heat away from the body 25 times faster than air because it has a greater density (and then a greater heat capacity). The skin of dolphins contains the same number of layers as that of land mammals although there are obvious differences (Fig. 2.1). They generally


2.1

do not have hair (with the exception of a few short hairs along the lateral profile of the rostrum which are quite visible in young specimens) and a series of thin epidermal layers cover the subcutaneous adipose tissue. Both the arteries and veins are disposed in such a way as to reduce heat dispersion (see the chapter concerning the circulatory system).

The thin superficial layer or epidermis of marine mammals is made up of keratinized epithelial cells which make the skin waterproof. These epithelial cells have a rapid turnover (of just a few hours in the bottlenose dolphin).

The underlying layer contains the papillary dermis, with fingerlike extensions reaching the epidermis, and the deeper reticular dermis. The papillary dermis encloses the blood vessels and the most superficial nerves, and sensitive terminations are found within it. Even the germinal cells which rest on the dermal papillae have a rapid turnover, partially because of the extended surface. The whole surface and the rapid turnover of the superficial layers of the skin ensure a smooth, hydrodynamic external surface.

The thickness and the general aspect of the adipose tissue under the skin (blubber) are an indicator of the state of health of a specimen, considering also that it represents a major water reserve for a marine mammal. Adipose tissue can make up a considerable percent of a marine mammal's total body weight (even more than 30%). While fat is not a good insulator compared to the fur coats terrestrial mammals wear, it is unequalled in the water environment. The thickness and the fat content of this layer depends on the water temperature, the animal's state of nutrition, the season, possible pathologies and other factors. The size of the blubber affects the buoyancy of a cetacean. Blubber is made up of connective tissue fibers and vascularized fat cells which are more abundant than in land mammals; these make the hypodermis (or subcutaneous fat layer) a sort of semi-continuous fat layer in which collagen and elastic fibers act as a support for the tissue architecture. Adipose tissue is scarce or absent in the dorsal, pectoral, and caudal fins, and in part of the head where thermoregulation is regulated by adaptations of the circulatory system (see the chapter on the circulatory system).

The dorsal fin of cetaceans is made up exclusively of skin and blood vessels, while the caudal one contains the last caudal vertebrae along the median sagittal plane (Fig. 2.2). The melon, a specialized fatty structure located in the forehead of odontocetes, is described at greater length in chapter 2.17.3.

2.1.1 The Mammary Glands

The mammary glands of cetaceans are buried deep within the animal's

underbelly. A single short mammary slit can be seen on either side of the genital slit in most female cetaceans (Fig. 2.3) (more than two nipples have occasionally been reported). The milk produced during lactation is characterized by a high fat content and is generally dense and slightly yellowish.


2.2

2.2 Osteology

The skeleton of a cetacean is that of a mammal that has adapted to life in the water. Acquiring a hydrodynamic form required marked changes in the anatomy of the skeleton, bearing witness to the extreme evolutionary adaptation of complex organisms which have equipped themselves for a totally aquatic life . The head is elongated, the neck almost inexistent, the forelimbs have become fins, there are no hindlimbs and the vertebral column terminates in a caudal fin. The bones of the face are elongated and form a rostrum, the cervical vertebrae are unable to move in an independent manner, the curves of the spine disappear giving way to an arched profile with a ventral concavity. The thoracic girdle has become a paddle-like flipper and the pelvic girdle has gradually been transformed into externally invisible remnants. It should also be remembered that the caudal fin is horizontal (and not vertical like that of most fishes) and lacks the support of bones with the exception of the last minuscule caudal vertebrae. The tail flukes move powerfully by contractions of the back muscles whose tendons terminate in the elastic connective tissue of the fin. Some cetacean families show specific skeletal differences depending on the depth of the waters they swim in, their hunting habits and the speed at which they move.

The general characteristics of the skeleton are, however, common to all cetaceans including large baleen whales. The major differences in the skeleton of mysticetes can be found in the form of the skull, which has become adapted for the insertion of baleen plates, the presence of residues of the femur, and in the remarkable dimensions of the hand.

The bones of all dolphins and whales have a high fat content (making them float), are quite spongy with a finely woven bone matrix (to increase their resistance), and the long bones lack a medullary cavity.


2.3

2.2.1 The Skull

The form of the dolphins' skull is notably different from that of a large percentage of land mammals. In synthesis, the maxilla and the mandible are elongated to furnish a position for long rows of teeth of similar size (see the chapter on the mouth and the upper digestive tract). The nose of a cetacean is almost vertical with respect to the jaw, with small nasal bones outside the bony nasal cavity, which is lined on the crown of the skull mainly by maxillary, premaxillary and ethmoid bones. In these animals the elongated maxilla is not linked to mastication, as cetaceans capture their prey and swallow it whole. It is possible that cetaceans have an elongated muzzle because they require numerous teeth to seize their prey and also because it provides the space necessary for the melon which is located dorsal to the upper jaw. They have no real bony orbit.

The hyoid apparatus with its tilted placement serves as an attachment point for many of the muscles and ligaments subserving breathing, swallowing and sound production.

2.2.2 The Vertebral Column

The vertebral column of cetaceans is notably different from that of land mammals both with regard to the form as a whole and the morphology of the single vertebrae. The spine of cetaceans is distinctly curved ventrally without lordoses or kyphoses. The vertebral formulae for some delphinids are listed in the table below:

The bodies of the cervical vertebrae have become fused and form a single mass incapable of rotation. The transverse processes are only in part fused. In effect, whales and dolphins basically lack mobility of the neck (with the exception of Delphinapterus leucas). The thoracic vertebrae present elongated spinous processes facing backwards towards the so-called anticlinal vertebra (one of the last thoracic vertebrae), in which the inclination of the spinous process is inverted. The number of thoracic vertebrae is subject to intra-specific variations. The latter and the position of the ribs, which are not always inserted onto the corresponding vertebra, are two important variables to consider. The lumbar vertebrae can be defined by the position that follows the thoracic ones and precedes the appearance of haemal arches. Just as in land mammals, the lumbar vertebrae have ample transverse processes. There are no sacral vertebrae and the caudal vertebrae are not easily distinguished from the lumbar ones. The principal differentiating characteristic is that of a double ridge positioned on the ventral side of the vertebral body which is balanced by a small Y shaped bone (the chevron bone or haemapophysis). Together, the ventral surface of the

caudal vertebrae and the haemal bones form a channel for the great vessels which flow ventrally to the caudal vertebral column.

species	Cervical vertebrae	Fused cervical vertebrae	Thoracic vertebrae	Lumbar vertebrae	Caudal vertebrae	Total number
Bottlenose Dolphin	7	1-2 and or 3-5 (also 6-7)	12-14	17	26-27	63-64
Striped-Dolphin	7	1-3	15-	18-22	32-35	74-79
Common-Dolphin	7	Complete fusion	14	21	31-35	70-75
Risso's Dolphin	7	-	12-13	18-19	30-3	68-69
Long-finned pilot whale	7	1-5/1-6	11	12-14	28-29	58-59

Tab. 2.1: Vertebral formulas of some dolphins' skeleton

2.2.3 The Thoracic Region

The ribs are flat and flexible (allowing the rib cage to collapse under high pressure) and the first ones do not articulate directly with the sternum but trough sternal ribs. Bottlenose dolphins (*Tursiops truncatus*) possess 12-13 pairs of ribs (sometimes 14, 5-6 of which are bicipital), striped dolphins (*Stenella coerulaeoalba*) have 15-16 pairs, short-beaked saddleback dolphins (*Delphinus delphis*) have 13-15 pairs, Risso's dolphins (*Grampus griseus*) have 12-13 pairs, long-finned pilot whales (*Globicephala melas*) have 11 pairs. The sternum is usually in the form of a cross and can present a central foramen that tends to close with age. In fact, the degree of ossification of the sternum is indicative of a dolphin's age.

2.2.4 The Thoracic Limb

All cetaceans possess a thoracic limb. Wide and flat, the shoulder blade adheres to the trunk through powerful muscles and are joined to a short, thick humerus. There is no collarbone. The shoulder joint is the only mobile articulation of the limb and carries out its function for the most part by modifying the inclination of the flipper during swimming . The bones of the arm, the forearm, and the hand are connected to

one another and are incapable of independent movements. Whales' flipper also has a radius and an ulna which are both flat. These articulate with a series of small carpal bones followed by some long metacarpal bones and phalanges. There are five fingers (except in rorquals which have four) but the number of phalanges for each finger varies and can be much more numerous with respect to the three commonly seen in land mammals. The architecture of the thoracic limb is not perceptible externally as the bones are fixed to one another by a strong semi-rigid connective tissue. The long bones of the thoracic limb do not have a medullary cavity. Patterns of fusion of the epiphyses provide information about the specimen's age in the case of dolphins. Bone density also changes with age.

2.2.5 The Pelvic Girdle

While the thoracic limb is present, although markedly transformed, cetaceans have only reduced pelvic bones. The coxal bone (pelvic residual) is only a small remnant whose function is that of being an anchor point for some muscles such as those relative to the penis (in the male). In fact, the pelvic remnant is located deep in the ventral musculature of the abdomen. The occasional presence of remnants of other bones of the pelvic girdle is to be considered a vestigial feature, even if in some mysticetes there are remnants of the femur bone.

2.3 Myology

The entire bodies of cetaceans have become adapted to life in the water and even the muscles have become modified. The greater part of the muscular system is dedicated to movement and thus to work. Since there are no pelvic limbs, the powerful muscles of the vertebral column are the main engine for locomotion through vertical movements of the caudal fin.

It is important to note the powerful muscle bands that are wrapped around the back muscles of cetaceans. All the principal muscle groups of land mammals (among which the m. multifidus, m. longissimus, m. semispinalis and intercostalis) can be identified. Besides these, other muscle groups such as those linked to the movement of the caudal fin (m. extensor caudae lateralis, m. extensor caudae medialis, m. intertrasversarius caudae dorsalis, m. intertrasversarius caudae ventralis, m. hypaxialis lumborum) have undergone an extraordinary development which has taken place only in cetaceans.

Dolphins and in general all cetaceans do not have mimic muscles for facial expressions. Some muscles used for chewing such as the temporal

are well developed while others such as the masseter are only vestigial. This should perhaps be viewed in relation to the fact that the chewing muscles of dolphins are used to grab and not to chew.

The ventral thoracic and neck muscles are involved in lowering and rotating movements of the head. The muscles of the thorax and of the abdomen follow the same plane present in land mammals. The ventrolateral muscles of the abdomen (internal and external oblique muscles, transverse and rectal muscles of the abdomen) are extremely powerful and contribute to propulsion. The diaphragm is quite inclined and as a result it greatly increases the thoracic part of the abdominal cavity.

The muscles of the thoracic limb are linked for the most part to the rotation or stiffening of the shoulder articulation. Since the radius and the ulna are unable to rotate along the same axis but are both stiffened by ligaments and tendons, there do not seem to be any muscles in the forearm. The wrist and the hand are covered by connective fibers which contribute to make the pectoral fin rigid. As there are no pelvic limbs, their relative muscles are also lacking. The muscles which are inserted into the pelvic remnants are connected to the genital apparatus.

2.4 Angiology

2.4.1 The Heart

The heart of all mammals is the organ that pumps oxygenated blood to the body cells and deoxygenated blood to the lungs where the exchange of oxygen and carbon dioxide takes place. This process is more complicated in mammals which dive and remain underwater for extended periods. In fact, the time interval spent at the surface during which a cetacean breathes may be quite short with respect to the time spent submerged . Many factors have contributed to the many adaptations that the cetacean circulatory and respiratory systems have undergone to be able to survive underwater. Some of these factors are linked to gross evolutionary aspects (the blood volume of marine mammals often exceeds 12% of their body weight, while it accounts for approximately 7% in humans and slightly more in other land mammals) or particular physiologic (such as the well-known extreme bradycardia) and/or biochemical (high hemoglobin affinities) aspects.

The heart of bottlenose dolphins (Tursiops sp.), wrapped within the pericardium, can be found at the third lower level of the second to fifth intercostal space on the left side of the thorax and in the third to fifth intercostal space on the right. The pectoral fins cover only a part of the heart region as the heart is positioned with its greatest dorso-ventral axis

rotated obliquely in a caudal direction. The organ's apex is, thus, not covered by the pectoral fins. Cetacean lungs have a dorsal position with respect to the heart, and a more lateral-dorsal placement just as in the large part of land mammals. The heart-lungs relationship is, thus, modified. The heart is in a certain sense "suspended" under the lungs and rotated to one side. The windpipe crosses the thorax in a cranio-caudal direction dorsal to the heart. The phrenic nerves on both sides rest on the external surface of the pericardium which covers the atria.

The heart of dolphins has the same external shape and internal organization as that of land mammals with a few differences that merit mention. Grossly the heart of cetaceans is similar to that of land mammals (Fig. 2.4 and Fig. 2.5). Generally it is wider and flatter than that of quadrupeds because of the form of the thorax and of the external pressure they must endure when they dive. Like the hearts of all other mammals, those of marine mammals internally have four chambers; the arteries have thick, muscular walls and there is a large number of trabeculae, which are the muscular bundles that cross the ventricles.

The thorax of a bottlenose dolphin tends to be wider at the base furnishing more space to the ventral part of the heart. It is known that the heart of cetaceans (and in particular that of dolphins) is slightly bigger (heavier) generally speaking than that of other land mammals. In the bottlenose dolphin the weight of the heart is approximately 0.6-0.93% of its body weight. The heart of an adult male bottlenose of 300 kg weighs less than 3 kg. The heart of a striped dolphin and of a common dolphin is slightly smaller than that of a bottlenose dolphin but it is in every other way comparable. In mysticetes the heart can reach immense dimensions, even if the relative weight of the heart remain the same. The heart of a fin whale (*Balaenoptera physalus*), a blue whale (*B. musculus*) and a humpback whale (*Megaptera novaeangliae*) can weigh a few hundred kilograms.

2.4

2.5

As in the majority of mammals, the drainage of the lymphatic circulation takes place in the right ventricle. Even if no anatomic studies have been carried out on the innervation of the heart in dolphins, physiologic investigations in living animals and comparative observations indicate that the sympathetic and parasympathetic innervation are the same as those in land mammals, humans included.

2.4.2 Arteries of the General Circulatory System

The entire general plan of the arterial circulatory system in marine mammals has adapted to the metabolic responses of a diving animal. Diving into shallow waters (10-200 meters) as well as into depths below 1,000 meters requires a particular kind of circulatory apparatus and vascular architecture. As the animal dives to ever greater depths, the temperature falls rapidly and the pressure and the level of carbon dioxide increase.

The marine mammals' thermoregulatory homeostasis has become accustomed to diverse conditions, including a thick layer of subcutaneous fat. The organization of the peripheral blood vessels contributes to this mechanism and prevents heat loss. In fact, arterial circulation is limited to the peripheral sectors of the body and, more importantly, the peripheral arteries of the fins are surrounded by a series of small veins facilitating the countercurrent heat exchange (Fig. 2.2).

During immersion, blood is carried to the thorax, to the brain and to the other vital organs. Blood is directed especially to the large reserves of the retia mirabilia. As liquids are incompressible, the presence of a sufficient quantity of blood in the thorax prevents irreversible collapse of the heart and lungs. The ribs, which are extremely compliant especially in the smaller cetaceans, yield to a certain extent to the external pressure, contributing in this way to pushing the blood through the thoracic rete mirabile.

How the muscles and brain are oxygenated is discussed elsewhere (see the relative chapters) and the reader is invited to consult specific works on physiology. It should be noted that the brains of dolphins and whales might be probably able to work in anaerobic conditions without damage, which is a unique, difficult-to-explain characteristic in mammals. The rete mirabile at the cervical level aids to regulate the pressure pulsing directly on the brain during long immersions.

a. The Structure of the Arteries

The arteries of cetaceans have the same architecture as those of land mammals. The arteries of the retia mirabilia (a complex counter-current heat exchanger system) have


2.2

different width depending on the sector. Even the elastic components within the artery wall vary in the different retia mirabilia. Even though there is little data in the literature concerning the innervation of the arteries in dolphins, the cervical rete mirabile is richly innervated, which is probably necessary to promote the regulation of the rhythm of arterial flow directly to the brain, especially during long immersions. In contrast to bottlenose dolphins, the arterial systems of striped and common dolphins (*Delphinus sp.*) have not yet been studied in detail. There may be noteworthy differences between members of the *Delphinidae* family. Small cetacean species such as the harbour porpoise (*Phocoena phocoena*) do not possess a true functional carotid artery after birth, but they have a well developed carotid rete mirabile joined to the ophthalmic network, out of which branches vascularizing the brain arise.

The general architecture of the vascular system of large odontocetes and mysticetes is more or less similar to that in dolphins, but there may be significant intraspecies differences. The *retia mirabilia* in mysticetes are in some way less developed than those in odontocetes in general and of dolphins in particular.

b. The Aorta

The ascending aorta commences in the left ventricle. It is wrapped in a thick pericardium and after giving rise to the coronary arteries, it immediately forms the aortic arch with the brachiocephalic trunk and thoracic aorta which go on to connect to the abdominal aorta. The aorta of an adult bottlenose dolphin has a diameter of approximately 3 cm at its origin but then becomes smaller. The aorta of the common and of striped dolphins has a smaller diameter with respect to the bottlenose dolphin.

The subclavian and carotid arteries arise from the brachiocephalic trunk. The subclavian arteries rapidly diminish as they penetrate the thoracic region. The common carotid arteries are quite short (remember that dolphins have virtually no neck) and soon divide forming the internal and external carotid arteries. The internal carotid artery of cetaceans has practically no direct connection with the brain vascularization, travelling for the most part on the outside of the brain and nourishing the dorsal cervical rete mirabile terminating in the ophthalmic region as a solid but no longer functional cord. The vascularization of the brain is, instead, provided by the cervical rete mirabile through the occipital foramen.

The great descending aorta carries blood to the thoracic region and to the abdomen, furnishing several intercostal and dorsal vessels. As in other mammals, the arteries reach the internal part of the thorax and the abdomen from the aorta. The intercostal and the dorsal thoracic arteries, which originate at the descending aorta and the brachiocephalic trunk,

furnish blood to the vast thoracic and spinal retia mirabilia. Arterial branches that contribute to the heat exchange mechanisms for the kidneys, testicles, uterus and ovaries also arise at the descending aorta. There are no vessels journeying to the absent pelvic limb with the exception of pelvic residuals. As in quadrupeds, there are no external iliac arteries, and there is a great ventral vertebral artery that vascularizes the caudal fin.

c. Arteries of the Head

Without a real cerebral arterial circle (the polygon of Willis), the spinal meningeal arteries which vascularize the brain originate at the most cranial part of the carotid rete mirabile. The internal carotid artery reaches the cavity of the middle ear and remains small after birth and throughout the animal's lifetime. As it travels along its path it gives rise to collateral branches directed to the cervical rete mirabile. The external carotid artery moves toward the most ventral part of the head and furnishes blood to the face, just as the face artery and its branches do in land mammals. The internal maxillary artery vascularizes the fibrovenous plexus of the air sacs along its ventral and lateral course with respect to the skull.

The brain, as already pointed out, receives oxygenated blood from the spinal meningeal arteries (well innervated) which arise from the retia mirabilia of the cervical and thoracic spine. Under close observation, it is also possible to identify the counterparts of the cerebral arteries (anterior/rostral, median and posterior/caudal) of the human species and of other mammals even if their origin is different from that of cetaceans.

d. The Arteries of Lung Circulation

Lung arteries of dolphins are quite similar to those of land mammals with a slightly modified path due to the more "dorsal" placement of the lungs.

e. Retia mirabilia

The specific characteristics of a rete mirabile have been described in some scientific works. It is defined as a network of small blood vessels that is formed by the branching of a large vessel (or more than one vessel) and that usually reunites into a single trunk and which is in effect the direct continuation of the artery which generated the network. It is believed to have an oxygen-storing function. In birds with webbed feet, the rete mirabile in the legs and feet transfers heat from the outgoing (hot) blood in the arteries to the incoming (cold) blood in the veins.

2.4.3 Veins

Just as in land mammals, the venous system in cetaceans follows the arterial one. Evolutionary adaptations to life in the water brought a series of changes in the general architecture of the venous circle of dolphins and other cetaceans.

The principal characteristics of dolphins' venous system are:

- a marked dilation of the caudal vena cava together with a specific muscle sphincter. Two or three principal hepatic veins are quite dilated and have their own muscular sphincter as well. This disposition is not found in mysticetes and perhaps not even in the great odontocetes such as sperm whales (*Physeter macrocephalus*) or hyperoodons (*Hyperoodon sp.*);
- spinal veins without valves are the primary drainage of the brain and of sinuses of the skull base;
- two well developed large veins located ventrally to the vertebral canal are connected to the cranial and caudal vena cava by the costocervical and intervertebral veins, respectively;
- a series of retia mirabilia accompany the corresponding arterial structures. The veins of the networks are generally without valves (so blood can probably flow in both directions). These veins, moreover, do not have a muscle layer. The arteries and veins of the network are interconnected only by a few capillaries. The veins can also absorb shocks from the arteries of the corresponding rete mirabile;
- there are retia mirabilia in the lateral and ventral walls of the abdominal cavity composed only of veins which in turn drain the blood from the superficial veins coming from the caudal fin. These networks direct blood flow towards the caudal vena cava and perhaps act to balance the pressure differences between the thorax and the abdomen;
- a specific venous system envelops the arteries directed to the testicles (which are abdominal). The veins of the peri-testicular plexus communicate with the superficial vessels which originate in the dorsal fin and the animal's back. This system function is to encircle the testicles with a network of cooler venous blood, which cools by exchanging heat with the arteries directed to the testicles and the testicular parenchyma itself. Similar venous systems can also be found at the ovaries and uterus;
- the venous system of the nasal cavity is drained by the spinal veins through the cranial sinuses, by the large vessels that converge at the external jugular vein, and by the tiny branches which become a

single vessel that flows into the mandibular vein.

2.4.4 Lymphatic Vessels and Lymph Nodes

There do not seem to be any differences between lymphatic vessels in cetaceans and those in terrestrial mammals. Lymphatic vessels are notoriously difficult to identify in a carcass, except in particular conditions (death taking place immediately after a meal, diseases causing swelling of the lymphatic vessels, etc.) or unless specific injection methods are used. This is why lymphatic vessels are only rarely described.

According to Slijper (1979) there are many lymph nodes that are relatively larger than those found in the greater part of terrestrial mammals. That author also noted that the lymph nodes of cetaceans participate in destroying old red blood cells. The structure of the lymph nodes of cetaceans is similar to that in pigs in which peripheral cords and sinusoids encompass the cortex of the follicles located at the center of the organ. Smooth muscle fibers have been described in the capsule and in the trabeculae of visceral lymph nodes of bottlenose dolphins.

a. Lymph Nodes of the Head

The lymph nodes of the head are difficult to identify. Dolphins have well developed tonsils which lie at the back of the oral cavity, slightly under the laryngeal folds (laryngeal tonsils).

b. Lymph Nodes of the Thorax

Lymph nodes of the thorax are similar to those found in cattle. Some lymph nodes are grouped together at the base of tracheal ramifications near the lung hilum. A lymph node of notable dimensions is evident beneath the ventral border of the lungs. A very large, elongated lymph node is present in the dorso-caudal mediastinum in the space between the two lungs. This particular lymph node is very similar to the corresponding one in cattle.

c. Lymph Nodes of the Abdomen

Abdominal lymph nodes, some of which very large, follow the insertion of the mesenteric lymph nodes. In this case as well, the dimensions and the distribution are similar to those of cattle lymph nodes. There are both nodular and diffuse lymphatic plaques in the walls of all of the post-diaphragmatic gastrointestinal tract. Even if there is not a vermiform appendix as in humans and rabbits, some tracts of the terminal part of the intestines of young bottlenose dolphins have a similar structure. Both odontocetes and mysticetes have large tonsils connected to the distal part of the intestines and called anal tonsils. The bottlenose

dolphin's anal tonsil is in effect a complex lymphoepithelial organ made up of a cluster of lymphoid tissue and of epithelial ducts (crypts) at times associated to mucus glands. The anal tonsil appears to be most active in young animals and apparently becomes involuted with age. This tonsil could carry out a role similar to that of the human vermiform appendix. Several lymph nodes are present in the female genital apparatus.

d. Lymph Nodes of the Thoracic Limb

No lymph nodes have been described in the modified thoracic limb in dolphins but some must be present even if of reduced dimensions. Prescapular lymph nodes, a group of lymph nodes found deep in the cranial border of the scapula, have been noted. These lymph nodes are the only readily accessible ones in these animals.

2.5 The Thymus

The thymus is a primary lymphatic organ that undergoes involution linked to senescence.

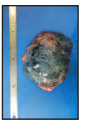
The thymus of dolphins is a tan or red-brown lobular structure found in the anterior mediastinum just behind the sternum and close to the thyroid gland. On the basis of the study carried out by Cowan (1994), the thymus can overlap with the thyroid.

According to some authors (Rommel and Lowenstine, 2001), in contrast to the medullary part, the cortex of the thymus is lymphocyte-rich. The tissue becomes atrophied with age and the lobules degenerate, plaques of mineralized tissue are formed, the thymic tissue is invaded by adipocytes, and Hassall's corpuscles become visible. The thymus of an adult bottlenose dolphin can be difficult to find. It is made up for the most part of adipose lobules in which islands of thymic tissue with Hassall's corpuscles and cysts may be clearly evident only at the microscope. As the specimen ages, the cysts can become filled with colloid substances and enlarged, to the point that they can substitute the entire thymus.

2.6 The Spleen

The spleen of dolphins is surprisingly small compared to that of other artiodactyls. It has a disc-like form and a reddish-blue color (Fig. 2,6). In adult striped and common dolphins the diameter of the spleen is no greater than 6-8 cm. In the bottlenose dolphin it is slightly larger.

a. Position and Topographic Relations with Other Organs


2.6

The spleen is connected to the great curvature of the first stomach by a specific ligament and although mobile it is generally positioned to the right side of the abdominal cavity (Fig. 2.7). Accessory spleens, which are small discs of splenic tissue also called haemolymph nodes, are often observed (Fig. 2.8). If present, they are distributed along the greater omentum in the peritoneal cavity. Accessory spleens are a frequent finding in dolphins.

b. Gross and Microscopic Anatomy

The spleen is surrounded by a thick capsule with a fibrous external part and an internal muscle layer. The muscle cells lead to the trabeculae. The general architecture of the spleen does not differ from that of terrestrial mammals and the organ is composed of two types of tissue: white pulp and red pulp. According to Zwillenberg (1956, cited by Slijper, 1979) the white pulp in dolphins corresponds to 30% of the total organ mass due to the large number of lymphatic corpuscles. That author hypothesized that the relatively low mass of spleens in cetaceans is due to mechanisms that control blood volume and pressure during immersions. These mechanisms are based primarily on the control of blood flow through the retia mirabilia and thus a bulky deposit/reserve of blood is not necessary in the spleen.

There are, however, indications according to which the spleen of cetaceans is slightly different from that of terrestrial mammals. The splenic red pulp of short-finned pilot whales (*G. macrorhynchus*) and bottlenose dolphins is formed by two distinct venous layers, an internal and an external one (Nakamine et al., 1992; Tanaka, 1994). The internal one has an undefined vascular architecture lacking lymphoreticular tissue development. Since the internal layer is homologous to the intermediate zone of more primitive mammals, some authors (Nakamine et al., 1992; Tanaka, 1994) speculated that in cetaceans the splenic vascular system has not become modified over the course of evolution and is similar to that of vertebrates belonging to other classes.


2.7


2.8

c. Intraspecies Differences

According to Slijper (1979) the spleen of rorquals (family

Balaenopteridae) is flatter and thinner than that of dolphins and weighs from 3 to 10 kg (or 0.02% of the total body weight, with respect to 0.3% of the body weight of most terrestrial mammals) with a diameter of 60 cm. The white pulp of mysticetes has less mass with respect to what is found in the spleen of odontocetes (Zwillenberg, 1956, cited by Slijper, 1979).

2.7 The Respiratory Apparatus

2.7.1 The Blowhole and the Upper Airways

The nostrils of cetaceans migrated from the face to the top of the head where it became a blowhole which is unique in odontocetes and bipartite (divided into two parts) in mysticetes. The blowhole is normally closed by a nasal plug and the cetacean opens it by contracting the muscular flap which covers it.

The blowhole is contiguous to the upper airways (nasal cavities) which are associated to 4 pairs of communicating air sacs:

- vestibular (near to the blowhole);
- tubular or nasofrontal (the nasal plugs have a node or lip that bulges into these sacs);
- connecting or accessory (communicating with the tubular sacs);
- premaxillary (situated under the melon lying adjacent to the upper surface of the premaxilla).

2.7.2 The Larynx

The larynx is formed by epiglottic and arytenoid cartilages which become stretched forming a tube that projects rostrally and dorsally from the floor of the pharynx. Its distal extremity rests in the nasal cavities. This tubular spout, which has been described in several species, forms a specialized air passage. Some mucous glands can be found between the epithelial-lined folds and the cartilage. The laryngeal tonsils are evident at the base of the larynx corresponding to the laryngeal folds (Fig. 2.9).


2.9

The larynx is connected to the lungs by a trachea equipped with a cartilage scaffolding which in cetaceans generally projects to the less important bronchioles at times furnished

with myoelastic sphincters.

2.7.3 The Trachea

The trachea in cetaceans is rather short: it can measure a few centimeters in some dolphins while its diameter can exceed 30 cm in some mysticetes.

It is a robust organ with an ample lumen made up of numerous tracheal rings composed of intermittently attached hyaline cartilage. There is no tracheal muscle as in terrestrial mammals. There is a system of vascular structures in the submucosa, richly innervated by parasympathetic fibers, that form erectile tissue whose function may be to modify the lumen of the trachea during some stages of immersion. The trachea is the only dead space of a cetacean's organism and this has a series of implications during pressure changes in the water column.

2.7.4 The Lungs

The lungs of cetaceans are not subdivided into lobes and are not segmented. They are positioned dorsally to the diaphragm with a more horizontal position with respect to that found in terrestrial mammals. This positioning favors the rapid expulsion of large quantities of air (from Fig. 2.10 to Fig. 2.12).

The airways of cetaceans present some characteristics that are meant to favor rapid energetic air exchanges. These include a short trachea positioned between the larynx and the lungs and equipped with a continuous cartilaginous scaffolding extending to the lesser bronchioles, equipped with myoelastic sphincters. These sphincters could be an adaptation to fluctuations in air pressure in the lungs during rapid, frequent dives and ascents, especially during violent inspirations and expirations of smaller odontocetes.

Cetaceans which commonly carry out long dives have comparatively small lungs which collapse at shallower depths during immersion compared to more superficial divers. This apparently contradictory situation aims to reduce the risk of decompression sickness (the bends) connected to the diffusion of gas (particularly nitrogen) to the bloodstream because of the immense hydrostatic pressure.

Cetaceans which normally dive for brief periods, such as many dolphin species, have relatively larger lung volumes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comparable to those of terrestrial mammals.

2.7.5 Adaptations of the Respiratory Apparatus to Deep Diving

To sum up, the respiratory apparatus of cetaceans present numerous adaptations to deep diving:

1. the trachea is strong, short, robust and surrounded by thick continuous cartilaginous rings which can form a sort of armor that reaches the lesser bronchioles;
2. the thoracic cavity is very flexible and can collapse under water;
3. there is a thoracic rete mirabile and a complex system of veins and venous sinuses;
4. the lungs are extremely elastic and generally small in deep divers; there are myoelastic sphincters in the branches of the bronchial tree that can compartmentalize the minute ramifications of the terminal parts of the respiratory tree.

2.8 The Digestive System

2.8.1 The Mouth and the Digestive Tract

The mouth of cetaceans is very different from that of other mammals, as dolphins and whales do not chew their food but grab it and swallow (dolphins) or filter it (whales). Their teeth and face muscles have, thus, been appropriately modified. The isthmus of the fauces located at the end of the oral cavity is constructed in such a way as to let food pass through the pharynx while contemporaneously limiting the passage of water towards the esophagus and the stomachs.

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The elongated mouth of dolphins becomes a long beak (rostrum) which is primarily formed by maxillary (upper jaw) bones. Their teeth are numerous, sharp, and pointed and are distributed in both the upper and lower jaws. The teeth of odontocetes are all of the same size (this is the reason they are called homodont, that means “equal teeth”) without any distinction between incisors, canines, etc. They also present a single dentition (they are also monophyodont , meaning “with only one set of teeth”) (from Fig. 2.13 to Fig. 2.15).

The lips of cetaceans, which are without an independent mobility, are thin; the jaw hinge opens only one way without any possibility of lateral movement (the only cetaceans that have a certain mobility are the beluga whale and rorquals). This is due to the reduction or absence of the mandibular coronoid process. In any case, the lips, the tongue, and the entire mouth of dolphins are extremely sensitive to touch because of numerous innervations. Dolphins interact with the world using their mouths.

The teeth are composed of a thin layer of cement which covers the dentine layers, and the enamel covering the crown.

a. The Lips, Tongue and Oral Cavity

The lips are rigid and keratinized and in the internal face they are linked to the whitish mucous membrane of the labial vestibule. The labial vestibule itself is attached to the right and left buccal vestibules (cheek vestibules). The mucous membrane of the vestibular apparatus continues on the medial side with the gums that surround the base of each tooth. The facial and the ventral part of each buccal vestibule constitute, respectively, the dorsal and ventral fornix. The labial vestibule is only slightly accented.

The tongue is short and wide and its apex is mobile; young bottlenose dolphins have rostrolateral papillae which disappear later in life (Fig. 2.16). Fringed caruncles are visible on the frenulum placed on the ventral surface of the tongue of these dolphins. The taste buds are greatly reduced or absent.

b. The Parotid and Other Salivary Glands

There are no greater salivary glands in dolphins, as animals which swallow their prey whole do not for the most part need a relevant amount of saliva. The mouth is, moreover, continuously open underwater and the saliva would be largely washed away.

A minimum quantity of saliva is in any case produced, probably by the lesser salivary glands located perhaps in the medial and caudal part of the tongue itself. Small quantities of saliva can be utilized to determine some hormone levels and dense saliva of the mouth may be interpreted as a symptom of gastritis in animals kept in captivity.

2.16

c. The Pharynx and the Esophagus

The pharynx of cetaceans moves dorsally to permit food to enter the esophagus through combined movements of the genioglossus, hyoglossus, and styloglossus muscles. In bottlenose dolphins an oval shaped tonsil is located in the submucosa of the pharynx (Fig. 2.17).

The esophagus has the typical structure of that in other mammals. It is a long tube composed of both smooth and skeletal muscles wound in a spiral whose function is to push food from the pharynx into the first stomach chamber. The esophagus of bottlenose dolphins has a thin mucosal robe without submucosal glands leading to a thick longitudinal *muscularis mucosae* of smooth muscle in the most caudal part of the organ.

The striated muscle constitutes the external part of the organ and has a tangential direction in the first third of the esophagus, with an internal circular layer and a smooth external layer which begin to appear in the middle third of the organ. It is to be remembered that the ingested food is practically alive as no mastication is carried out in the oral cavity. Small dolphins prey on herrings and other small fishes, but killer whales (*Orcinus orca*) prey on seals and even small dolphins (even attacking large mysticetes and sperm whales for the tongue and other parts they prefer). The esophagus of larger dolphins allows passage of large volumes of food which are literally pushed into the stomach. It has to me remembered that the esophagus is penetrated by the larynx, and as a result it is divided into two pharyngeal canals (*piriform sinuses*), of which the right one is mostly involved in swallowing, being the largest of the two.

d. Intraspecies Differences

Odontocetes have quite different numbers of teeth; fewer teeth in beaked whales (such as *Ziphius cavirostris*, *Hyperoodon ampullatus,* etc.) is clearly correlated to the fact that prey are sucked into the animal's mouth and not seized.


2.17

The monodontid narwhals (*Monodon monoceros*) present sexual dimorphism in the number of teeth they possess. The male has a single long tooth or tusk that erupts from the upper left gum which can grow to become 3 meters

long. Sperm whales (*P. macrocephalus*) have teeth only in the lower jaw.

Mysticetes do not have teeth but use baleen plates to filter their food (principally small crustaceans) from the water. Baleen plates are made up of a tough corneous substance which hangs from the upper jaw having a hairy fringe along the inner edge. The fringes on the plates overlaps and create a mesh-like strainer inside the whale's mouth. The whale uses this strainer to trap its prey (usually small schooling fish, crustaceans or plankton) while it filters out the sea water, which it cannot drink in large quantities. Some baleen whales feed by gulping large quantities of prey and water and then using their tongue to force the water out in between the baleen plates. Other whales move slowly through the water with their mouths open, letting the water flow in the mouth frontally and out in between the baleens. Along the way, tiny plankton is trapped by the baleen hairs. (Fig. 2.18). The great mysticetes such as the bowhead whale (*Balaena mysticetus*) have high, curved baleens which can grow to even 4 meters.

2.8.2 Stomach

Mammals have stomachs which are located in the abdominal cavity and are generally composed of three tissue layers (tunics). Dolphins are poligastric animals, which means that they possess more than one stomach (three in bottlenose, striped, and common dolphins) (Fig. 2.19 and 2.20). As all these stomachs derive from a single dilatation of the primary digestive tract, some sustain that they can be defined as a stomach with more than one chamber. Other odontocetes have even more stomachs (or have stomachs with diverse structures as in the case of the Cuvier's beaked whale, *Z. cavirostris*). This anatomic structure must compensate for the absence of mastication by crushing and squeezing food before it is absorbed.


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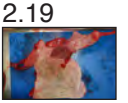
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a. Dolphins

The stomachs of dolphins (such as bottlenose – *Tursiops sp.*) are composed of:

- a first chamber or forestomach (which is devoid of glands and functions by repeatedly and energetically compressing food; there is probably no absorption at

this point);
- a second chamber (or main stomach with an epithelial cell lining and gastric glands);
- a series of small connecting chambers (difficult to identify) which terminate with a sphincter that leads to
- a third, oval shaped chamber (pyloric stomach). The pyloric stomach ends with a thick muscular sphincter that communicates with
- another chamber (the duodenal ampulla) which has a wider form.
- There are no sphincters between the forestomach and the main stomach so gastric enzymes apparently pass from the second stomach to the first one to contribute to food digestion.

b. Cuvier's beaked whales (*Z. cavirostris*)

The stomach of Cuvier's beaked whales has the largest number of gastric chambers. Immediately after the esophagus there is:
- a first chamber (main stomach) that is not a non-glandular forestomach (there are none in this species) but a real, comparatively large glandular stomach. Then there are:
- a series of minor glandular stomachs (called accessory or distal stomachs) the number of which varies depending on the species (beaked whales have 8-9, the first two are small and difficult to detect), and at the end of which there is a sphincter which marks the passage to the pyloric stomach;
- the pyloric stomach that has a relatively spherical form and ends with a sphincter;
- the duodenal bulb that is relatively small;
- Other ziphiids belonging to the Mesoplodon genus may present fewer gastric chambers positioned after the main stomach.

c. Dimensions

It is difficult to find reliable data concerning the capacity of the stomachs of bottlenose, striped and common dolphins. Based on dissections of numerous individuals of these three species, it can be estimated that:
- bottlenose dolphins (calculations based on an adult specimen living in captivity and weighing 250 kg) have a 3 liter capacity for the non-glandular forestomach, a 3 liter capacity for the main glandular stomach and 1.5 liter capacity for the pyloric stomach.
- The striped dolphin (calculations based on an adult specimen approximately 200 cm long) has a 2 liter capacity for the non-glandular forestomach, a 1.5 liter capacity for the main glandular stomach and 1 liter capacity for the pyloric stomach.
- The common dolphin (calculations based on an adult approximately

175 cm long) has a 1.5 liter capacity for the non-glandular forestomach, a 1.5 liter capacity for the main glandular stomach, and a 1 liter capacity for the pyloric stomach.

- It has been estimated that every day a bottlenose dolphin in captivity eats from 4 to 6 kg of fish, while free-ranging specimens grow much bigger and eat as much as 25 kg of fish a day.

d. Position and Topographic Relations with Other Organs

The stomachs are located immediately after the diaphragm, close to the liver in the intra-thoracic abdominal cavity, and is thus protected by the rib cage. The abdominal cavity, which has an elongated oval form, is extremely small in proportion to a dolphin's body. Since the diaphragm itself is very convex and is quite forward in the body, the three major stomachs are located practically one behind the other, positioned dorsally with respect to the liver. The pillars of the diaphragm run along the dorsal surface of the stomachs (mainly the forestomach) and can contribute to gastric mobility.

The kidneys are positioned dorsally and caudally with respect to the stomachs. A voluminous mesenteric lymph node is positioned caudally to the stomachs (and in particular caudally to the pyloric stomach and to the duodenal bulb) immediately below the rostral pole of the kidney. The small oval spleen is adjacent to the first gastric chamber.

The greater omentum extends from the greater curvature of the stomach (generally positioned ventrally and in a caudal direction). The lesser omentum connects the dorsal extremity of the liver with the rostral extremity of the first two gastric chambers (lesser curvature of the stomach).

2.8.3 Intestines

An interesting feature with regard to the intestines of cetaceans is the absence of external subdivisions between the various segments. After the stomachs, the intestines can be described as a continuous convoluted tube that terminates at the anus with minimum variations in the diameter and external specializations. There are, in fact, no evident distinctions between the small and large intestines and there is no blind-ending or vermiform appendix. This simplified disposition is apparently due to their diet composed almost entirely by protein, all of animal origin. In effect, the diet of odontocetes is composed principally of cephalopods and fish (and in the case of the killer whale and of the false killer whale – *Pseudorca crassidens* - of other marine mammals). Mammals whose diet is composed entirely or partially of vegetables need specialized sectors of the intestinal tract in which bacterial fermentation can degrade

cellulose, a process which obviously does not take place in odontocetes. Cetaceans furnished with baleens can instead use the gastric chambers for microbial fermentation.

It is impossible to note gross distinctions between the various parts of the intestine once topographic references are removed (for example, when the intestine is removed during a necropsy and there are no longer any organs or ligaments) (Fig. 2.21).

Even the microscopic anatomy of the intestine varies only slightly from the duodenum to the rectum.

The feces are rather liquefied and can contain undigested food residuals especially in the case of mysticetes, whose diet is based on krill (which is made up of crustaceans belonging to the order Euphasiacea).

2.9 The Liver

The liver is the largest organ in the body and carries out a key role in animal metabolism; damage to the hepatic structure can seriously compromise the animal's health and possibility to survive. This is one of the reasons why the study of pollutants (caused, for example, by heavy metals) found in the liver of cetaceans is so important as it helps to expand knowledge about the causes of strandings and the marine environment in general.

The liver is situated in the peritoneal cavity, adjacent to the visceral face of the diaphragm. Subdivision into lobes is not very distinct in cetaceans. The peculiar characteristic of the liver in dolphins is the convexity of the diaphragmatic face of the liver and of the dorsal border, due to the form and to the position of the diaphragm and to the relatively reduced volume of the peritoneal cavity. The liver of bottlenose dolphins can be found at the level of the inferior third of the 6°-9° intercostal space in the right hypochondriac, epigastric, and left hypochondriac regions in the intrathoracic abdominal cavity.


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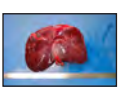
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The liver of dolphins is grossly divided into two lobes by a slightly pronounced dorsal indentation and by a deeper ventral one. The diaphragmatic face of the liver is completely in contact with the surface of the diaphragm and follows its curvature (Fig. 2.22). The visceral surface (Fig. 2.23) presents the organ's hilum which permits the passage of

the hepatic artery, the porta vein, the lymphatic vessels and the nerves.

In cetaceans, just as many terrestrial species, the gallbladder is absent, and bile passes continuously from the liver stroma to the duodenum. The absence of an extra-hepatic deposit for bile is probably related to the continuous assumption of food resulting in an almost constant presence of content in the proximal intestine. The general vasculature of the dolphin's liver is similar to that of terrestrial mammals. The centrolobular veins in the liver are well marked and easy to identify, while the strips of tissue connecting the two lobes are not particularly marked and at times lacking.

The mean weight of a dolphin liver can reach 2-3% of the animal's total body weight. The liver of an adult male bottlenose dolphin which weighs approximately 300 kg can weigh between 6 and 9 kg. According to Slijper (1979), the liver of cetaceans (and of some dolphins) is decidedly larger and heavier than expected in view of the anatomy of terrestrial mammals. The liver of the great whales and of the larger dolphins is relatively small with respect to dolphins belonging to smaller species

2.10 The Pancreas

A large, complex gland located in the peritoneal cavity, the pancreas performs both exocrine and endocrine functions. The pancreas in mammals is adjacent to the visceral face of the liver, encased by the mesoduodenum which terminates in the cranial flexure of the duodenum. The pancreas in cetaceans is very similar to that in land mammals. The exocrine pancreas produces enzymes necessary for the digestion, while the endocrine pancreas produces insulin, glucagon and other hormones regulating carbohydrate metabolism (See the section on the endocrine apparatus).

The pancreas of bottlenose dolphins is located in the epigastric region in the intra-thoracic part of the abdominal cavity. The organ is located so deeply that it is impossible to examine it externally or to take samples for a biopsy.

The pancreas of dolphins is flat and has an irregular form. Its two faces correspond to the laminae of the proximal part of the mesoduodenum directed towards the cranial flexure of the duodenum. The pancreas is therefore linked to the small omentum and connected to the cranial part of the duodenum. The liver hilum is always relatively close to the body of the pancreas and represents a landmark during a necropsy. The greater part of dolphins possess a single pancreatic duct which connects the pancreas with the duodenal lumen, but there are numerous exceptions. From a microscopic point of view, the only difference worthy of note

with regard to the architecture of the dolphin's pancreas are relatively few islets of Langerhans. The pancreas in dolphins weighs on average between 0.1 and 0.2% of the total body weight. The pancreas of a male adult bottlenose dolphin weighing 300 kg can weigh from 0.3 to 0.6 kg. The pancreas of large mysticetes is comparatively smaller than that of dolphins in proportion to body weight. In any case the absolute weight can be noteworthy (even 40 kg in a fin whale).

2.11 Excretory System

The urinary apparatus of cetaceans presents the same components detectable in land mammals. Two important characteristics (besides the kidneys and bladder) concern the thickness of the ureters and their low position at the base of the bladder. The presence and the position of testicles sometimes make it difficult to distinguish the ureters as they are similar to the ampoules of the deferens of some land mammals. Even the walls of the vas deferens are often thick and short as the distance they travel is limited in view of the intra-abdominal location of the testicles. The ducts of the vas deferens on both sides cross the ureters to reach the urethra (Fig. 2.24).

2.11.1 The Kidneys

The kidney is the organ that filters blood and produces urine. As blood flows through the kidney, it passes through glomerular capillaries located within the cortex (outer zone of the kidney); the urine is filtered in the Bowman's spaces and tubules in which ions and salts are concentrated or diluted depending on the homeostatic needs. Urine is thus collected in the renal pelvis and reaches, through the ureters, the urinary bladder.

Life in salt water creates several metabolic problems for mammals needing to maintain a hydric equilibrium and saline excretion at acceptable levels. This is particularly true considering that dolphins drink minimal quantities of salt water (dolphins living in rivers are an exception). Numerous factors including diet and hydric metabolism must be considered if the metabolic processes of cetaceans


2.24

are to be understood. Larger dolphins (like long-finned pilot whales), in fact, ingest large quantities of squid whose salt content is higher with respect to average content in other fish (mackerel, herring) which bottlenose dolphins prey on. Further information concerning this aspect can be found in the books and articles listed in the bibliography.

The kidneys of dolphins are lobulated, that is their external surface is divided into smaller spherical units called *reniculi* (or *renculi*) that have a diameter of approximately 10 mm (Fig. 2.25). The disposition of the renal parenchymal mass is not at all unique to cetaceans and can also be found in other marine mammals including polar bears, ruminants such as cattle, and in children. The human adult kidney presents hardly any traces of that initial subdivision. The magnitude of the subdivision of the renal parenchymal mass into separate subunits is, however, unique in cetaceans and interests all species including large mysticetes.

Mammals which have kidneys in a form similar to that in dolphins are characterized by greater cortical surface areas. Some years ago several authors (the most important Slijper, 1979) suggested that an increase in the cortical surface was justified by the greater amount of work required by the kidney of marine mammals whose life in salt water requires an intense filtering activity. This hypothesis has continued to be debated as some marine mammals (such as the harbour porpoise) have much smaller kidneys. Other studies suggest that the subdivision into renculi does not increase the parenchymal mass or glomerular volume but makes it possible to keep the length of the tubules limited. In very large mammals (except cetaceans) the absence of this division into lobules makes it necessary to have renal tubules with dimensions that increase the resistance of the intraluminal flow. The sporta perimedullaris, a fibromuscular layer which is surrounded by medullary pyramids, is present between the renal cortical and medulla of cetaceans (and of pinnipeds). Smooth muscle fibers in this band could favor the expulsion of urine.

The renal artery in bottlenose dolphins penetrates the kidney near the rostral pole, more cranially with respect to land mammals, while the ureter exits in a more caudal position near the caudal pole. The renal vein travels near to the corresponding artery.

2.25

2.11.2 The Bladder

The bladder of dolphins is relatively small with respect to its body mass but this is not surprising, given the relative narrowness of the abdominal cavity and since urinary retention is not important for the delimitation of the territory.

The bladder of dolphins is a sac-like, elongated organ that is partially covered by the peritoneum. Two long lateral ligaments unite the bladder to the abdominal walls. The thick ureters run in a dorsal-ventral, rostral-caudal direction originating from the kidneys and terminating at the base of the bladder, a position that is lower with respect to that in land mammals (and especially other artiodactyls, the closest relatives of cetaceans). The ductus deferens crosses the ureters at the base of the bladder to enter into the urethra.

The dimensions of the bladder are so small that at times the organ is difficult to find during a necropsy and appears lost in the intestinal mass. The bladder is usually empty at the post-mortem exam.

The structure of the bladder of cetaceans does not differ greatly from that normally found in other mammals.

2.12 The Reproductive Organs: the Male

The male reproductive apparatus of cetaceans is similar to that of ruminants such as the domestic cow (*Bos taurus*) and presents a fibroelastic penis which, when retracted, curves in an s-shaped loop. A relevant diversity with respect to the anatomy of ruminants is, however, noted with regard to the position of the gonads, that are intra-abdominal rather than externally in the scrotum (Fig. 2.24).

The physiologic cryptorchidism made thermoregulating mechanisms necessary, and are made up of a complex system of lombo-caudal venous plexuses positioned juxtaposed to the arterial ones that supply the testicles. The venous plexuses receive blood directly from subcutaneous veins in the dorsal and caudal fins, and the lower temperature with respect to body temperature gives rise to a thermic exchange that cools the gonads. As the genital organs are located internally, it is not possible to distinguish between males and females simply by examining the

2.24

animals externally, unless looking at the relative position of the genital and anal slits. In males they are clearly separated, whereas in females they form an almost continuous one, which is also flanked on both sides by two mammary slits. The similarity between individuals of different sexes makes it generally difficult to distinguish between the two in dolphins of small dimensions in the wild. Big sized odontocetes such as the killer whale and the sperm whale are sexually dimorphic, with males larger than females; in mysticetes instead females are slightly larger than males.

2.12.1 The Testicle and the Epididymis

The testicles of dolphins are situated within the abdominal cavity. This evolutionary adaptation perhaps took place because male dolphins and whales need to maintain an extremely hydrodynamic form. Besides obvious hydrodynamic needs, it implies marked modifications of the vascular bed of the gonads. In fact, the temperature of the seminiferous tubules must remain below that of the surrounding organs for sperm maturation to occur. Other mammals including the elephant have internal testicles.

Some data indicate that, just as in land mammals, during some phases of the development the testicles in cetaceans are in the scrotum as the fetal gubernacula, described in some species including the common dolphin and the harbour porpoise (van der Schoot, 1995). This finding suggests that in the fetal life of dolphins and whales there is still a vestigial mechanism of testicular descent. The hypaxial muscles of the caudal region contribute to impeding the development of a complete vaginal process necessary for the testicular migration (van der Schoot, 1995). There are no data available about the length of the epididymis in dolphins.

Similar to those in all mammals, the testicles of dolphins are elongated and cylindrical in shape. The interstitial cells are generally thin and less evident than those in some land species (see the chapter on the endocrine system).

2.12.2 The Vas Deferens

The vas deferens moves away from the testicles in the caudal direction, passes near the ureter and enters the penis. The vas deferens has a quite convoluted and to some extent twisting course. In contrast to many land mammals, the vas deferens of cetaceans does not present ampullary dilations, and it courses craniocaudally along the principal peritoneal axis, rather than the ventrodorsally from the scrotum.

2.12.3 Accessory Glands

The only accessory gland of the male reproductive system of cetaceans is the prostate, made up of prostatic tissue distributed in the walls of the urethra.

2.12.4 The Penis

Dolphins and whales have a fibro-elastic penis similar to that in ruminants. The organ at rest shows an “S” form due to the retractor muscle and reaches erection through blood flow into the cavernous bodies, relaxation of the retractor muscles and the consequent distension of the sigmoid flexure. The spongy body of the penis surrounds the penile urethra. There is not a well defined glans in cetaceans. The cranial part of the penis is cone shaped and contains the external urethral ostium.

One of the principal advantages of an “S” formed fibro-elastic penis is that the reposing organ is completely retracted, an extremely useful characteristic from a hydrodynamic viewpoint. The penis is extracted for mating and also during sex games with other young males. There are no bones (baculum) in the penis.

The penis of large stranded cetaceans frequently becomes everted as a consequence of gas production in the abdominal cavity during decomposition of the carcass. Just as all fibro-elastic penises, the organ of dolphins has a thick, tough tunica albuginea while the cavernous bodies are relatively exiguous.

2.13 The Reproductive Organs: the Female

2.13.1 Ovaries and Uterine Tubes

The ovaries of the dolphin generally have an oval form but at times are folded having a “U” shape. Age and pregnancies cause modifications. In bottlenose dolphins the left ovary is generally more active and responsible for two thirds of ovulations, at least during the first part of the animal’s life. In mysticetes, instead, both ovaries are contemporaneously active throughout life. The corpus luteum, when present, is clearly evident and the consequent corpus albicans may leave permanent scars that can be counted to estimate the animal’s age at the time of the necropsy (even if the presence of a accessory corpora albicantia can bias the estimate).

Ovulation continues even at an advanced age, even after 50 years in bottlenose dolphins and in short-finned pilot whales.

The ovaries are located dorsally in the abdominal cavity, in the bottlenose dolphin in the angle formed by the junction of the epaxial

muscles with the hypaxial ones, and held in place by their respective ligaments. This topography in this species can vary in animals which have had numerous pregnancies. The ovaries are wrapped in a venous plexus which also envelops the uterus and is functionally comparable to the one that covers the testicles. The flexuous uterine tubes have a diameter which is much smaller with respect to the uterine horns.

2.13.2 The Uterus and the Cervix

The uterus of cetaceans in general and in dolphins in particular is bicornuate (two-headed) and is very similar to that of the cow. The cranial part of the uterine body is divided into two uterine horns (Fig. 2.26).

The residue of a primitive double uterus can be detected in a septum which runs along the axis of the uterine body and which divides the cavity into two part. It is to be remembered that some species of ruminants with two-headed uteruses have twin births, while this possibility is extremely rare in cetaceans. In the bottlenose dolphin the fetus is located in the left uterine head in two thirds of all pregnancies.

The uterine cervix links the uterus to the vagina and its form is similar to that of the human or horse cervix. The vaginal wall presents a number of mucosal folds which some authors term a pseudocervix, distal to the true one. The cervix has numerous internal folds and frequently contain mucous. A countercurrent heat exchange device, composed of lombo-caudal (the cool part) and uterine vessels (the hot part), maintains constant the uterine temperature and avoids its overheating that would be induced by the continuous action of the powerful muscles of the vertebral column. The endometrium is modified in conjunction with the phases of the reproductive cycle and proliferates during ovulation and pregnancy. However there is no menstrual cycle with blood loss and sloughing of the endometrial cells as in the human species, as the placenta is of epitheliochorial type. Lactation reduces the width and the glandular activity of the endometrium and myometrium.

2.13.3 The Vagina, the Vestibule and the Clitoris

2.26

Linked to the cervix, the vagina is the organ in which the penis is placed during mating. Muscular and relatively

short, it is similar to that of a cow and other ruminants. The vestibule is short and characterized by an opening of the urethra. A hymen separates the vestibule of the vagina from the vaginal vulva. The clitoris is relatively large and has specific folds. It is unclear if these folds correspond to the lips of land mammals.

2.13.4 The Vulva

The vulva is a slit-like aperture with labia majora and minora (inner and outer folds, or “lips”). A thick strand of tough, fibrous tissue with a powerful underlying muscle is found at the front end of the vulva. This is obviously an adaptation to aquatic life. The nipples are situated in slits on either side of the female genital slit.

2.14 The Placenta

Cetaceans have a placenta that closely resembles that of the other Artiodactyla. The umbilical cord has two veins, two arteries, and the urachus in the central position. All of these structures are visible macroscopically. The podalic position is commonplace at birth (Fig. 2.27).

2.15 Endocrine Glands

It is difficult to define the endocrine system. According to the traditional anatomic definition, the endocrine glands pour their hormones directly into the blood stream. In recent years, however, the concept of endocrine organ has been extended to the single cells, such as those of the diffuse neuroendocrine system which produce many hormones. Various parts of the body, including even the intestines, are thus recognized as a part of the endocrine system.

The endocrine system of cetaceans has not been studied in a homogeneous manner. Some organs have been described exhaustively while others have not received much attention from the scientific community. Lack of specific knowledge about some endocrine glands such as the parathyroid or the pineal gland makes it impossible to answer some questions about the physiology of cetaceans


2.27

and may prevent opportune therapy of sick animals.

2.15.1 The Hypophysis

The anterior lobe of the hypophysis (adenohypophysis) of cetaceans is separated from the posterior one (neurohypophysis) by a thick connective tissue septum, making them de facto isolated. The adenohypophysis is much larger than the neurohypophysis. There is no intermediate part and the hormones specific to that part of the anatomy are produced by the pars distalis. A large rete mirabile is situated at the dural fold that contains the pituitary gland.

2.15.2 The Pineal Gland

The pineal gland (*epiphysis cerebri*) of mammals is a conical structure belonging to the epithalamus situated above a recess in the third ventricle. There is apparently no pineal gland in cetaceans (and in elephants) with the exception of the humpback whale (*M. novaeangliae*) in which a tiny pineal gland (approximately about 1 cm3) has been described. The organ is present during fetal development in the blue whale (*B. musculus*). The pineal gland has recently been described in the bottlenose dolphin. It is possible that cells similar to pinealocytes found caudally to the splenium of the corpus callosum might be able to produce melatonin hormone in cetaceans.

2.15.3 The Thyroid

The thyroid is composed of two lobes situated to the sides of the larynx and connected to one another by a bridge of thyroid tissue. This endocrine gland is generally well developed in dolphins. In the bottlenose dolphin the thyroid can be present in various forms. In some specimens the bridge between the two lobes develops so much that the gland becomes a single organ situated centrally to the windpipe . There are also intermediate forms with respect to the one described. In the bottlenose dolphin, some studies have reported high plasma levels of thyroid hormones. In general the thyroid is more developed in marine mammals than in land ones, even if this is not to be considered linked to the content of iodine in the food. It is important to remember this fact to avoid formulating a mistaken diagnosis of goiter or thyroid hyperplasia in dolphins.

The follicles are small with a higher content of colloid in young specimens, and in older ones the colloid dehydrates and assumes a dark pink color. The dimensions of the thyroid follicles are smaller in cetaceans with respect to humans. The presence of parafollicular cells can be considered quite probable even though there may be confusion

with the walls of follicles gathered tangentially in the section plane (Simpson and Garner, 1972).

2.15.4 The Parathyroid Glands

Few studies have been carried out on the parathyroid gland of cetaceans. It has been noted that parathyroid glands can occasionally be found within the capsule of the thymus (Cowan, 1994).

2.15.5 The Adrenal Glands

Adrenal glands in dolphins are flat corpuscles situated in the retroperitoneum rostral to the kidneys, near the pillars of the diaphragm not far from the median sagittal plane.

The adrenal glands of cetaceans are partially subdivided into lobules (pseudolobules) by short connective tissue septa which penetrate at a right angle the entire width of the cortex. The capsule is often surrounded by numerous nervous bundles and by vessels situated within a connective tissue stroma. The zona fasciculata is generally the thickest part of the cortex but there are noteworthy individual differences (Fig. 2.28).

The medulla contains more vessels than the cortex. As in the greater part of mammals, the cells form cords separated by connective septa in which the vessels travel.

2.15.6 The Endocrine Pancreas

The islets of Langerhans, known as insulin-producing cells, are dispersed within the pancreatic parenchyma of cetaceans. The pancreas has already been described in the section concerning the digestive system. It is important to remember that many studies have noted that there are relatively few pancreatic islets within the parenchyma and that these are of small dimensions (with only a few cells for each islet).

Quite a few years ago in times preceding the synthesis and industrial production of insulin, the great whales were at times captured to extract insulin to be used for therapy in humans.


2.28

2.15.7 Gonadic Endocrine Function

The interstitial cells of the testicle produce male sexual steroids. These interstitial cells are not always easily

identifiable as they are more tapered with respect to those in land mammals.

As in other mammals, the ovaries of dolphins produce female sexual steroids.

2.15.8 The Diffuse Neuroendocrine System

There are only a few studies on the diffuse neuroendocrine system in cetaceans. According to these works, the intestinal mucosal tunic of striped dolphins contains neuroendocrine cells that produce neuropeptides just as land mammals.

2.16 The Nervous System

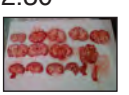
2.16.1 The Brain

The central and peripheral nervous system of cetaceans has evolved to favor their adaptation to aquatic life. Cetaceans have the biggest brains on the planet. Some species of large dimensions such as humpback, sperm, and blue whales have the largest, heaviest brains that have ever existed on earth. The heaviest brain ever recorded (9.2 kg) belonged to an adult sperm whale (Kojima, 1951).

The brain of bottlenose dolphins weighs between 1,550 and 2,030 g (the human brain weighs on the average approximately 1,400 g). Any comparison with regard to form, volume, and number of lobes between the brains of various species must take into consideration various parameters such as the relationship between brain and body weight. Some dolphin species have a higher brain/body weight ratio with respect to anthropomorphic monkeys, with values similar to those found in humans (from Fig. 2.29 to Fig. 2.31).


2.29


2.30


2.31

The brains of cetaceans are wider, higher, shorter and more curved along their transverse axis.

The large telencephalic hemispheres present an impressive development of cerebral convolutions, that hide a large part of the cerebral cortex . In effect, the entire cerebral cortex of a bottlenose dolphin is more expanded with respect to the human one even if the width of the latter is double. The overall cortical volume of this dolphin can reach approximately 80% of the human one. Even if the cortex is markedly evolved, its structure is apparently

"monotonous" as the characteristics of the layers do not change in the different lobes, but tend to reproduce the same conformation throughout the entire brain. The fourth layer (the internal granular layer) is difficult (if not impossible) to identify. The presence of a repetitive and monotonous cortex and the relative absence of internal granule cells are used by some to argue that the brain of cetaceans is incapable of carrying out superior cortical functions typical of anthropomorphic monkeys and of man in particular. The great brain size of cetaceans has been explained as a function of adaptation to an aquatic life. Sirenians and pinnipeds have, nevertheless, small brains which are only slightly convoluted or in some cases without any convolutions, having a rather thick cortex. According to one theory based on neurohistologic data, the brain of cetaceans is a sort of "primitive" yet quite elaborate one that conserves many of the characteristics with regard to the cortical plate of insectivores and chiropterans.

In dolphins one can identify the cerebral lobes of land mammals including the olfactory lobes (which in any case disappears during embryogenesis). A tiny terminal nerve with the relative ganglion has been described in odontocetes.

The limbic lobe (but not the hippocampus) is extremely well developed. On the medial face of the hemispheres near the limbic lobe, there is a paralimbic lobe which is unique to cetaceans. The visual cortex is not localized in the occipital lobe but in the dorsal medial part of the parietal lobes. The development of the auditory cortex is one of the possible factors responsible for the development of the modified brain cortex in cetaceans. The auditory cortex does not seem to be localized in the temporal lobe but in the laterodorsal part of the parietal cortex. The corpus callosum in dolphins is comparatively smaller with respect to the human one even if the absolute weight of the brain is greater. This may be due to the reduction in the number of interhemispheric fibers or to a reduction in the thickness of the myelin sheath. Both factors can be important in increasing the independence of the hemispheres. The basal ganglia are very well developed and are clearly evident in the transverse sections (coronal). The thalamus is also well developed. The mammillary body (or bodies) is not visible on the ventral face but mammillary nuclei have been described.

The pons is large, while the mesencephalon and the medulla oblongata are bent, short, and thick. The elliptical nucleus also present in elephants is clearly noted in the mesencephalon. Its functions are obscure and perhaps linked to regulating the musculature of the nasal sacs (in a certain sense equivalent to the proboscis of elephants). The inferior (caudal) colliculi are more important with respect to the superior ones (rostral), a fact that may be linked to the importance of sound perception in the

aquatic environment (see the section on the ear that makes mention to the acoustic way including the structures relative to the brainstem).

The cerebellum, which is also well developed, presents lateral hemispheres which stand above and surround the more sunken vermis, a characteristic also common in man, monkeys and elephants. Paleontological findings indicate that the cerebellum of the first cetaceans (archaeocetes) was larger (or at least wider) than the telencephalon, again due to an adaptation to the aquatic life.

The nucleus interpositus (formed by the fusion of the emboliform nucleus with the globular nucleus) is extremely well developed.

Neurophysiological data on dolphins are scarce and not recent; neurophysiological studies on the great cetaceans do not exist. It is important to remember that dolphins, which are voluntary breathers, sleep with only one hemisphere of their brain at a time, an ability that is needed to avoid drowning. There does not seem to be REM phases during sleep although the matter is controversial. A growing body of evidence indicates that the brain of dolphins might be able to switch to an anaerobic metabolism during deep dives.

2.16.2 The Spinal Cord

Generally speaking the morphology of the spinal cord of dolphins and whales is similar to that in land mammals. The reduced thoracic swelling (because of the transformation of the thoracic limb into a fin) and the absence of a lumbosacral swelling (due to the disappearance of pelvic limbs) are some of the most particular, prominent characteristics.

The histological examination of the spinal cord shows that the medullary canal is absent and there are two arteries in its place. The dorsal horns are diminished while the ventral ones are rather large. The corticospinal tracts are crossed and of reduced dimensions.

2.16.3 The Cranial Nerves and the Peripheral Autonomic Nervous System

The olfactory nerve disappears during embryogenesis and is absent in the adult. The vomeronasal organ and nerve are absent. The terminal nerve and its relative ganglion are visible on the anterior brain face, at which point they cross the dura mater and the cribriform plate of the ethmoid bone going towards the nasal sacs.

The optic nerves cross almost completely at the optic chiasm in such a way that virtually all the fibers coming from the retina of one side are destined to form the optic tract which terminates in the contralateral visual cortex (see also the description of the eye).

The existence of a parasympathetic nucleus of the oculomotor nerve

is debated also because of the existence of an elliptical nucleus that occupies part of the corresponding position (see also the description of the mesencephalon).

The trigeminal nerve is the largest, most evident nerve after the acoustic one. It travels close to the internal and middle ear. There are some findings indicating that besides its own functions the fifth pair of cranial nerves of dolphins contains fibers moving to the cavernous body of the middle ear and regulates its vascular filling.

As mimic muscles do not exist, the somatic driving component of the facial nerve principally innervates the musculature of the blowhole and of the upper airways.

The sensitive and vegetative fibers of the facial and glossopharyngeal nerves are reduced because of the disappearance or marked reduction of salivary glands and taste buds in the oropharynx.

The enormous auditory nerve contains more fibers than that in humans.

The vagus nerve continues to be the major vegetative nerve in the body.

There are not many descriptions of the peripheral nervous system in cetaceans. The brachial plexus is clearly evident. The greater part of the motor fibers which belong to it are probably directed to muscles that rotate the humerus and the shoulder blade or, on the contrary, stiffen the scapular-humeral articulation.

The ganglia of the sympathetic chain are easily detectable at the sides of the spinal cord. The communicating branches are well distinguished within the two series of ganglia. The sympathetic ganglia are encased by retia mirabilia.

2.17 Sense Organs

2.17.1 The Eye

Dolphins and cetaceans in general have large eyes situated to the sides of the head and, as we have seen, the greater part if not all of the fibers of the optic nerve which originate in the retina on one side cross over so that the optic pathway terminates in the contralateral visual cortex.

The position of the eyes and the organization of the fibers of the optic nerve make stereo vision impossible in the different species. The lateral position of the eyes permits ample visual fields and consequently a greater control of the surrounding environment. The eyes can move independently and movements are not conjugate. There are rods in the retina just as in land mammals but only L-type cones.

As in the large part of mammals (but not in humans) there is a well

developed retractor muscle of the ocular bulb.

A well developed lobulated Harderian gland (an accessory gland associated with the third palpebral in land mammals) with sexual dimorphism has been described in the bottlenose dolphin. In the form of a band, it surrounds the ocular globe. Mucous glands are also present in the palpebra.

2.17.2 The Ear and Acoustic Features

The perception of sound in a watery environment requires some modifications of the auditory apparatus. In cetaceans there is no externally visible auricle and even the acoustic meatus is not immediately visible. An ossicular chain is present in the middle ear even if the elements that constitute it are connected with one another in a more rigid manner with respect to what is found in land mammals. A cavernous body is found in the middle ear.

The cochlea is long and thin. There are many more neurons present in the spiral ganglion with respect to the corresponding human ganglion.

As sound travels four times faster in water than in air, the structures which discriminate sounds such as the superior olivary complex (rostral) must be well developed to be able to distinguish where the sound is coming from (right or left).

The inferior colliculus (caudal), as already mentioned, is much more developed with respect to the superior (rostral) one. The nuclei of the lateral lemniscus and the acoustic radiation directed towards the cortex are large and highly developed.

The auditory responses of the brainstem and the studies conducted on myelinated fibers of the acoustic nerve indicate that the auditory system of cetaceans is specialized in rapidly processing acoustic signals.

2.17.3 The Melon

The melon in odontocetes is not a true sense organ (in effect, it is part of the integumentary system) (Fig, 2,32). We chose to deal with it in this section because we thought that those not familiar with cetacean anatomy might be prompted to look for the melon among the sense organs, given its close relationship with the highly specialized auditory apparatus. It is a structure composed of fat and

2.32

connective tissue that is used to focus sounds produced by generators situated near the upper airways and modulated in the diverticles connected to the nasal sacs. It acts as an acoustic lens for the sonar and also to produce sounds that can stun prey.

The sperm whale was named after the milky white substance, the spermaceti, whose density depends on the quantity of heat produced by the enormous arteries that surround it. Changes in the state of fluidity influence the body's buoyancy and contribute to the whale's movements and stabilization during deep immersions (of even thousands of meters).

3

NECROPSY PROTOCOL FOR SMALL ODONTOCETES

Sandro Mazzariol, Cinzia Centelleghe

Before beginning *post-mortem* 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.

3.1 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.

3.1.1 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 Postitioning 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.

3.1.2 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. More specific information on specific protocols and procedures will be detailed in chapter 5.

3.1.3 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.

3.1.4 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.

3.2 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 table 1 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)

Code	State of conservation	Description	Possible investigations
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), itology(PCR), contaminants (limited) biology, aleopathology (on the skeleton) (life history),gene ticsparasitology(P CR), 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)

Tab. 3.1: Conservation code category in strandings animals

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

3.2.1 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 (Fig.3.1).


3.1

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.

3.2.2 Categories 2-3

In these cases the carcass can still furnish useful information about the cause of death for both health and conservation purposes (Fig. 3.2 and Fig. 3.3). 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.

3.2.3 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 (Fig. 3.4) to the appropriate centers can delegate personnel


3.2


3.3


3.4

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 *aleicephalea 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 crocephalus*	300	350 -500		670	>2	670	11-13 F 15-18 M

Tab. 3.2: Some correlations between lengths and age classes

to collect the samples.

3.3 Physiological parameters estimation

3.3.1 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) (Fig. 3.6). The exemplar'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.

3.3.2 Weight estimation


3.6

It is important to estimate the weight of stranded animals for therapeutic purposes (to calculate drug dosages and

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

Tab. 3.3: Relationship between total length and maximum extimated weight

Total Length (m)	Maximum estimated weight (kg)		
	Striped Dolphin Common Dolphin	Bottlenose Dolphin Risso's Dolphin	Long-finned pilot whale
Family	Sex	a	b
Myticetes	M	-7.347	2.329
	F	-7.503	2.347
Odontocetes	M	-8.702	2.382
	F	-9.003	2.432

Tab.3.4: Principal linear regression coefficients according to family and sex.

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.

To have more precise estimate it is possible to resort to a linear regression according to the loge Mmedia 0 a + b loge Lmax 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 x L2.74). The table below indicates the coefficients for the various typologies.

3.4 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 (Fig. 3.5). 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.

3.5 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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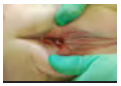
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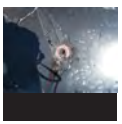
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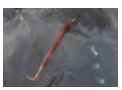
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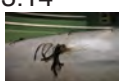
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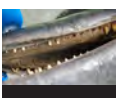
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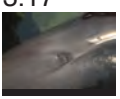
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in the male. A single short mammary slit can be seen on either side of the genital slit in most female cetaceans and occasionally males also possess this feature (Fig. 3.7). One of the simplest ways to determine the sex in a cetacean is by blunt-probing 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 (Fig. 3.8).

3.6 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 (from Fig. 3.9 to Fig. 3.15). 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 (Fig. 3.16).
- The color and amount of discharge from the blowhole as well as the presence of parasites and/or obstructions must be noted (Fig. 3.17 and Fig. 3.18). 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 (Fig. 3.19).
- 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.


fig.3.19

- 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.

3.7 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.

3.7.1 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


fig.3.20


fig.3.21

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 (Fig. 3.20 and Fig. 3.21). 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 interface 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 (from Fig. 3.22 to Fig. 3.24). 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 (Fig. 3.25 and Fig. 3.26). Samples of the blubber and of the subcutaneous tissue should be collected for histology and for analysis of contaminants (from Fig. 3.27 to Fig. 3.29). 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.


fig.3.22


fig.3.23


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3.7.2 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 color 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 lateralspinal 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.

3.8 Internal examination

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

3.8.1. 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.

3.8.2 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 cavernosus.

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.

3.8.3 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 proceeds 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 (Fig. 3.30).

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.

3.8.4 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 (Fig. 3.31). 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.

3.30

3.31

The pharyngeal mucosa should be examined and possible color and appearance alterations of every lesion, foreign

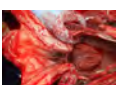
body or exudate should be noted. One penetrates with a scissors the epiglottis lumen continuing the cut on the dorsal side between the two arytenoids highlighting the pharyngeal tonsil and continuing to cut the tracheal wall until reaching the bronchial bifurcation (Fig. 3.32). Luminal contents (foam, fluid, blood, puss), the appearance of the mucous and of the folds of the laryngeal tonsil (hyperemia, edema, hemorrhage, petechiae, erosions) must be examined (Fig. 3.33). Samples should be collected for histology.

3.8.5 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.

3.8.6 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 ilet, 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.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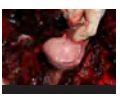
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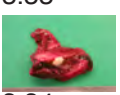
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3.8.7 The tracheobronchial (TB) lymph node

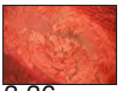
The TB lymph node is located along the distal cranial ventral surface of the lung proximal to the bifurcation of the trachea. It can easily be located by reflecting the cranial lung tissue away from the cavity and palpating the connective tissue between the lung and anterior to the

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.


3.35

3.8.8 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 linger (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 (from Fig. 3.35 to Fig. 3.44).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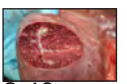
Serial, parallel cuts perpendicular to the long axis of the body into the tissue should be made using a long knife and single sweeping movements to examine the parenchyma. The parenchyma should be examined and its color pattern and texture noted. A sample in formalin for histology, microbiology, molecular and ancillary investigations should be collected from the cranial lobes of both lungs (4 sampling sites).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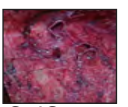
3.8.9 Heart and vessels

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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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 semilunars and the aorta whose wall can be cut following the first bifurcations. Operators must look for signs of thrombi, endothelial plaques, whitish mineralizations, aneurysms, or breaks and the consistency of the ductus arteriosus should be evaluated. The other alternative is to precede as in the right part of the heart, 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.

3.8.10 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 (Fig. 3.45).

3.8.11 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 (Fig. 3.46 and Fig. 3.47). 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 noted and the organs should be sampled for histology and secondary investigations.


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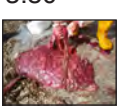
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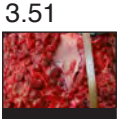
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3.8.12 The kidneys and the ureters

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 (from Fig. 3.48 to Fig. 3.52).

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, microbiology, molecular and ancillary investigations should be collected.

3.8.13 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 (Fig. 3.53). At the same time, the bile ducts should be examined for the presence of parasites. Samples for contaminants, histology, microbiology, molecular and ancillary investigations should be collected. Note that all cetaceans lack a gall bladder.

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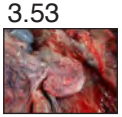

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3.8.14 The pancreas

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 (Fig. 3.54). 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.

3.8.15 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 (from Fig. 3.55 to Fig. 3.59). 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


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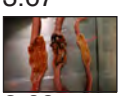
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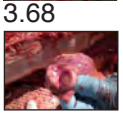
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the fore stomach is composed of squamous tissue and is usually white. The wall of the main stomach is stratified and usually thicker than that of the fore stomach and the mucosa is usually dark red. The pyloric stomach tends to be thin walled, glandular, 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 (from Fig. 3.58 to Fig. 3.65). The stomach should be weighed empty and samples of each compartment should be taken for histology.

3.8.16 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 (Fig. 3.66). 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 5 to 10 longitudinal cuts about 20-30 cm long. The color, 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 (Fig. 3.67 and Fig. 3.68). Samples should be taken for histology. Feces should be collected for biotoxin analysis.

3.8.17 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 (from Fig. 3.69 to Fig. 3.72). 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.


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3.8.18 The bladder


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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 (Fig. 3.73 and Fig. 3.74). A sample of the cranial tip of the bladder should be taken for histology.

3.8.19 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 (Fig. 3.75 and Fig. 3.76).


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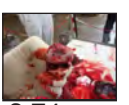
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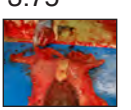
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If a fetus is present but is too small for a sufficient individual necropsy, the abdomen should be incised and microbiology and molecular samples should be taken and the fetus should be preserved whole in formalin. If the lung tissue floats in formalin 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.

3.8.20 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 (Fig. 3.77). 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 (from Fig. 3.78 to Fig. 3.83). 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.


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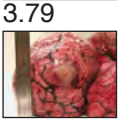
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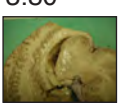
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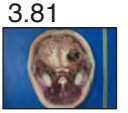
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3.9 Samples management

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.

3.9.1 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.

3.9.2 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.

3.9.3 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.

3.9.4 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.

3.9.5 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.

3.9.6 Sampling for contaminants

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 bioaccumulate 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.

3.9.7 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.

3.9.8 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 placed 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.

3.9.9 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.

3.9.10 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 cm3 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 Lymphnode Blubber lesions Prostate Testiclesi Epididymus Uterus Placenta	Aseptic sample	Refrigerated, +4°C
Hystopathology	All organs and lesions	1 cm3 of tissue	10% Formalin
Parassitologia	Parasites		70% Ethanol
Parasitology	Intestine Liver Lung Organs with parasites	5 cm3 of aseptic sample	Freeze, -20°C
Age estimate	Gonads	At least one	10% Formalin

DIAGNOSTIC INVESTIGATION	ORGAN OR TISSUE	COLLECTION MODE	CONSERVATION MODE
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	MuscleTessuto Fat tissue Liver Sleen	15x20 cm of aseptic sample	Freeze, -20°C
Algal biotoxins	Stomach contents Urin Feces	Plastic box	Freeze, -20°C
Life history and morphometric studies	Skeleton, skull		Freeze, -20°C
Genetic	Muscle	1 cm3 of aseptic sample	Freeze, -20°C

Tab. 3.5 : Check-list for sampling collection to use during a necropsy (from “Protocollo di intervento sui cetacei spiaggiati morti sul territorio nazionale”, Istituto Zooprofilattico Sperimentale of Piemonte, Liguria and Valle d’Aosta).

4

MAIN DISEASES IN MEDITERRANEAN CETACEANS

Giovanni Di Guardo, *Massimiliano Pennelli*, *Federica Marcer*, *Sandro Mazzariol*

One of the consequences of climatic variations and environmental degradation is more numerous and more serious health emergencies in the human and animal world. Emerging diseases can cause epidemics and can play a zoonotic role depending on other variables such as anthropic pressure determined by pollution, genetic factors and weakened immune systems. Marine organisms represent, thus, health sentinels of the aquatic ecosystem acting as a barometer permitting us to study, characterize, and manage the impact of those events affecting the state of health of humans and animals and the ocean environment on a long-term scale.

Marine mammals are the first sentinel species because of their long life expectancy, are coastline residents and feed at the highest level of the ocean food chain and possess a unique adipose deposit which can be transformed into a poison tank. They consume the same food as human beings and can be affected by the same public health problems. They are, moreover, exposed to the same environmental stressors such as chemical agents and algal biotoxins and recurring as well as emerging pathogens. Marine mammals are, moreover, part of a charismatic megafauna that habitually evokes a sympathetic response on behalf of humans.

4.1 Viruses

Twenty-five years ago few viruses were known that could infect marine mammals causing at times even mass mortalities. The incapacity to isolate viral agents from these animals was partially due to the difficulty in gathering tissue samples in a rapid, appropriate way and then sending them off to the next stage of laboratory analysis.

Interest in these pathogens increased considerably as numerous morbilliviral epidemic outbreaks took place in European waters between 1987 and 1992, with thousands of Lake Baikal seals (*Pusa siberica*) having also been killed between 1987 and 1988; while about 20,000 common seals (*Phoca vitulina*) died in the North Sea and 2,500 striped dolphins (*Stenella coeruleoalba*) in the Mediterranean. Additional morbilliviral disease outbreaks and cases have been reported in the North-Western Atlantic, in the Black Sea, and in the North Pacific.

4.1.1 Morbillivirus

The *Morbillivirus* genus belongs to the *Paramyxoviridae* family of viruses in the order *Mononegavirales*. Many members of the genus cause diseases such as rinderpest and measles and are highly infectious. *Dolphin Morbillivirus* (DMV) is tightly related, from genomic and antigenic points of view, to *Porpoise Morbillivirus* (PMV) isolated in 1988 for the first time in Ireland.

Beginning in the second half of the 1980's and until now approximately ten distinct morbilliviral epidemics have taken place in several species of marine mammals in various corners of the planet. Some of these, such as the 1988 and 2002 epidemics among common seals (*P. vitulina*), or the one that took place between 1987-1988 in bottlenose dolphins (*T. truncatus*) along the Atlantic coast of the United States and another in 1990-1992 involving striped dolphins (*S. coeruleoalba*) in the Mediterranean, were particularly dramatic provoking the death of hundreds or thousands of specimens and had in some cases negative consequences on the precarious state of survival of species already risking extinction as in the case of the Mediterranean monk seal (*M. monachus*), whose most numerous colony living along the Mauritania coast in Africa was stricken in 1997 by an epidemic that cut its population in half. As far as the Mediterranean basin is concerned, an ulterior epidemic event caused by a strain of DMV that was quite similar in genomic and antigenic terms to that responsible for the dramatic epidemic in 1990-1992, took place towards the end of 2006 in the population of long-finned pilot whales (*G. melas*) in the Strait of Gibraltar and the following year in the striped dolphin population living on the Spanish coastline, in particular in young

and subadult animals. That infection reached the Sardinian and French coasts between 2007 and 2008 and then went on to cause some strandings along the Italian peninsula beginning in 2009. In 2010-2011 another epidemic took place in the Mediterranean basin. The epidemic started out, it seems, once again on the southern Spanish coast and the Strait of Gibraltar, striking this time only newborns and extremely young individuals, probably those born after the last epidemic. The epidemic arrived along the Italian coastline involving species that until that time had been untouched, as in the case of two fin-whales (*B. physalus*) found stranded in the area around the Pelagos Sanctuary. Additional cases of infection were responsible for chronic damage only to the brain, thereby giving rise to a condition similar to "old dog encephalitis", caused by *Canine Distemper* Virus (CDV), or to human "subacute sclerosing panencephalitis", caused by *Measles Virus* (MeV). A phylogenetic study of the viral strain(s) analyzed demonstrated a mutation of the virus, in particular in the gene codifying for protein F, with respect to viral strains responsible for the precedent epidemics. The other difference with respect to precedent epidemics was the season of onset: in fact, while the epidemics in 1990-92 and 2006-08 took place primarily in spring-summer, this one occurred primarily during the winter months. *Morbillivirus* infections can have long term effects on cetacean population dynamics both as endemic infections and as well as and even more as recurring epidemics. In the latter case, the greater impact depends on the number of sexually mature specimens that are involved.

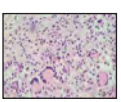
Macroscopically, the main *post-mortem* findings noted in striped dolphins in the Mediterranean at the beginning of the 1990s was pneumonia, often associated with extensive areas of atelectasis, subpleural emphysema and ectasia of the subpleural lymphatic vessels. Pulmonary lymph nodes were frequently edematous and increased in volume (Fig. 3.69). An infestation of variable entity by nematodes was also noted in the airways. Some dolphins had necrotic and haemorrhagic lesions in the cerebral cortex caused by secondary fungal infections (*Aspergillus spp.*), frequently seen - as in the case of other biological agents - in infected animals because of severe immunosuppression primarily induced by the morbilliviral agent (Fig. 3.83). At times a concomitant ulcerative stomatitis was also noted.


3.69


3.83

In the bottlenose dolphins studied during the epidemics that took place in 1987 along the eastern USA coastline and between 1993 and 1994 in the Gulf of Mexico there were frequent signs of septicaemia including edema of internal organs with accumulation of large quantities of sero-haemorrhagic fluid in the pleural and peritoneal cavities. Liver fibrosis, pancreatic, myocardial and pulmonary lesions could be also noted, with these pathologic changes bearing witness to the subacute/chronic progression that morbilliviral infection tends to take in cetacean victims, differently from what generally occurs in pinnipeds.

Histologically, the most important lesions were found in the lungs, which showed a subacute-to-chronic bronchiolo-interstitial pneumonia. In particular, hyperplastic type II pneumocytes, accompanied by exudation of large mononuclear cells with a clear macrophagic-histiocytic morphology, were observed in the bronchial, bronchiolar and alveolar lumina. An extremely peculiar finding – similar to what commonly reported in natural infection models already amply characterized in humans and in other terrestrial mammals - was represented by the numerous, voluminous, intra-bronchial, intra-bronchiolar and intra-alveolar multinucleated syncytia (of the “Warthin-Finkeldey” type) (Fig. 4.1). These cells frequently contained, similar to macrophages and the bronchial, bronchiolar and alveolar epithelial cells mentioned above, eosinophilic intranuclear and intracytoplasmatic inclusion bodies. Actually, the viral inclusions and the syncytia were often focal or even difficult to identify due to the modifications in lung architecture following serious lesions induced by secondary infections caused by opportunistic fungi or bacteria. Fibrosis of the alveolar septa denoted, moreover, a chronic infection progression. Severe lesions of a generally multifocal, non-purulent encephalitis associated with concurrent gliosis (microgliosis and astrogliosis/astrocytos) and neuronophagia were all observed in 69% of the striped dolphins analyzed in the Mediterranean Sea (Fig. 4.2). Demyelination, particularly of the cerebellum, was occasionally noted. Intracytoplasmic and intranuclear acidophilic inclusions could be also detected in necrotic and degenerating neurons and glial cells, as well as in brain syncytia. The severe virus-induced immunosuppression was, likewise, the basis for the fungal or protozoal superinfections as in the case, for example,



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4.2

of *Toxoplasma gondii* infection. A more or less consistent lymphocyte depletion was noted in the lymph nodes and in the spleen, particularly in the cortical area, associated with the presence of syncytia in both locations.

Intracytoplasmic and intranuclear acidophilic inclusions in the aforementioned syncytia, as well as in lymphocytes and macrophages, were also noted in 34% of lymphoid tissues from the infected animals. A concomitant secondary bacterial lymphadenitis was also frequently found. At times there was a concurrent hepatic degeneration in which the hepatocytes showed the presence of eosinophilic inclusions and hyaline globules; it was also possible to note, although only occasionally, necrosis of the biliary duct epithelial cells associated with moderate pericholangitis. Intracytoplasmic and intranuclear inclusions were, moreover, noted within the epithelial cells of the pharynx, esophagus, stomach, intestine, biliary ducts, renal pelvis, ureters, penile urethra, oviduct, vagina, lacrimal and mammary glands, as well as in cells of the skin and of the exocrine pancreas.

In 1995 Domingo and coworkers described a non-purulent encephalitis associated to acidophilic intracytoplasmic and intranuclear inclusions in the neurons and in other brain cells of some striped dolphins found stranded on the Spanish coastline both in the context of and following the dramatic epidemic of 1990-92 in the Mediterranean Sea. Other lesions attributable to the virus were not found, nor there was immunohistochemical evidence of morbilliviral antigen in other tissues from those animals. In view of the apparent “confinement” of the virus (and of its pathogenic activity) to the brain tissue, it can be hypothesized that the infection was a chronic form similar to “subacute sclerosing panencephalitis”, a well-characterized neurologic sequela of human MeV infection. A similar infection’s pattern seems to have been followed also during the 2006-2008 morbilliviral epidemic.

4.1.2 Herpesvirus

Herpesvirus is another infectious agent frequently found during *post-mortem* investigations in some species of stranded cetaceans from different corners of the world including the Mediterranean Sea and adjacent waters. In particular, *alpha* and *gamma-Herpesviruses* have been identified secondary to other infections or as primary pathogens.

Reports of infections linked to this virus are not frequent in cetaceans but have been increasing over time: the investigations carried out during strandings along the Spanish and Japanese coastlines have allowed to identify the presence of *alpha* and *gamma-Herpesviruses*, the first related to the human virus (*Herpes Simplex Virus*, types 1 and 2) and mainly responsible for skin and encephalic lesions; the second related to

Rhadinovirus and found in mucous membrane samples.

These pathogens have a tropism particularly for epithelial tissues determining a circularly-shaped, necrotizing dermatitis characterized by a slight central depression and a lighter peripheral area. These macroscopic lesions were confirmed by histopathologic examinations showing necrosis of the spinous layer; the necrotic keratinocytes exhibit picnotic nuclei with eosinophilic inclusion bodies, along with cytoplasmic vacuolization.

Herpesviruses have been recognized as pathogens of the oral and genital mucous membranes determining disepithelization and papillomatous/plaque-like lesions especially in males. *Herpesviruses* were, moreover, responsible for a severe non-purulent encephalitis both in porpoises (*Phocoena phocoena*) in the North Sea as well as in Mediterranean cetaceans. In these cases the virus replicates in neurons of the cerebral cortex. In 2001 two new *alpha-herpesviruses* responsible for systemic forms of disease and determining necrotizing lesions both macroscopically and histologically were detected in many internal organs from stranded bottlenose dolphins. Systemic infections were described during the last morbilliviral epidemic in the Mediterranean Sea, which was characterized by a coinfection between the two viral agents probably due to immunosuppression caused by the *Morbillivirus* pathogen.

4.1.3 Poxvirus

Dolphin Poxvirus (DPV) infections have been described in various species and are caused by viral agents which seem to belong to the new subfamily *Chordopoxvirinae* but having a common ancestor with *poxviruses* of the *Orthopoxvirus* genus with a specific affinity for the *Cow Poxvirus*. These viruses determine a pathological condition in cetaceans called “tattoo skin disease” (TSD) because of its typical irregular gray, black or yellow lesions appearing on the skin and resembling tattoos.

Recent studies carried out in three oceans and adjacent seas highlight similar patterns in the endemic form of TSD mainly involving young subadult specimens with respect to newborns/calves (*poxviruses* induce a strong humoral immunity in these subjects) and adults. When endemic, TSD does not cause high mortality rates but can provoke death of weaker subjects, in particular of newborns and calves, thus modifying population dynamics. Even if environmental degradation has not been studied in relation to this infection, polluting agents and high levels of chronic stress due to social interactions seem to produce immunosuppressive effects predisposing for high levels of contagion or recurring forms in adults.

Typical lesions may appear in every part of the body but their distribution seems to depend on the species; the lesions seem to persist for months

or years even if localized predominantly on the dorsal part of the body or on the pectoral, dorsal or caudal fins. The lesions appear as solitary or coalescing, at times slightly raised irregular areas that are gray, black or yellowish with sharply demarcated edges. Advanced ring lesions may take the form of black, punctiform, stippled patterns characterized by discrete, tattoo-like black lines. On close examination, the lesions are composed of numerous small pigmented dots. Microscopically, the lesions are characterized by vacuolar degeneration of the cytoplasm of the cells of the intermediate epidermal layer, just above the basal skin layer. Small round or irregular eosinophilic viral inclusions are found in the cytoplasm of the cells of the spinous layer. The epithelial cells adjacent to the aforementioned areas show an altered, shrunken morphology with their major axis perpendicular to the epithelial surface. The corneal layer exhibits hyperkeratosis and parakeratosis of various degrees associated to more or less deep cracks sometimes reaching the dermal layer. The cause of hyperpigmentation is still unknown: this may be due to a reaction to the viral action on behalf of the melanocytes located at the dermo-epidermal junction.

This pathological condition does not seem to be of zoonotic relevance, but field operators should take appropriate precautions in the case of cetaceans with suspect poxviral lesions.

4.1.4

Genital *Papillomavirus* (PV) infection has been described in many species of cetaceans and in particular in the Atlantic and Pacific waters of the American continent. Papillomaviral lesions have been reported even in controlled environments. Recently, three types (1,2,3,) of Bottlenose *Papillomavirus* (TtPV) have been identified. *Papillomavirus* generally causes exophytic growths on the genital mucosa which can have a negative effect on mating. Similar lesions can be found also on the skin, tongue, mucosa of the pharynx and first stomach chamber. Their aetiology has been confirmed by immunohistochemical and biomolecular analyses. Macroscopically, easily identifiable masses with a wart-like central formation made up of small filamentous papillae have been described. Histologically, the papilloma develops as an exophytic bowl-like mass with a marked epithelial proliferation accompanied by a slight fibrovascular proliferation; numerous arborizing projections form penetrating into the submucosal layer. Proliferating epithelium consists of a layer of flat basal cells often made up of 3 to 15 cells covered with mature epithelium. Single elements or small groups of cells undergoing hydropic degeneration can be found amidst the cells of the hyperplastic/ neoplastic epithelium.

4.1.5

Reports of this infection in cetaceans are rare. Seropositivity in some species of baleen whales is more frequent. A serotype of *Marine Calicivirus* was isolated in 1982 from several species of cetaceans (grey whales, sei whales, and bottlenose dolphins of the Pacific), which experimentally induced vesicular exanthema in pigs. The reservoir for that type of infection seems to reside in rudderfish - *Girella nigricans* -, an ocean fish.

The lesions which appear during the course of *Calicivirus* infection typically are small vesicles, mainly on the dorsal side of fins due to the accumulation of purulent exudates between the basal and the spinous cell layers.

4.1.6 Adenovirus

Until now the only two reports of this viral infection in cetaceans involved a sei whale – (*Balaenoptera borealis*) - captured by a Japanese whaleboat and a whale from Greenland. This is also the first DNA virus to be ever isolated from marine mammals. The most important macroscopic lesions were found in the liver: hepatomegaly, slight to moderate jaundice, with a colour ranging from yellowish to deep red due to marked congestion. Splenomegaly and mesenteric lymphadenopathy may be present. Microscopically, the liver may show a severe panlobular necrosis, particularly around the centrolobular veins. A slight, diffuse infiltration of inflammatory cells (lymphocytes, macrophages and neutrophils) may be also noted around the hepatic vessels and the necrotic areas.

4.1.7 Influenzavirus

Some influenza *type A viruses* may be responsible for haemorrhagic pneumonia with necrotizing bronchitis/bronchiolitis and lymphadenopathy (making it necessary to distinguish this from morbilliviral infection) in some cetacean species. The serotypes identified (H1N3, H13N2 and H13N9) are antigenically related to avian influenza viruses and it is thought that they originated from birds, in particular seagulls, which continue to be susceptible to infection. Both A (H1N1, H3N3, H3N8, H4N5, H4N6, H7N7) and B type viruses, similarly to the human ones, have been also identified in seals.

4.1.8 Other viruses

Besides these widely studied and described infections, there have been occasional reports, in aquatic mammals, of infections caused by viruses of public health concern. In this respect, descriptions of viruses belonging to the *Orthomyxoviridae*, *Rhabdoviridae*, *Hepadnaviridae*, *Retroviridae*, *Astroviridae*, *Adenoviridae* and *Papovaviridae* families have been made in both free-ranging individuals and in some animals in captivity.

4.2 Bacteria

Pathogenic bacteria, alone or in combination with parasitic agents, appear to be one of the most serious problems faced by cetaceans, with special reference to those living in captivity. These are in fact more prone to inhaling or ingesting pathogenic bacteria with respect to free-ranging specimens. Pathology reports involving bacteria regard Gram-negative organisms such as those belonging to *Vibrio*, *Edwarsiella*, *Aeromonas*, *Pseudomonas*, *Pasteurella* and *Klebsiella* genera. Besides determining organic lesions, these bacteria are often responsible for septicaemic or endotoxemic syndromes. Some of these bacteria such as *Vibrio spp*. are frequently isolated from the blowhole and genital mucosa as part of the normal host's microflora, highlighting their role as opportunistic pathogens in that they are able to cause disease only in the presence of predisposing factors such as immunosuppression due to concurrent viral infections or high loads of environmental pollutants. There are also reports of infections caused by Gram-positive bacteria (*Streptococcus spp. Staphylococcus spp., Erysipelothrix spp*.).

Cetaceans living in coastline waters can be exposed to pathogens that are normally associated to humans or domestic animals, especially in urbanized areas. Bacteria such as Salmonella spp. have been linked to some disease episodes but they have not been identified in apparently healthy subjects. New strains and species of *Brucella spp*. have been also isolated from free-living cetaceans in north-European, Mediterranean (including Italian) and USA waters. While these infections are probably not fatal (with the exception of *Brucella ceti* infection in striped dolphins, which frequently develop a severe form of meningo-encephalitis), they can lead to a number of chronic disease conditions which make animals more susceptible to other pathogens, or prevent them from feeding in an effective manner.

4.2.1 Integumentary system

Bacterial infections involving the integumentary system and its underlying structures share a loss of continuity of soft tissues as their common denominator to gain access into the host's body. The main cutaneous lesions due to bacteria are centrally ulcerating, crater-like areas, which in some cases are well demarcated with respect to the adjacent intact skin. In others they are characterized by the presence of crustous proliferations and necrotic peripheral material due to exudation and to proliferation of the nearby epidermis. The lesions can be small, multifocal or coalescent, forming an ample necrotic area. They are normally localized on the skin of the dorsal fin or on the lobes of the caudal fin. Less frequently, these lesions determine the formation of abscesses localized in the pectoral fins.

Histologically, the first changes detected in bacterial lesions of cetaceans are hydropic degeneration and necrosis of the squamous epithelial surface, together with a diffuse infiltration of neutrophils originating from dermal capillaries. This can later cause an erosion of the epithelium with subsequent exudation and formation of a crusty surface associated with a pyogranulomatous inflammation in the dermis. The intact surrounding epidermis can be characterized by hyperplasia. When dealing with highly pathogenic bacteria, the dermatitis can progress deeply, resulting in cellulitis, myositis or deep abscesses.

The bacteria predominantly involved in the development of ulcerated and/or necrotic lesions usually live in water and behave as opportunistic pathogens such as *Pseudomonas spp*. (*P. pseudomallei* and *P. aeruginosa*), *Edwardsiella spp*., *Vibrio spp*., (*V. vulnificus*, *V. holisae* and *V. alginolyticus*), *Photobacterium spp*. (*P. damselae*), *Aeromonas spp*., and *Clostridium spp*. These latter can produce deep necrosis associated with gas production, abscesses, and toxemia. Other bacteria normally live on the skin of animals or humans, such as *Staphylococcus spp*., *Streptococcus spp*. (*alpha-* and *beta-haemolytic streptococci*), *Proteus spp*., *Pasteurella spp*., and *Mycobacterium spp*. (*M. marinum*). These latter, together with *Pseudomonas spp*., are the main bacterial agents isolated from abscesses.

4.2.2 Respiratory apparatus

Another site often affected by bacterial infections is the respiratory system and, in particular, the lung parenchyma. Pneumonia and bacterial pneumonia, at times associated with parasitic infestations, are often caused by microorganisms such as *Pseudomonas spp., Salmonella spp., Staphylococcus spp.* (Fig. 3.44), *Streptococcus spp*., and *Aeromonas spp*.


3.44

Macroscopically, the lungs may show a marble-like appearance with gray-whitish areas alternating with deep red ones. They are generally hyperaemic and the superficial vessels appear congested. Their consistency is normally increased and, on cut surface, purulent exudates can be seen within the bronchi and bronchioles. In some cases, abscess formations containing a white creamy material can be also noted.

Histologically, classical bronchopneumonic lesions may be observed, with presence inside the bronchi and bronchioles of inflammatory cells (neutrophils and mononuclear phagocytes in the acute-subacute phase; a predominance of macrophages is found in the chronic phase), along with mucus and bacterial aggregates; the bronchial and bronchiolar epithelial cells can also appear hyperplastic.

4.2.3 Septicaemia

Signs of septicaemia are often noted in stranded cetaceans that are characterized by severe and evident histologic lesions. It is quite common to see necrotic and haemorrhagic lesions associated with inflammatory infiltrates of variable composition and bacterial aggregates in various organs, especially in the liver, spleen, lung and heart. Necrosis and lymphoid depletion of the follicles of the lymph nodes are associated at times with histiocytosis of the sinuses. The most frequently isolated bacteria are *Salmonella spp., Corynebacterium spp.*, *Edwardsiella spp.*, *Streptococcus spp.*, and *Erysipelothrix spp*. The latter is in particular a Gram-positive microorganism as well as a zoonotic agent causing systemic disease conditions characterized by square or rectangular skin erythematous plaques associated with wide areas of desquamation and necrosis. There is usually only a moderate lymphadenopathy, particularly of the mesenteric lymph nodes. Bacteria can be histologically detected both in the extracellular matrix as well as in monocytes, neutrophils and macrophages.

4.2.4 Brucellosis

Several studies have reported *Brucella spp*. infection

in cetaceans. Genetic studies have revealed that these bacterial agents probably originated from *B.ovis*, meaning that they distinguished themselves in ancient times, thus following the evolution of their respective host (seals, porpoises and dolphins). Two species of *Brucella spp*. have been recognized on the basis of their biological and molecular characteristics: the Brucella strains infecting cetaceans, classified as *B. ceti*, and those infecting pinnipeds, classified as *B. pinnipedialis*. *Brucella spp*. isolates obtained from cetaceans were later distinguished into *B. delphini* and *B. phocoenae*. The disorders associated with *Brucella spp*. infection in cetaceans include placentitis, orchitis, abortion, non-suppurative pneumonia and meningo-encephalitis (Fig. 4.3). In some populations as in small cetaceans from Peruvian waters the infection appears to be endemic, in particular infecting sexually mature young individuals and thus representing a potential threat for the growth of resident cetacean populations. The zoonotic potential of these microorganisms must be also carefully evaluated and some cases of human infection have been reported: Brucella-infected individuals complain about sinusitis, weakness and headache, symptoms that last for a week if treated with antibiotics. Two cases of neurobrucellosis associated with osteomyelitis were also reported in human patients with a *Brucella spp*. genotype ST27 infection.

4.2.5 Helicobacter spp.

Recent studies have identified new Helicobacter genus members which provoke gastric ulcers and are not associated with parasitic infestation in the stomachs of common dolphins (*Delphinus delphis*), white-sided dolphins (*Lagenorhyncus acutus*) and beluga whales (*Delphinapterus leucas*). *Helicobacter spp*. are Gram-negative, microaerophilic, curved to spiral-shaped motile bacteria which are able to cause a superficial, slight to moderate lympho-plasmacytic gastritis in the main stomach chamber. The pyloric stomach is less involved and the bacteria do not penetrate deeply into the underlying glands. Isolated bacteria are genetically related to *Helicobacter pylori*, the leading cause of human ulcerative gastritis.

4.3

4.3 Fungal agents

With the exception of dermatophytes, lesions caused by fungi are often serious and can be systemic involving several organs. Fungal agents are not generally considered contagious; in fact, there is no evidence that transmission takes place between an infected and a healthy animal; it is believed that infection occurs following accidental contact with fungi that grow as saprophytes in the ground or in organic material. In the case of primary lung colonization, the infection may begin through inhalation of fungal spores, while in cutaneous mycoses spores can penetrate through preexisting skin lesions. These are generally secondary, opportunistic pathogens and determine infections principally when there are predisposing conditions causing immunosuppression as in the case of captivity or malnutrition.

From a diagnostic point of view, fungal infections can be distinguished into two types: deep or systemic, which cause severe granulomatous lesions, and superficial ones that are confined to the skin and are responsible for relatively mild lesions.

Even if nocardiosis, actinomycosis and dermatophytosis are caused by actinomycetes and not by fungi, they are in any case considered as a fungal infection because of the lesions they provoke, which are similar to those caused by fungi.

4.3.1 Candidiasis

This is a pathological process caused by *Candida albicans* yeasts, which is characterized by the occurrence of gray-whitish plaques both on the skin and on the mucous membranes of the gastrointestinal (especially the oral cavity and the esophagus) and urogenital apparatuses. It can also cause a severe ulcerative dermatitis. In systemic candidiasis, whitish lesions due to necrosis and leukocyte infiltration can be also observed.

Histologically, acanthosis and pseudo-epitheliomatous hyperplasia can be noted. Aggregates of septate hyphae, pseudo-hyphae and blastospores characteristic of *Candida spp*. can be additionally found.

4.3.2 Sporotrichosis

Determined by the ubiquitous and saprophyte fungus *Sporothrix schenckii*, sporotrichosis is a pathological condition involving a primary skin infection. Once fungal bodies have penetrated into the subcutaneous later, they can disseminate through the lymphatic vessels, thereby

determining subcutaneous nodular lesions and with a frequently reported subsequent colonization of loco-regional lymph nodes and of other organs. A primary lung infection may develop following inhalation of fungal spores. An inflammatory granulomatous process, characterized by a large number of histiocytes and small clusters of neutrophils, is microscopically observed. The pathogen can be free or engulfed by histiocytes. This condition generally involves the lymph nodes, where it is possible to observe a severe granulomatous and/or necrotic lymphadenitis.

4.3.3 Cryptococcosis

Caused by *Cryptococcus neoformans* and *C. gattii*, which in nature are saprophytic fungal microorganisms, cryptococcosis is a pathological process which usually affects the lung as its primary infection site and can spread to other organs, particularly to the central nervous system and to the fetus. Macroscopic lesions have almost always the appearance of nodular formations of a viscous consistency. Histologically, a slight to moderate histiocytic response is found.

4.3.4 Aspergillosis

Being mainly caused by *Aspergillus fumigatus* and less commonly by other species, aspergillosis generally affects the respiratory system determining the formation of nodules of relevant dimensions, which protrude into the tracheal lumen. A more or less severe pneumonia may also develop, this being the result of a necrotizing and granulomatous inflammatory process showing a multifocal distribution, often associated with a necrotic-purulent lymphadenitis. Additional lesions can be found in the placenta as well as in the gastrointestinal tract, skin and brain, where the fungus causes a severe, multifocal, necrotizing encephalitis with extensive malacic areas. Together with the presence of multinucleated giant cells, septate *Aspergillus spp.* hyphae, which easily infiltrate the mucous membranes, the lung parenchyma, the nervous tissue and the lymph nodes, are histologically observed. They easily penetrate, moreover, the major blood vessels provoking thrombotic lesions.

4.3.5 Zygomycosis

The fungal agents responsible for zygomycosis are linked to pathologies caused by *Absidia spp.*, *Rhizopus spp.*, *Mucor spp.*, *Basidiobolus spp.*, *Conidiobolus spp.*, *Hyphomyces spp.*, *Mortierella spp.*, *Cunninghamella spp.*, and *Saksencaea spp.* infections. They are opportunistic organisms

that can give rise to localized lesions of the skin and of the subcutaneous tissue. These agents cause pyogranulomatous lesions harbouring fungal bodies and hyphae. The latter can invade and proliferate even within blood vessels, thus provoking thrombosis, emboli, ulcerations and localized infarctions in various organs and tissues.

4.3.6 Nocardiosis

The respiratory system is probably the primary infection site for *Nocardia spp*. microorganisms, with subsequent dissemination through blood and lymphatic vessels to other organs such as the brain.

Multifocal and diffuse granulomatous pneumonia, sometimes associated to pleuritis, tracheitis, fibrino-haemorrhagic bronchitis and granulomatous lymphadenitis, have been found in *Nocardia spp*.-infected cetaceans; similar lesions of have been also reported in subcutaneous tissue, skeletal muscle, kidney and brain in association to multiple abscesses both in the thoracic and abdominal organs. Necrotizing inflammatory granulomatous lesions, generally with a perivascular location and associated with microbial bodies, have been also observed; sometimes the vessels show coexisting thrombotic lesions.

4.3.7 Lobomycosis

Lobomycosis is a blastomycosis, a fungal infection of the skin caused by *Lacazia loboi*. It is characterized by dermal and subcutaneous whitish to slightly pink granulomas and yeast-like bodies connected in chains by a small tubule. Lesions in dolphins occur on the dorsal fin, head, flukes, and peduncle. Histologically, the squamous cell layer of the epidermis is characterized by acanthosis and cell proliferation; granulomas predominantly formed by histiocytes, which can fuse into multinucleated giant cells, may be found in the dermis. The fungal bodies can be phagocytized by macrophages and/or by multinucleated giant cells, or they can be otherwise seen free in the dermal stromal tissue.

4.3.8 Others

Some species of cetaceans may develop firm erythematous skin nodules on the head, trunk and on the caudal region of the body. Histological examination of the above lesions has revealed the presence of hyphae similar to those of ascomycetes. Bothryomycosis may cause the occurrence of multiple ascesses involving the skin and the subcutaneous layer in various body regions.

4.4 Parasites

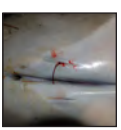
Parasites appear to be a more serious problem for aquatic with respect to land mammals. Helminths, for example, can cause death in these animals and are one of the primary causes of single and mass strandings of odontocetes.

In view of the particular, difficult environment in which hosts live, many parasites have developed an indirect biological life cycle. Therefore, infection of the final host takes place mainly by oral ingestion of intermediate/larval forms of the concerned parasite.

The lesions and the pathogenesis of parasitic infections of cetaceans are infrequently described or mentioned in the literature, since those investigators who carry out research on these agents are not directly interested in the alterations provoked in host organisms. Here below is a list of the main parasites and of the lesions induced by them in cetaceans.

4.4.1 External parasites

Lesions due to ectoparasites have been observed only with regard to *Pennella spp*., consisting in a local inflammatory reaction of the blubber and of the muscle. Such lesions are found with greater frequency near the hips and umbilical area (from Fig. 3.13 to Fig. 3.15) . Other parasites such as *Syncyamus spp.* (skin), *Conchoderma virgatum* (teeth), *Lepas spp*. (teeth) and *Xenobalanus globicipitis* (caudal fin lobes) do not cause appreciable lesions either macroscopically or histologically but when odontocetes that normally swim rapidly begin to swim slowly the parasite, especially *Lepas* or *Xenobalanus* on the fins, the dorsal fin, or on the labial commissure, can be suspected.



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Ciliated protozoa near the blowhole have been isolated from cetaceans in association with ulcerative lesions probably due to traumas and/or bacterial infections; these do not seem to invade the surrounding tissue or lungs. They are considered opportunistic microorganisms.

4.4.2 Internal parasites

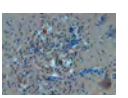
a. Protozoan parasites

A severe, suppurative pneumonia especially affecting the alveoli and associated with haemorrhage and with

the presence of protozoa in the lung parenchyma may be caused by *Holotricha spp. protozoa*. The larynx, the trachea and the bronchi can show large quantities of solidified exudate. Ciliated protozoa of an undetermined species may also induce a suppurative, histiocytic dermatitis and ulcerative cellulitis. Some cases of vasculitis, thrombosis and the presence of ciliated protozoa within the vessels have occasionally been reported.

In some cetaceans *Sarcocystis spp*. infection has been also reported, in which the protozoa cause cystic formations in skeletal myofibers containing groups of zoites bounded by a wall of variable widths depending on the parasitic species. No inflammatory response is generally observed. A fatal hepatic sarcocystosis caused by *Sarcocystis canis* was diagnosed in a striped dolphin on the basis of detailed anatomo-histopathological and ultrastructural investigations carried out on this animal. The main macroscopic findings were jaundice, subcutaneous haemorrhage and hepatic congestion. The main microscopic lesions consisted in multifocal to coalescing severe acute necrotizing hepatitis with cholestasis and protozoan organisms within the lesions.

The recent reports of several cases of severe, multifocal non-suppurative meningo-encephalitis in striped dolphins from the Ligurian Sea caused by *Toxoplasma gondii* are noteworthy. In contrast to what was seen during the morbilliviral epidemic in 1990-'92 in striped dolphins in the Mediterranean Sea, in this case the protozoa in question carried out the unusual role of being the primary pathogenic agent. The presence of parasitic bodies and zoites was, moreover, documented in earlier studies at the encephalic (with the simultaneous finding of a mild inflammatory reaction) (Fig. 4.4 and Fig. 4.5), lung and lymph node levels (in the latter site a moderate to serious necrotizing lymphadenitis was also apparent). The presence of protozoa has been also documented in pelagic species of large dimensions such as the sperm whale (*Physeter macrocephalus*) and the fin whale, generally as the consequence of either virus- or pollutant-induced/related immunosuppression, or cachexia/starvation. The number and the nature of infections caused by T. gondii underlines how this agent has spread in coastline waters likely affected by anthropic pressure and by coastline changes, along with the prolonged resistance of protozoan oocysts even in sea water.



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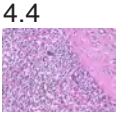

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b. Cestoda

Cestoda is the name of a class of parasitic flatworms, commonly called tapeworms of the *Phyllobotrium delphini* and the *Monorygma grimaldi* species. Larval cestodes of the pleroclercoid *Phyllobothrium spp.*, in particular *P. delphini*, are usually found localized in the blubber and muscles of the abdominal wall, with special emphasis on the uro-genital region (Fig. 3.25 and Fig. 3.26). Larval formations approximately 0.5 cm wide are often associated with tortuous paths and serpiginous tunnels but the parasites do not migrate once they have entered the host's blubber tissue.

Monorygma spp., more commonly called *M. grimaldi* larvae, stimulates the production of a very thin, smooth, uniform fibrous capsule approximately 1-3 microns wide, thus determining a characteristic cystic formation with a fluctuating consistency. These structures, which provoke a slight inflammatory response, are predominantly infiltrated/surrounded by neutrophils and eosinophils, being preferably sited in the abdominal cavity, attached to the visceral or parietal peritoneum of the gastrointestinal tract, of the abdominal wall and of the gonads.

It is thought that these infesting forms are *Scolex pleuronectis* larvae. The presence of "small" (mainly located in the terminal part of the colon and of the pyloric region of the stomach) and "large" individuals (located in the anal crypts) is generally associated with inflammatory granulomatous lesions of various degrees of intensity of the pyloric stomach as well as of the anal canal. The infiltration of eosinophils in the gastrointestinal tract wall is a frequent response to parasitic infestation and in this case it does not seem to have clinical relevance.

Previous studies suggest that *Scolex pleuronectics* penetrates through the mucosa of the various internal parts of the gastrointestinal tract and then migrates through the circulatory and lymphatic system and finally reaches the subcutaneous blubber and the muscles of the abdominal wall, thereby giving rise to encysted larval forms, respectively *Phyllobothrium delphini* (in the case of "small" Scolex forms) and *Monorygma grimaldi* (in the case of "large" Scolex forms). This type of migration has been hypothesized because *S. pleuronectis* has been detected in blood and lymphatic vessels.

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The only cestode that seems to infest adult cetaceans through the intestine is *Strobilocephalus triangularis*, which fixes its scolex deeply in the mucosal layer of the colon causing an intense inflammatory response.

c. Trematoda

The Digestive System

The stomach fluke *Braunina cordiformis* is a trematode that can be found in the mucosa of the second stomach chamber. It is not an invasive parasite but can cause focally - at the insertion point of the chamber - a slight inflammatory response with occasional presence of eosinophils.

Pholeter gastrophilus is another trematode which preferably colonizes the second stomach chamber. This trematode localizes in the gastric submucosa causing the occurrence of hard reactive nodules whose diameter may reach even several centimeters that can be felt at palpation (Fig. 3.64). Some of these can be located near to the ostium muscle that divides the two chambers and can impede the progression of food or in any case can cause an alteration in the organ's secretory and motor functions (Fig. 3.65). Histologically, a marked fibrous tissue reaction and a more or less consistent inflammatory infiltrate made up mainly of neutrophils and a few eosinophils (which instead seem to be numerous in association with the parasite eggs) are usually observed. Once the eggs are released they tend to migrate to the submucosal and mucosal layers until they are shed into the gastric lumen. The mucous membrane overlying the parasitic lesions generally appears to be intact.

Campula spp. causes instead hepatic and pancreatic lesions. The parasite migrates from the intestine to the pancreatic and hepatic parenchyma through the biliary and pancreatic ducts. The presence of segmental spikes causes hyperplasia of the ductal epithelium, associated to fibrosis around the ducts. This later causes sclerosis of the smaller ducts and the dilation of the larger ones. A population of inflammatory cells made up predominantly of macrophages, lymphocytes, plasmacells and few eosinophils can be noted in the adjacent interstitial tissue. The pancreatic lobules can also undergo atrophy and hyalinization.

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3.65

Respiratory System

Nasitrema spp. trematodes can be found in the nasal

sinuses, especially in the lateral pterygoid sinuses of many odontocetes. These parasites and their eggs are also found in the internal ear complex. *Nasitrema spp*. are associated to the presence in the nasal sacs of a reddish-brown exudates, while mild to moderate inflammatory reactions are seen microscopically. The mucosa in the auricular cavity appears macroscopically edematous, while a severe degeneration of the eight pairs of cranial nerves, distributed like a mosaic, can be observed microscopically associated to hypoplasia or loss of ganglion cell bodies linked to the degenerated nerve. Occasionally, there are trematode eggs in the folds of the mucosa or submucosa of the oval window, which are associated to the degenerated areas of the eighth pair of cranial nerves. An inflammatory infiltrate characterized by neutrophils and macrophages can be noted there. Rarely malacic lesions also on the dorsal surface of the brain and of the cerebellum involving the leptomeninges have been noted. Necrotic tissue containing pigments from the parasites or haemosiderin can be observed microscopically in the area corresponding to the lesions; a severe meningitis, characterized by neutrophil and macrophage infiltration and by the presence of perivascular lymphocytic cuffs associated to necrotic foci in the brain tissue, may be additionally found. This parasite is considered to be the cause of some strandings of single specimens as well as of mass strandings because it seems to interfere with the animals' echolocation system.

d. Nematoda

Digestive System

Anisakis simplex and *Contracaecum spp*. are the main nematodes that can be found in cetaceans, where they can induce the formation of crater-shaped gastric ulcers (whose diameter is between 1 to 5 cm) in the mucosa of the first and third stomach chambers (from Fig. 3.58 to Fig. 3.60). Gross infestations of adult parasites and larval forms are rarely noted in the absence of ulcerated lesions; in this case a number of parasitic elements including both adult and larval forms can be considered an indicator of good health in that it indicates that the animal constantly takes nourishment. Larval migration towards regional lymph nodes, which appear increased in volume, is possible. The ulcers can break through the wall of the organ, thus causing

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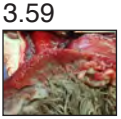

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a transmural gastritis with secondary focal peritonitis. Histologically, the lesion appears similar to a reaction to a foreign body characterized by severe peripheral fibrosis and by a predominant mononuclear inflammatory infiltrate containing neutrophils and rare eosinophils. The ulcer is superficially characterized by necrosis and by a possible secondary bacterial colonization.

Respiratory System

The main nematodes found in the respiratory system of cetaceans stranded along the coast of the Mediterranean Sea belong to the following species:

Pseudalius inflexus: This is the largest nematode parasite found in the lungs of cetaceans. It is generally found in the bronchial tree and occasionally in the blood vessels of the lung where larval forms are more frequently found. Infestation takes place during the first twelve months of life and after the animal remains infected for the rest of its life.

Torynurus convolutus: This nematode is similar to the former as far as location is concerned, being mainly found in the bronchi. It can be vertically transmitted.

Halocercus spp.: These nematodes are mainly located in the small bronchi and bronchioles. Of all parasites only the presence of Halocercus seems to be negatively correlated with the nutritional status of the animal, evaluated by the width of the blubber and morphologic parameters. *H. tauricus*, *H. invaginatus*, delphini/lagenorhynchi are among the most common species: the first seems to cause a more intense immune reaction in the host, characterized by an encapsulation reaction of the parasite. This is supported by the lower prevalence of *H. tauricus* in adult specimens. Finding *Halocercus spp.* in young individuals suggests there may be a transmission of the parasite through vertical migration.

Stenurus minor: Besides inhabiting the lung parenchyma, this nematode can also be found in the middle ear. The fact that the parasite is found in the middle ear with greatest frequency seems to suggest that the lung is only an occasional habitat. The particular distribution of the parasite makes us suppose that migration is through the blood.

Skrjabinalius ovatus: This parasite results in similar findings and similar locations with respect to the one above. The macroscopic lesions caused by lung nematode infections vary consistently from one individual to another. The main findings are multiple hard nodules with a 1-3 mm diameter distributed throughout the lung. It is often possible to find foci of consolidation of the parenchyma associated with focal to diffuse emphysema. There may also be signs of lung congestion and serous exudate in the lumen of the trachea and the bronchi. The lung and bronchial lymph nodes may be increased in volume.

The microscopic changes associated with the presence of nematode infestation of the bronchi include chronic bronchitis characterized by an inflammatory infiltrate made up both of lymphocytes and eosinophils and by hyperplasia and squamous metaplasia of the bronchial and bronchiolar epithelium, mild to severe hyperplasia of the mucous glands and interstitial fibrosis. In association with *P. inflexus*, microfoci of necrosis of the mucosa together with calcifications are noted in small bronchi and bronchioles.

The lesions associated with the vast majority of lung nematodes predominantly have an alveolar localization and include subacute or chronic multifocal granulomatous interstitial pneumonia, associated with interstitial thickening due to a lympho-plasmacytic infiltrate of a variable entity and moderately diffuse interstitial fibrosis. Mononucleated cells, mainly macrophages, and occasional multinucleated giant cells can be found multifocally inside alveolar lumina. Atelectasis or emphysema and parasitic microgranulomas with a necrotic or calcified center, associated with a moderate to severe, chronic inflammatory reaction, have been also reported. These findings are often described in the absence of parasites. Due to cuticular spines, *Halocercus spp*. causes an initial acute phase reaction involving also the bronchial and bronchiolar epithelia, which appear desquamated and infiltrated by neutrophils.

Cardiocirculatory System

Nematodes belonging to *Crassicauda spp*. are parasites that are found both within and outside the blood vessels, causing lesions especially in the kidneys, mainly in fin and beaked whales. They may subsequently cause inflammatory granulomas characterized by a fibrous reaction that tends to delimit them and produces an inflammatory infiltrate predominantly made up of eosinophils. In the veins and in particular in the arteries these parasites are known to induce a severe diffuse necrosis of the endothelium that can spread and involve the entire wall (from Fig. 3.50 to Fig. 3.52). The inflammatory reaction associated with the necrotic process is largely due to the eosinophils and to the occasionally present histiocytes. In Risso's dolphins, these parasites is often found in the pterygoid air sac, where it provokes erosions of the pterygoid bone, as well as in the tympanic bulla and in the brain.


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4.5 Non-infectious spontaneous pathologies

Pathologies of a biological, nutritional or metabolic nature, degenerative and neoplastic diseases and traumatic events are considered in this section. Lesions due to anthropic causes will be described in a later section. These events are in any case occasional and only rarely involve large numbers of animals.

4.5.1 Algal Toxins

Harmful algal blooms (HAB) refer to the proliferation of toxic algae that have a negative impact on marine mammals and in general on all aquatic organisms and man. Negative effects have been associated to direct ingestion, inhalation and absorption of toxins including the powerful domoic acid, saxitoxin and brevetoxin neurotoxins. Reports concerning domoic acid poisoning incidents following ingestion of algae and infected prey have been made available with reference to California sea lions (*Zalophus californianus*), common dolphins, sea otters (*Enhydra lutris*), and whales. Brevetoxin has been suspected in the deaths in dugongs (*Dugong dugon*) as well as in dolphins which inhaled or ingested infected food. Paralytic Shellfish Poisoning (PSP) has been found in whales which ingested contaminated copepods. No episodes have as yet been reported in the Mediterranean Sea.

Since the toxins are lipophilic, from a clinical point of view they can cause nervous manifestations (convulsions, paralysis, respiratory alterations) that are more or less serious depending on the concentration and quantity taken by the animal, as the central and peripheral nervous tissue are the target districts which are reached by different mechanisms. From a necroscopic viewpoint, poisoning can be suspected in cases of mass strandings concurrent with sea weather and atmospheric conditions predisposing for algal growth. Often during necropsy the stomach cavity is found to contain undigested food, suggesting a sudden death. Macroscopic and histologic investigations reveal multi-visceral congestion possibly associated with haemosiderosis and haemorrhage. Degenerative, necrotic or inflammatory lesions may be found at the level of the central nervous system, while there may be degenerative heart lesions and acute myocardial necrosis. The final diagnosis can be made by direct analysis of the toxin in the serum, urine or feces.

4.5.2 Neoplasia

The prevalence of neoplastic disease conditions reported in the literature

concerning marine mammals appears rather low. This may, however, be affected by various factors: many deaths may have gone unnoticed and necropsies are often not carried out. Post mortem autolytic changes that may be present may alter the tissues and affect the diagnosis. In fact, the cases described often make reference to animals kept in captivity, whose history and clinical information is known.

Benign and epithelial tumors are found more frequently in mysticetes and odontocetes. Squamous carcinomas in dolphins are the malignant neoplasms that are seen with greater frequency, while lymphosarcomas are the most numerous mesenchymal tumors. These forms appear to affect animals exposed to high levels of polluting substances as in the case of the beluga whales in the Gulf of St. Lawrence, Canada.

4.5.3 Nutritional, metabolic and degenerative pathologies

One of the main disease conditions not having an infectious cause and which is often found in the urogenital system is the presence of mineralization; sometimes there are discrete mineral deposits within the tubules present in each single renal papilla. These can probably be caused by an excess of phosphorus in the diet, which can alter the calcium-phosphorus ratio. Again with regard to renal tubules and the urinary tract, urolites which can occasionally partially or totally fill the renal pelvis may cause an alterations in normal renal function. In some species such as the common dolphin, single or multiple kidney stones is/are frequently found in the vaginal lumen. The vaginal sphincter in a dolphin possesses, in fact , an effective closure system of the vaginal lumen and it is thought that any uterine or vaginal material or seminal fluid that is trapped there can act as an aggregation nucleus. At the cut surface these structures appear concentric. These stones are predominantly made up of calcium phosphate similar to that of mammalian bone tissue.

Degenerative pathological processes have been described in cetaceans, many of which are to be considered senile diseases. Cases of atherosclerotic lesions and of intimal fibrous plaques both at the aorta and coronary arteries have been reported.

4.5.4 Traumatic lesions following intra- and inter-specific interactions

In view of their dimensions, there are only few predators of the great baleen whales which represent a threat to young individuals or weakened animals. These possible predators include the killer whale (*Orcinus orca*), the false killer whale (*Pseudorca crassidens*) and the white shark (*Carcharodon carcharis*). Few reports of attacks by these species have, nevertheless, been recorded and only a limited number of

cases of bites noted on carcasses of stranded animals have been registered suggesting that such events are relatively rare.

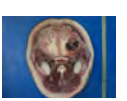
Some reports have been made with regard to young or subadult victims of attacks which may not cause death immediately but are, nonetheless, a predisposing cause. Lesions can at times be found in newborns due to aberrant maternal behavior or attacks by members of their own group.

Signs of attacks by sharks are easily recognizable as these are thin, deep parallel lacerations. Lesions by cetaceans are, instead, contusive (poorly visible at the external examination and appreciable as haemorrhage of subcutaneous tissues) as well as scratches which are typically parallel and continuous (Fig. 3.82). In this case it is usually possible to form hypotheses about the species of the aggressor depending on the number of linear lesions. Lesions associated to physical trauma also include rib fracture, perforation of the parietal pleura and pleural haemorrhage.

4.5.5 Diseases related to life in marine waters

A large number of epidermal lesions are correlated to low sea water temperature and to high salinity levels and since these factors can vary depending on the latitude, the alterations can depend on one of these factors or on their association. Low salinity can cause cell damage to the epidermis altering the normal electrolyte equilibrium and weakening the non-specific host's defenses towards infectious agents present in the surrounding waters. Low temperatures can limit bloodflow to the skin and thus reduce immune protection or epithelial regeneration.

Although cetaceans possess anatomic and physiological adaptations which permit immersions to deep waters, they are in any case subject to pulmonary barotrauma which can cause pneumothorax and bubbles in the pulmonary parenchyma. These bubbles can play an important role or be a complication in the formation of a pneumothorax or they can be totally independent. Barotrauma occurs when there is a break in the pleura under pressure at great sea depths when lung volume, under notable hydrostatic pressure, is reduced and the thoracic cage collapses. As the animal ascends the volume of gas increases in accordance with the law of Boyle-Mariotte, thereby causing bubbles to form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and provoking a pneumothorax. The bubbles have a diameter of a few centimeters and their own gray colored wall. Hard nodules and yellowish plaques are found.

All adaptations of cetaceans that have made them successful in the water make them slow and vulnerable when they are stranded: hopes for survival of a stranded animal diminish in fact with time. A cetacean has difficulty dissipating heat even in water and a large (and heavy) individual can have difficulty breathing as the thoracic cavity is compressed under its own weight. Lung lesions associated to live strandings are severe hypostatic congestion with incomplete collapse and emphysema of the contro-lateral organ. Some cetaceans show signs of shock or cardiovascular collapse within a few hours of being stranded and this may lead to circulatory failure and altered function of tissues such as skin, skeletal muscle, liver and kidney. Compounds normally metabolized by the liver such as metabolites and hormones can accumulate, thus reaching critical levels. Whatever the reason causing the stranding, the shock profile could be so serious as to compromise the possibility of intervening and of returning the animal to the sea even if it appears to be in good condition.

Studies carried out on stranded whales and museum findings with regard to these species have highlighted the presence of proliferative and degenerative lesions with rarefaction of bone tissue of the vertebrae, a picture compatible with chronic decompression despite the anatomic and physiological adaptations developed by these animals to face the effects of underwater pressure.

4.5.6 Old age lesions

Partial or complete erosion of synovial cartilage with subsequent proliferation of the subchondral bone has been found during some necropsies. Localized especially in the atlanto-occipital and/or humerus-scapula articulations, the lesions become affected over time with ankylosis. That disorder clearly causes notable difficulties in swimming and in all vital functions connected to it. Spondylosis without ankylosis of the vertebrae or arthrosis of the shoulder linked to aging have also been described.

Pathologies of the adrenal gland such as cortical hyperplasia, bilateral cortical cysts and nodular hyperplasia of the spleen have been identified. These lesions could be due to normal aging processes. Cortical lesions could instead be due to stress or adreno-corticolytic xenobiotics, while hypoxia or xenobiotic estrogens or estrogen-like compounds could be at work at the medullary level.

4.6 Non-infectious pathologies linked to anthropic activities

Many of the most relevant and most documented examples of factors which can disrupt homeostasis and provoke alterations in the organs and tissues of cetaceans are linked to anthropic activities such as accidental capture in fishing nets or exposure to environmental pollution. A survey carried out on 111 beached specimens along the Italian coastline from 1995 and 2005 showed that 17% of the mortality was due to direct anthropic causes (accidental capture, direct killing, collisions) without considering the possible predisposing action caused by neurotoxic and immunotoxic polluting agents. Further information and indications for an appropriate diagnostic approach in these cases, especially when direct responsibility of humans is clearly evident, may be found in Chapter 7.

4.6.1 Interactions with fishing hardware

The accidental capture of cetaceans in fishing nets or gillnets has been and in some cases continues to be the main cause of human-induced mortality for cetaceans in the western Mediterranean. Gillnets are still being used by many Moroccan, French, Italian and Turkish fisheries. The estimates of capture before the 90s for European fisheries are appalling (5000-15000/year Italy), at least until the practice was banned in international waters at the end of 1992. At the moment the Country with the largest number of accidental captures is Morocco (1555-2092/year), which continues to use gillnets. With regard to other types of nets, the data available are less systematic but indicate that there is a high mortality of striped dolphins due to traps, bottom trawling and ghost fishing. As in the case of striped dolphins, the bottlenose dolphin is threatened directly or indirectly by fishing activity. The only part of the Mediterranean basin that has certain records/data on this issue is the Northern Adriatic Sea area; there the resident population has undergone a 50% decrease over the last 50 years due to a campaign to reduce fish/food competition, accidental capture in nets and overexploitation of fishing resources. The conditions of the north-western Mediterranean are comparable. In the coastline areas, moreover, bottlenose dolphins are exposed to a wide range of anthropic factors which are coupled to accidental or unplanned capture (by-catch), the effects of chemical substances and xenobiotic activity, direct or indirect disturbance due to boats or other types of acoustic sources, undersea constructions or demolitions, other forms of degradation and destruction of the marine habitat. By-catch does not usually cause immediate death in large animals which can usually break free, but they can become entangled in lines, nets and other fishing gear which can remain attached to the

animal's body, generally at the fins, the caudal peduncle or at the mouth.

A careful post mortem examination makes it possible to identify drowning as the main cause of death. Individuals exhibit a good state of nutrition and when the stomach chambers are opened signs of a recent meal are found confirming their good standing. The presence of after-death abrasions and linear or circular injuries is noted at the external evaluation, in particular on the rostrum, the fins and on the tail, while contusions and haematomas are mainly found around the blowhole and the jaws. The lungs appear edematous and congested and a whitish to pinkish foamy fluid material is found in the upper airways. Pleural and sub-epicardial petechiae are found in individuals which have just recently died but these are often masked by post mortem autolytic alterations. A moderate lung congestion associated with edema and intra-alveolar haemorrhage and the presence of edematous fluid with gas bubbles and erithrocytes due to hypoxia are the main histological findings in the terminal airways and in the pulmonary parenchyma.

There are also cases of direct killing by fishermen who directly kill the animals either voluntarily or to free them from the netting. In this case, cut wounds by a bullet or a knife wound, at times amputations, loss of substance and haemorrhage are found. If there are cut or bullet wounds it is possibly to identify the passage the bullet made in the body and the damage caused to internal organs. In particular, when injury is caused by firearms the bullet can be found and the distance from which the shot was fired can be determined by the characteristics of the entrance hole.

4.6.2 Collisions with boats

Collisions with vessels (ship strikes) affect both large whales as well as small cetaceans although in different ways and in different areas.

The fin whale is the most frequently reported victim of vessel strikes. Vessels that are at least 80 meters long and travelling at velocities of over 15 knots are usually implicated. In a study carried out in 2006, Panigada et al. reported that out of a total of 287 fin whales stranded between 1972 and 2001, the cause of death in 16% of these was collision with a vessel. Calculating the number of collisions that have taken place over recent years, it was found that every year approximately 220,000 vessels travel the Mediterranean waters with even greater traffic in summer months, the period in which the highest percent of collisions are reported. These data demonstrate that over the time period considered at least 43 whales were killed following a ship strike and that only 9 survived a collision. There are also photos showing scars on individual animals probably caused by a collision. It is not clear why these animals were unable to avoid a collision although some hypotheses have been formulated: it is thought that whales are unable to perceive the presence

of vessels or that they do not have time to change their route as the vessels are moving at high speeds. This is why it would be appropriate to limit the speed that vessels can travel, at least in waters where large mysticetes are normally found. Collisions between cetaceans and vessels are therefore considered a major, not to be overlooked threat to fin whales.

The largest number of accidents taking place with regard to sperm whales is linked to the area around the Strait of Messina where strandings involving this species have been concentrated and where these animals usually pass during their seasonal migration from the Ligurian to the Ionian Seas. Lesions in these animals are often due to propeller blades which leave evident traumatic cutting lesions at regular distances on their back indicative of the vessel's dimensions. In view of their dimensions, contrary to what takes place in smaller odontocetes, those injuries may not be fatal and the animal may survive with possible onset of secondary infections or with scarring but may also return to a normal state of health.

One of the difficulties in diagnosing a lethal event caused by a collision with a boat is linked to the time taking place between death and the time the carcass is found, which subsequently undergoes putrefaction and fermentation as after-death processes. Often haemorrhagic post-traumatic lesions that developed when the animal was alive are difficult to interpret. It is therefore necessary to utilize forensic pathology techniques in order to ascertain the role of trauma. Signs of fat emboli in the tissues (with special reference to the lung parenchyma), caused by the liberation of fragments of adipose tissue following a traumatic injury to the blubber and/or possible fractures must be searched for.

4.6.3 Exposure to acoustic pollution

The impact of man-made noise on the marine habitat and on the conservation of the species has grown exponentially both at a national and international level causing increasing scientific and social concern in recent years. One of the most striking examples linked to this phenomenon are strandings of large cetaceans caused by sound emissions during military exercises. Beaked whales (*Ziphius cavirostris*) appear to be the species that are perhaps most affected and subject to strandings linked to the use of high-intensity, mid-frequency military sonar. Atypical strandings of individuals belonging to the aforementioned species were reported for the first time in 1963. Since that time mass strandings have been reported in various corners of the world but with the exception of a case in the Bahamas (2000) in which three animals were partially analyzed and diagnosed as affected by an acoustic trauma, no complete investigations were carried out until 2002. Until that time the hypothesis of acoustic pollution was basically the only accredited one.

In 2003 and in 2005, following the seminal work carried out on beaked whales found stranded in the Canary Islands in 2002 during military exercises, Antonio Fernández and Paul Jepson raised for the first time the “non-acoustic” hypothesis and provided a possible explanation of the relationship between active sonar and strandings and whale deaths. The necropsies carried out on 10 out of the 14 stranded cetaceans involved in this atypical mortality event indicated that there were no inflammatory nor neoplastic processes, with no infectious pathogen having been identified in these animals. Macroscopically, the whales had diffuse congestion and extensive haemorrhagic lesions, especially around the acoustic jaw fat, the middle and internal ear, the brain and kidneys (Fig. 4.6) Gas bubble-associated lesions and fat embolism were observed in the vessels and parechyma of the vital organs.

It was hypothesized that in vivo bubble formation associated with sonar exposure can modify the whales’ behavior during immersion causing supersaturation of the tissues above a threshold value normally tolerated by the tissues (as occurs in human decompression sickness). Alternatively, the effect that sonar has on tissues that have been supersaturated with nitrogen gas could be that of lowering the threshold for the expansion of in vivo bubble precursors. These mechanisms, alone or in combination, could enhance and maintain bubble growth or initiate embolism causing cardiovascular collapse, death and stranding. This new pathologic entity, termed “gas and fat embolic syndrome” or “decompression-like syndrome,” appears to be induced by exposure to mid-frequency sonar affecting in particular deep, long-duration, repetitive-diving species like beaked whales.

Diagnosis of this syndrome is made based on the evaluation of haemorrhagic lesions in various organs and tissues and in particular in the periotic regions, in the “acoustic” fat and within the jaw branches, as well as on the basis of the analysis of the gas bubbles and verifying if there are fat emboli in the lung.

Evaluating the gaseous component is complex and implicates collecting gas samples of the blood in vacutainer test tubes and carrying out gas chromatography analysis. The investigation must include a careful description of the vessels and tissues in which bubbles form and must exclude anaerobic bacteria that may have spread after death. The


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presence of fat emboli can be easily demonstrated in various tissues (lung, kidney, liver) through histochemical techniques which selectively stain the lipid tissue components (Sudan black or Oil-Red-O) on frozen tissues or using a complete secondary fixation (post-fixation) in osmium tetroxide of formalin-fixed tissues. These emboli can then be quantified by microscopically observing suitable lung tissue sections of the concerned animal(s).

Tympanic membrane bubbles must be also collected and fixed in formalin by injecting a minimum quantity also through the tympanic window. After treatment with a rapid hydrochloric acid decalcifier for at least 24-48 hours depending on the species, a macroscopic and histologic evaluation of the internal tissues can be carried out. These investigations make it possible to detect haemorrhages or emboli in particular of a fatty nature which may develop following exposure to sonar/acoustic waves.

An ultrastructural investigation to verify the occurrence of sub-microscopic changes in the tissues of the internal ear following exposure to sonar/acoustic waves of relevant intensities, may not be easy to arrange and it is feasible only in animals which should have died a few hours earlier.

4.6.4 Exposure to chemical substances

Among the most insidious causes of death are those relative to pollutants. As cetaceans are at the top of the marine food chain they accumulate organic compounds and other polluting agents when they ingest their prey. The consequences are not clear but basing ourselves on our knowledge of other species and on the limited studies carried out on marine mammals living in highly polluted waters, these could cause endocrine and reproductive disturbances and greater sensitivity to illness secondary to immunosuppression. There are notable differences in the bioaccumulation patterns between species and the differences in accumulation within the same species are linked to some parameters such as age and sex. Contrarily to what takes place in other parts of the world where coastal cetaceans are affected to a greater extent by the degradation of the habitat and by biological and chemical contamination of the sea, cetaceans living near the coast in the Mediterranean Sea and pelagic fish seem to be affected in a similar manner. Even epidemics such as morbilliviral outbreaks can have an effect on the xenobiotic substances but it is not known whether these events may be the cause or the result of high tissue concentrations of given environmental pollutants.

The mechanisms which cause immunosuppression by polychlorinated biphenyls (PCBs) in aquatic mammals are probably linked to their metabolites. They seem to be able to bind to transthyretin, a protein

carrier, inhibiting transfer of retinol and thyroxine to the epithelial tissues during an immune response, thereby making them more susceptible to infections. It has been seen that human PBC exposure can be negatively correlated with plasma immunoglobulin concentrations and blood tumor necrosis factor (TNF)-alpha levels, while there is a positive correlation between the depletion of lymphoid organs such as the thymus and levels of organchlorines which seem to alter the release of cytokines, thus increasing the incidence of secondary infections. Another important lesion is the lymphoid depletion in lymphatic organs which can be caused at least in part by environmental pollutants such as PCB and DDT but also by heavy metals which seem to lower the immune response determining a reduction in circulating immunoglobulins and an atrophy of lymphoid tissues, thereby making the host to become more susceptible to infections of various types. In order to better characterize the effects of these environmental stressors, researchers have considered the possibility that a modification of the ratio between the lymphocyte subpopulations in lymphoid organs occurs in porpoises exposed to more or less consistent levels of persistent organic xenobiotics, thereby demonstrating phenotypic changes such as depletion of immature thymocytes and bone marrow B cells, along with a reduction of T lymphocytes in the spleen.

In view of the fact that an immune response can be reduced in situations of prolonged pathologies or cachexia, a correlation between the presence of chronic infections, spleen lymphocytic depletion and an increase in interleukin IL-10 blood levels has been demonstrated.

Further studies have, moreover, demonstrated a reproductive toxicity with the formation of multiple luteinic cysts. It was hypothesized that PBCs alter the hypothalamic-pituitary axis or have an effect on the response capacity in the ovary, altering the progesterone balance and disturbing the implantation of the blastocyst. Even DDT and its analogues have an effect on the reproductive system as they show an estrogen-like activity and inhibit (at least in vitro) uterine estradiol receptors. It should be highlighted once again that living cetaceans with high tissue levels of these polluting substances that were subjected to molecular investigations to evaluate estrogen receptor expression, show higher expression levels in males with respect to females, thus confirming that such compounds disturb/alter endocrine and reproductive functions.

Other important effects of these and other pollutants such as polycyclic aromatic hydrocarbons (PAHs) become apparent following chronic prolonged exposure: it seems, in fact, that these compounds are linked to carcinogenesis, increasing the incidence of neoplastic pathologies. It has also been seen that cetaceans that live in particularly polluted waters present higher rates of neoplasms and in particular carcinomas

of the reproductive system, lungs and liver, as mentioned in point 4.6.2.

With regard to the effect of other pollutants such as heavy metals, a tight correlation between an increase in mercury (Hg) and nutritional status degeneration has been demonstrated. Pathological alterations of the lungs have also been reported. No pathological lesion has been primarily related to direct or indirect Hg poisoning. In fact, cetaceans seem to have developed a detoxification mechanism which is carried out by depositing inorganic mercury in the form of mercury selenide (Hg/Se) granules in the hepatocytes, in Kupffer cells and in the renal tubule epithelial cells. Organic mercury (methyl-Hg) seems to accumulate in the muscle as it awaits the detoxification process. The ratio between Hg and Se – generally 1:1 -, along with the percentage of methyl-Hg (the neurotoxic form) in the single tissues and the animal's age are all important toxicological parameters. A condition of cachexia, pregnancy, or prolonged fasting can in the end affect the organic form of Hg present in skeletal muscles, thereby facilitating the neurotoxic and immunotoxic action of this element that can act in synergy with the organochlorine substances harboured by the same or by other host's tissues.

5

LARGE CETACEAN NECROPSY PROTOCOL

Sandro Mazzariol, Cinzia Centelleghe

Performing a necropsy on a large cetacean is a complex, difficult, costly, challenging endeavor entailing quite a bit of coordination and rapid planning. The stranding of a large marine mammal is nevertheless an unrepeatable opportunity to learn more about these animals as well as human-induced mortality. This protocol outlined here describes methods to collect measurements and to describe internal and external body systems, lesions and/or injuries in a standardized way. It should aid the members of the necropsy team to collect specific data and to focus the work load necessary to complete a large cetacean necropsy.

5.1 Logistical aspects

Logistical planning begins at the time the first report of a stranding is made. Most frequently carcasses are reported as floating offshore by the Italian Coast Guard or Navy or an offshore fishing vessel. In all these cases it is opportune to arrange for it to be towed to shore so that a thorough necropsy can be carried out. The following steps – some to be taken concurrently – are all necessary before the necropsy begins.

5.1.1 Stranding Location

In most cases, necropsies of large whale carcasses can be conducted on-site. When the report of a carcass found floating at sea is made, the

report may include latitude and longitude coordinates and the locality where it was sighted. These of course must be updated before attempting to locate and to tow the whale to a suitable location for the necropsy. Pinpointing the area where the carcass is floating may also aid in identifying the species, the dimensions and its conditions.

5.1.2 Necropsy Site Location

At this point a site offering easy access for equipment and personnel and must be identified and permission to utilize it for the time needed for the necropsy and cleanup must be obtained. Particularly in remote locations, the ability to get equipment to the site should be weighed carefully as the highest priority (Fig. 5.1). This might require that the carcass is towed for a significantly longer period with respect to a straight line towards shore with longer times and higher costs.

Additional site parameters that must be considered include:

- Local authorities: the local municipality or provincial authorities must be contacted and authorize the possible site to carry out the necropsy taking into consideration all possible problems connected to the event. The city government is, moreover, responsible for the disposal of the carcass once the necropsy has been carried out.
- Heath authorities: the mayor of the locality where the necropsy will be carried out and the Local Veterinarian Health Authority must both be contacted and informed and permission for the necropsy must be requested and obtained.
- Law enforcement: the city police and the state police must be contacted in view of public safety, crowd formation and logistic support. The appropriate Port authorities will contact the specific government authorities and will furnish logistic support with land transportation. The Fire Department may have the necessary means to furnish support with land transportation.
- Public and private organizations: contact must be made for logistic and scientific support (museum to recuperate the skeleton etc.).

5.1

5.1.3 Transportation to shore and organizing the site

Transportation to shore can begin only when the necropsy site has been identified and arranged. Taking a large cetacean under tow can prove extremely stressful on a vessel, the captain and crew in view of its weight and adverse conditions that can complicate the operation. It is important to identify the best way to transport the carcass which is in any case by the caudal peduncle using ropes to secure the towing harness to the strongest attached piece of the vessel. A line should be passed under the peduncle with a float attached to the end or with a weight that can be retrieved with a boat hook on the opposite side of the peduncle. In this way the whale can be safely taken under-tow. One of the crew should be posted to watch and to monitor the condition of the towing equipment and the distance between the vessel and the carcass (from Fig. 5.2 to Fig. 5.4).

As soon as possible the team that will be carrying out the necropsy should begin the initial assessment of the carcass and measurements should be taken and photos should be taken on all sides.

To keep the beach as clean and organized as possible and to lessen the confusion of curiosity seekers wandering around, it is best to set up work-stations coordinated by a single person. One member of the team should take charge of all aspects of public relations. As the necropsy may be going on with teams working simultaneously at two or three locations on the whale at a time, it is important to set up a table where datasheets film, cameras, notes etc. can be organized. Parts and samples of the animal must be managed and processed for investigation, transportation and/or storage, description and classification.


5.2


5.3


5.4

5.2 Heavy equipment and the work team

5.2.1 Heavy Equipment

The ability to conduct a thorough necropsy of a large cetacean depends on the ability to deliver large equipment on site. A front-end/backhoe loader or a loader with clamshell/4-in-one front bucket is at the very least needed to attempt such an undertaking. The Fire Department or private

organizations can be contacted for this kind of support.

5.2.2 The work team

A team of a minimum of 5 to 12 persons is needed depending on the cetacean involved. These should be divided into 2-3 teams (made up of 2-3 persons); each team/person will each be involved in various specific aspects of the necropsy.

The necropsy of a large cetacean requires a number of key personnel:

- An off-site coordinator who manages the flow of information to/ among agencies/organizations, solves organizational and logistic problems, and coordinates the general process.
- An on-site coordinator who assigns roles and coordinates activities, calls and conducts briefing and debriefing meetings. He/she coordinates the flow of samples and tracking and organizes the necropsy site, the equipment, the disposal of the carcass.
- A necropsy team leader. This individual manages the necropsy crew and is responsible for samples and the data that is gathered and outlined. He/she supervises sample submission and analysis and drafts and completes the necropsy report. Together with the on-site coordinator, he/she is responsible for human safety. A veterinary who is familiar with the necropsy protocol and who can act as a liaison between the teams would be desirable. He/she could work in the team responsible for the thorax and thus be readily available to the other teams whenever necessary.
- Cutting Crews: A cutter, a hooker who lifts the parts of tissue, and another person who is responsible for safety issues and who takes samples to the sampling teams.
- Photographer: He/she photographs all external and internal points of interest identifying and registering the sections of every lesion. Images should bear an identifying number that links it to the sample ID and the necropsy record.
- Sampling team coordinator: He/she coordinates the activities of the 2-3 persons assigned to collecting samples and manages the sampling area set up near the warehouse.
- The coordinator of material or logistics: He/she manages the material and the equipment furnishing that whatever necessary to the single teams to carry out their work: He/she is responsible that equipment works (blades are sharpened and equipment is set up), sets up and manages the warehouse area and is responsible for bordering the site and its safety.
- Scribe: He/she records all gross necropsy observations and descriptions of samples and drafts the report.

If there are not enough persons for each of these roles, some can be grouped together in a single person depending on personal qualifications. For example, the necropsy team leader can also be the on-site coordinator and/or a member of the one of the teams; the sampling team coordinator can also photograph and/or be responsible for relations with the public, and/or coordinating the clean-up activities.

At the most, 4 crews should be set up (head and cervical region, thoracic, abdominal and logistics) each made up of 3 persons. The crews which work on the animal should be made be made up of a veterinary who evaluates necropsy aspects, cuts and makes morphological diagnoses, a a logistic and operational support technician, a technician or biologist who collects data and samples. The coordinators may or may not be involved in necropsy activities. The logistics team is, instead, made up of personnel in charge of photos, the warehouse and internal and external communications.

During the necropsy each member of the team must collaborate with regard to safety measures and care should be taken that knives and other cutting equipment are not left in the animal's tissues. Every member of every team must constantly monitor the situation and prepare an avenue of escape from the carcass should some of the tissue or carcass begin to cede or if a wave engulfs all or part of it.

5.2.3 Safety equipment (Individual Protection Measures)

Many large cetacean necropsies are conducted in remote locations. As a general rule then, all participants should be prepared for all problems ranging from small on site wounds to more serious injuries. The most frequent risks are lesions that are traumatic or due to physical agents such as cuts or abrasions, knife wounds, concussions, dislocations, fractures, hypothermia and sun stroke. These risks can be reduced by using facemasks, forearm guards or protective vests while using knives or electric cutting instruments.

It is also important to remember that cetaceans are bearers of potentially zoonotic infectious agents (*Brucella*, *Vibrio*, *Leptospirosis*, etc.) that can be transmitted by live animals or fresh carcasses. The risk in this case can be reduced by wearing facemasks, gloves and protective eyewear. Hand should be washed thoroughly and frequently of hands and instruments should be disinfectants at the end of each piece of work.

It is not recommended to conduct necropsies after dark or during the night but, despite well thought out plans, it is possible that a team will find itself out on the beach in the dark trying to secure a carcass or collecting morphometrics or cleaning up. In those cases it is useful to have a some commercially available lighting instruments although heavy equipment should be avoided.

In order to avoid slipping or crushing accidents, it is wise to use reinforced boots. Ice crampons (there are types with numerous, thin points) can be utilized to facilitate moving up and down safely without slipping on the carcass.

5.2.4 General equipment list

Here below is a complete list of instruments and equipment, besides those indicated (DPI and heavy equipment). Those items considered indispensable are marked with an asterisk.

- 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, coveralls 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 liter container of 10% buffered formalin with pour spigot;
- 10 liter container of 95% alcohol;
- 2 bread box size waterproof plastic boxes for gross tissue collecion;
- 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;
- Hito cassettes;
- 10 20 cc plastic syringes;
- 5 50 cc plastic syringes;
- Roll of aluminum foil;

Every person must be equipped with:

- Jumpsuit or apron;
- Protective eyewear;
- Disposable gloves;
- Boots or shoes;

The vets and biologists involved in carrying out the necropsy and collecting samples should be equipped with:

- Cut-resistant gloves;
- Work helmet;
- Facemasks;
- DPI in steel in case electric cutting instruments are used;
- The vet needs be equipped with:
- A complete set of knives;
- Steel diamond files;
- Pincers;

All other members of the team need at their disposal:
- Hooks;
- Rubber gloves;
- Swabs;

Those who write or take photos should remain distant from all cutting operations and thus keeping their hands clean. They should be equipped with:
- Clipboards and data sheets;
- Pens and markers;
- Rulers;
- Camera/videocamera;
- Containers for samples;

All other pieces of equipment should be managed and distributed by the head of the warehouse who is also in charge of keeping all cutting tools efficient and sharp. The material needed to take and fix samples will be managed by the head of the sampling team.

5.2.5 Preparations and preliminary data

The working site must be organized so that there is a clean changing room near the warehouse. A central sample area should be organized near the clean zone and the necropsy site (Fig. 5.5 and Fig. 5.6). The carcass should be divided into at most three sections and each team should be assigned a specific part of the body to disassemble.

Prior to commencing the necropsy it is important to take the time to prepare all involved and to conduct a briefing before necropsy for everyone's benefit. It is important to outline all of the procedures that will be carried out and the samples that must be collected. Safety measures should also be emphasized. In the same way, everyone should be aware of the dangers and risks that may be involved. All members of the team should try on rubber boots and practice duct-taping them so that fluids cannot drain in. Everyone should try on their eye goggles/safety glasses.


5.5


5.6

There should be numerous copies of data sheets and plastic clip-boards available for everyone. If possible, it is useful to have single-sided data sheets printed on Rite in the Rain paper, heavy bond cotton paper or on mylar. If sheets are splattered with biological fluids they should be sprayed with a mild formalin solution and left to dry in the sun. All tags should be made up of high quality mylar or heavy bond paper.

There should be an ample supply of trash bags and containers for waste and all garbage should be divided appropriately. When the necropsy has been completed and the carcass has been disposed of, the entire site should be inspected for any remaining equipment. Any pools of remaining fluid should be filled with sand or sawdust to prevent pet involvement.

5.2.6 Floating carcass

When a floating carcass is reported it is important to carry out an external examination before it is handled or moved. This will make it possible to differentiate between existing lesions and signs of human interaction from marks resulting from the towing, landing and transporting of the carcass. Photos and descriptions will almost certainly be the freshest ones concerning the carcass and may be critical for the final diagnosis.

The carcass must, of course, be towed to an area where it will be possible to carry out a thorough, unrushed necropsy and this is possible if the carcass is in good enough condition to be towed intact. This, of course, means that all logistical variables of towing – distance from the shore, the landing site, the disposal plan etc. - need be evaluated.

In general, the vessel should be significantly longer in length with respect to the length of the animal that will be towed. A towing bridle would make hooking up the carcass easier. Using a boathook the float ball can be pushed under the caudal peduncle until it emerges on the opposite side of the tail and then make it pass through the loop pulling the short head of the noose tightly.

The amount of power required to move a carcass to the place where the necropsy will be carried out depends on the nature of the site and the size of the whale. In order of increasing friction, it has been found that the easiest to hardest surfaces are: wet slab rock such as basalt, cobble, slick mud, gravel, sand. In the latter case track laying machines are better than wheeled ones. An adequate heavy rope is critical for dragging the carcass above the high tide line. It is important to have ropes, chains or cables of 90 ton breaking strain and that any bystanders be kept at a distance to avoid accidents in case a cable should break.

5.3 Necropsy Protocol

The necropsy protocol on large cetaceans does not present substantial differences from that on small ones with the exception of the impossibility of positioning the animal and its dimensions.

Before beginning the necropsy it is important that the history of the car-

cass, including information on when it was found including if possible a map or drawing, the persons who first sighted and reported the stranded animal, the condition in which it was found, the transportation that was organized and the disposal foreseen, is reviewed. The field number site will make it possible to identify the animal being studied and at a later date the animal will be given a specific identification code that will be registered in the national database.

5.3.1 Life history

It is important to obtain all data concerning the stranding and thus of the sighting, the place where it occurred, the persons who were involved, and the means the carcass was recuperated, transported and handled.

5.3.2 Site safety

Before beginning the necropsy, an on-site meeting of all personnel involved in the examination should be held. Everyone should be briefed on the plan for the necropsy and all other important issues, including and especially safety. The dangers and risks involved are those linked to the use of large machines and equipment and of large, sharp knives, as well as chemicals; there are also physical risks involved in moving large pieces of tissue/body parts. Everyone should, in particular, be aware of the risk of zoonotic diseases. All of the personnel should be aware of the importance of keeping track of all instruments and in particular hooks and knives which may have been left in the carcass. All injuries should be reported immediately to the on-site coordinator.

5.3.3 External Examination

Once the animal is at the necropsy site, another careful assessment of the external condition, and the presence of lacerations, scars, contusions and other external lesions should be noted and photographed. Photographs should also be taken of individual characteristics of the species that can be compared with photo identifications banks.

5.3.4 Human interaction and external observations

The investigation of human interactions with marine mammals is a recent development. In small cetaceans, the quality and interpretation of data collected is directly related to the condition of the carcass. A fresh, non-frozen carcass will yield the best interpretation of the potential for human interaction.

A thorough investigation of the external surfaces of the animal is required. Notes should be taken during the necropsy recording observations

concerning edges of appendages and of other areas where there may be signs of entanglement. The animal should also be investigated for fractures or abrasions that could be indicative of involvement in anthropic activities. It is to be remembered that cetaceans are literally wrapped in blubber, a thick layer of vascularized adipose tissue, which can act as a cushion to most blunt trauma injuries. A contusion consequent to a collision with a vessel may then appear at the external investigation as abrasions of the epidermis, while dissection to deeper body areas may uncover muscle tearing, hemorrhage and/or compound bone fractures. Generally speaking, the hypothesis of vessel strike as the cause of death of a large whale should never be ruled out until the entire carcass has been dissected down to the vertebral column.

All skin lesions must be localized and described on a separate data sheet and photographed. Skin samples should also be collected and immersed in 10% neutral buffered formalin (NBF) for the histopathological exam (2-3cm x1 cm tissue sample in a 10:1 formalin:tissue ratio). It is important to specify if the lesion is fresh (as in a wound) or healed (as in a scar). A section of epidermis fixed in a super-saturated salt solution of 10% DMSO in water should be collected for genetic investigations. Another section of epidermis should be collected as soon as possible and stored at -80°C in a freezer to determine hormone and environmental contaminant levels.

Photo and video documentation of the necropsy is of the utmost importance. It is important to ensure that all evidence of human interactions are carefully documented. Photographs should be clear and of high quality with identifying signs clearly visible. The presence of external parasites should be recorded and quantified in the data sheets concerning external parameters.

5.3.5 Morphometric data

At this point the external length and girth measurements should be taken and noted on a specific data sheet. All lengths should be made parallel to the long axis of the animal. Approximate measurements should be estimated even for a floating carcass as the towing and landing operations may alter its real ones. With a large whale, girth measurements can be made of half of the animal from the dorsal to the ventral midline and multiplied by 2. Another alternative is passing the tape under the animal during positioning and then taking the length measurement.

5.3.6 Internal examination

The first incision is the most dangerous moment of the necropsy. It is important that all members of the crew stand back while an experienced

cutter decompresses the abdomen and thorax to avoid being sprayed with liquid or gas discharges. Once that it is done, the blubber should be removed in circumferential slabs, allowing blubber thickness to be measured. All areas of hemorrhage, edema, swelling and suppuration should be noted and described. Patterns of change that are widespread and uniform are often post-mortem in nature. Histology samples of all identifiable as well as suspect tissues should be taken and can be discarded later if need be. The evaluation of other persons may confirm suspects or impressions. The teams should proceed logically through the entire carcass from organ to organ as described for small cetaceans.

5.3.7 Blubber thickness

Blubber thickness can be considered an index of the animal's nutritional status. Multiple measurements carried out along the animal's entire length will provide a more complete picture of body conditions as this lipid layer has different functions and depth profiles depending on its position. Nine landmarks along the length of the whale can be identified and measurements can be taken along the circumference. The basic points are: the nuchal crest behind the blowhole, axilla, midthorax (midway between the axilla and the cranial insertion of the dorsal fin) the cranial insertion of the dorsal fin, lateral to the dorsal fin, caudal insertion of the dorsal fin, anus, mid- keel (midway between the anus and the insertion of the flukes), and fluke insertion. Transverse cuts should be made from the dorsal to the ventral midlines through the epidermis to the muscle/blubber interface at each landmark (effectively forming half-girths). Blubber thickness should be measured to the nearest millimeter from the blubber/subdermal sheath interface to the blubber/epidermis interface at seven equidistant positions. Separate thicknesses should be recorded for the epidermis (the dark pigmented external surface) and blubber (the white fiber and lipid rich hypodermis). There is a data sheet in the appendices where measurements taking during the necropsy can be noted.

5.3.8 Gross necropsy report

All observations and notes made during the necropsy should be written here. If there are multiple teams collecting data during the necropsy, each should fill out its own part, but the final report should be made by a single person.

The report outline was designed to prompt the teams to make specific observations about all organ systems. If an organ/tissue appears normal, it should in any case be described and the fact that there are no visible lesions (NVL) should be specified. If an organ/tissue was not examined (N/E) that too should be noted and the reason that it was not

examined should be explained (e.g. autolysis). If the tissue or body part was missing (i.e. flipper removed by propeller cut), that should be noted as not applicable (N/A) and the reason it is not applicable should also be noted. These notes will help to make the report more homogeneous. All observations should be written as completely and carefully as possible and alterations in form, dimensions, surfaces, colors, number/quantities, and distribution should all be noted. The pages of the report and all added pages should be numbered and marked with the identifying number of the site (in the case of multiple strandings) and that of the team.

5.4 Disposal of the carcass

Once the necropsy has been completed, the carcass must be disposed of in accordance with health regulations outlined in EC Regulations N. 1069/2009 and 142/2011. As the necropsy event is technically complex and costly, it is important to contact the local authorities and the qualified technicians during the planning stages of the operation. The ways to dispose of a carcass of such large dimensions are limited and must be examined carefully by the city council and the health and law enforcement authorities bearing in mind all of the exemplar's individual characteristics. Often logistic conditions, and in particular the difficulty in reaching the stranding site with appropriate means of transportation make it impossible to follow the rules outlined by the European Parliament and a waiver must be requested.

Disposal options include:

- Surface Decay: If the necropsy was carried out on a remote, difficult to reach, isolated beach the fastest way to achieve disposal is by leaving the carcass open for maggots and other scavengers. In this case it is important to have good images of the state of the carcass when it was left at the site so it can be identified if a storm washes it offshore.
- Beach Burial: Whenever the ecology and human use of the beach allows, beach burial is the most affordable and easiest option. This should be done away from water supplies and other environmentally sensitive areas. This option avoids extensive transport costs/arrangements. A suitable backhoe, excavator or other heavy equipment is required. Ideally a hole is dug before beginning the necropsy beside the carcass and as flesh and blubber are stripped off they are examined, sampled and pushed into the hole. The hole, which should obviously be dug far from likely tracks of future vehicles, is then backfilled. If a museum has expressed interest in having the skeleton, the bones should be recuperated immediately to avoid

nighttime thieves. If the museum is unable to collect the bones right away but within 24-48 hours, covering the bones with large pieces of soft tissue waste may prove to be an adequate deterrent. If the bones will be collected much later, the carcass should be covered with a piece of metal to enable later use of GPS and a metal detector.

- Offshore Dumping: If the body and skeletal remains can be dumped offshore this is a good solution as the risk of material washing ashore is minimal. Local currents and tides will dictate how far and where to go. The Italian Coast Guard and the Agenzia Regionale per la Protezione Ambientale (the Regional Agency for Environmental Protection) should be contacted for guidance and assistance. It will probably be best to wrap the carcass and the skeletal remains in special fishermen nets and the incident should be reported to impede fishing in that area. Wastes must be disposed of following local regulations.
- Landfilling: This is often the only and best option. The administrators of the landfill must, of course, be contacted as the impact of a large quantity of organic material deposited at a landfill can alter the implant's equilibrium. The costs as well as the necropsy site must be considered when this option is being examined.
- Composting: there is a growing movement in the United States which is studying how to compost stranded marine mammals using a specific methodology similar to that is employed for livestock waste. More information can be found on the general principles of this composting system at: http://compost.css.cornell.edu/naturalrenderingFS.pdf

5.5 Examining a floating carcass

When a carcass sighted floating offshore cannot be towed to shore, a modified necropsy can be organized which is limited but nonetheless the source of important information. This kind of event requires careful coordination and activities should be planned utilizing satellite or air coverage (to relocate the carcass' position at sea).

5.5.1 Video and photographic documentation

The support boats should slowly circle the carcass and photographs and videos should be taken. It is important to carefully take note of body conditions, the state of conservation and scavenger damage. The body

condition (robust, emaciated et.) should be defined and photos of the fluke, in particular the caudal fin or other identifying signs, to help identify the exemplar by comparing photos contained in the identification bank. It is important to examine the carcass for evidence of human interaction (entanglement marks, scars, ship strike etc.). Any suspected evidence of human interactions should be documented using digital, video and still photos. Any object or lesion of possible or suspected human interaction should be collected for further investigation whenever possible.

The total length of the animal should be measured. This can be challenging at sea as some of the carcass will be underwater and the presence of sharks is probable. An estimate can be calculated by pinning the tape to one end and holding it in place while the tape is played out. Alternatively, a large vessel can be laid alongside the whale and the position of the head and tail marked on the vessel. In bad weather conditions a partial external examination can be conducted using aerial and underwater videos. Later, frame by frame analysis can uncover substantial information about rope marks, propeller wounds, lesions, etc.

5.5.2 Sampling

When sea conditions are relatively calm, it is possible to obtain internal samples to a limited degree from free-floating whale carcasses. Most carcasses present ventral side up, making access to tissues feasible. Often despite heavy shark scavenging internal organs are intact. Dissection is best carried out with an extra long handled flensing knife attached to a telescopic pole working from a moderate sized ship perhaps with the help of a platform nearby. It may be possible to anchor the vessel with ropes tied to the tail stock and flippers (which can be severed if necessary). If the boat is small two persons each with a whale hook on either end of the vessel (blow and stern) can hold the whale and the vessel together.

The animal should be opened with a long cut along the mid ventral line from sternum to anus. An exploratory cut is useful to determine the level of gas build-up inside the animal prior to proceeding with the real incision. Samples can be obtained from the colon, bladder, small intestine and stomach in addition to the skin, muscle, and blubber. While these investigations will not be useful to determine the cause of death, they will allow analysis for biotoxins, contaminants etc. As large whales are quite broad, making sample collections from a boat is quite challenging as most of the organs of interest are in the midline. Given the significant risk of unexpected shark attacks, it is not recommended to get out of the vessel. Work then should be done using long handled knives, hooks, bailers, and suction devices from a stable vessel platform and no one should stand on the carcass at an time.

5.5.3 Sample Preparation

The pre-labeled bags and containers should be prepared ahead of time as already described. If intestinal contents are scant, a large knife can be used as a scraper to remove contents adherent to the mucosa.

If possible, a blood sample should be collected in a red top blood tube. This can be done on male carcasses by severing the penis and collecting a sample as it bleeds out. In females (and males from which blood cannot be collected from the penis) blood can be collected from the flukes, behind the eye or snout.

It is important to collect a skin sample that is in good condition so it should be collected from a part, often immersed in water that is not epithelialized. A 10 x 10 x 5 cm piece of blubber should be collected and stored in a ziplock bag

If access permits the genital regions should be incised. A muscle sample should be taken. The bladder should be incised and urine which is bailed out with a centrifuge tube should be centrifuged before it is stored.

Continuing the incision cranially along the ventral midline to the sternum, it is possible to reach the other internal organs. The largest gastric chambers can be identified by moving the liver, if visible. Stomach should be incised and contents sampled if present storing them in appropriate containers. A 10 x 10 x 5 cm liver sample should be taken and placed in a ziplock bag.

If possible, collect a sample of the kidneys (which are the same size as the liver) which are visible along the body wall of the abdominal cavity.

If possible, sample any tissue showing signs of human interaction.

If possible collect an eye and store it in a ziploc bag.

5.5.4 Sample processing

Once back on land, the samples should be processed immediately. Bagged samples should be cut in half and the second half placed in another labeled bag. A part of the blubber, liver, and kidney should be wrapped in acetone-washed aluminum foil before dividing the samples.

All plastic containers should be dried and relabeled if necessary and closed with scotch tape taking care not to cover already written information.

Clean all equipment to avoid contamination, eliminating disposable material.

5.5.5 Quick sample collection guide

In cases of stranded or floating animals, the following samples should be taken (priority samplings are noted in bold face)

Collect skin, blubber, muscle, liver, kidney, etc. in ziploc bags

Collect urine, feces, stomach contents in centrifuge tubes
Collect blood in red top tubes.

6

HUMAN INDUCED MORTALITY

Sandro Mazzariol

Deaths of marine mammals directly linked to human activities such as fishing, commercial or military shipping or construction) have been described in chapter 3. Mention was made to the fact that a large proportion of strandings of cetaceans along the Italian coastlines are due to human activities. This estimate is based, of course, only on the cases brought to the attention of the authorities and we need to remember that many of the *Post-mortem* examinations that were carried were not conducted in a systematic way by appropriately trained veterinarians. Those diagnoses may have been made by individuals without a medical background or based on incomplete or fragmentary documentation. In order to standardize data on this issue and to avoid both underestimating and overestimating mortality rates caused by human intervention, the protocol being outlined here intends to provide appropriate guidelines for evaluating human interaction.

6.1 Evidence of entanglement in nets or by-catch

Direct or indirect interactions with man also include long existing and never resolved problems such as interaction with fishing. This problem generally threatens the lives of marine mammals in three ways:

- Accidental death of cetaceans which are caught unintentionally by fisheries and then discarded
- Direct killing of cetaceans as these animals are considered competitors or the cause of damage to fishing gear;

- Depletion of fish stocks due to excessive exploitation or illegal fishing practices.

The first of the three types of interaction, also called by-catch, is defined as the portion of a fishing catch that is discarded as unwanted or commercially unusable. By-catch represents one of the principal causes of the death of marine mammals. In the Mediterranean, in particular, pelagic species are prevalently involved while in the Black Sea the phenomenon affects coastal species. A cetacean captured in a fishing net often dies because it is not strong enough to disentangle itself or to reach the surface to breathe. Large cetaceans may be unable to break through the nets or may drag any remaining pieces for long distances despite debilitating lesions which may cause a slow death.

External lesions documenting the animal's encounter with fishing nets are the principal elements confirming an episode of by-catch. If an animal is found entangled in fish nets, the cause of death may, nevertheless, be different with respect to the immediate evidence: when in a state of debilitated health the exemplar is more likely to fall into a trap and be unable to escape. The absence of evidence of being captured in a net does not, on the other hand, exclude a priori the hypothesis of by-catch which is discernible in this case only by a thorough *Post-mortem* examination. Just as in human medicine and in other sectors of veterinarian medicine, even if the cause of death seems evident, all factors and possible contributory causes (biological, microbiological, parasitological, physical) must be carefully considered and analyzed. These investigations are all the more important in the case of beached or floating carcasses without any signs of interaction with fishing gear. Unless a thorough necropsy is carried out, death may be erroneously attributed to false causes.

One of the first things that needs to be estimated during a necropsy is the time between the moment of death and when the carcass was first sighted. This is done by evaluating the animal's internal temperature, the body's state of conservation, evident postmortem signs, the appearance of the eye, the presence of bacterial proliferation. Observations and data concerning the tides and wind current will make it possible to hypothesize when and where death took place and thus what human interaction may have taken place. It is necessary then to identify the mechanism of death or rather all of the structural and functional alterations which may have contributed to making an independent life impossible. All elements which can help to define how death occurred, and in particular those documenting direct and indirect human interaction such as fishing activities should be collected in the attempt to identify any criminal or illegal activities. All of these elements and the results of other collateral analyses may lead to the cause of death or pathology, damage or

alteration/s which alone or in association with other factors set off the sequence of functional disturbances which terminated in death.

One of the members of the response crew should be charged with all aspects of public relations. The degree of entanglement and consequently the severity of associated lesions can vary with the species and the type of net. Dolphins killed in fine-mesh seine nets, for example, become trapped in the folds of the net and often do show any evidence of what took place. In contrast, interaction with gill nets leads to lacerations or indentations from the net material.

6.1.1 Physical evidence of entanglement in gill nets

Any carcass with linear lacerations or indentations in the epidermis most commonly around the head, dorsal fin, flukes and flippers should be assumed to have died as a result of becoming entangled in gill nets.

Careful examination of the nature of these lesions may indicate in what type of net the animal was entangled and the size of the mesh. The type of laceration varies depending on the type of material with which the net was made. Lesions from monofilament twine generally appear as thin, distinct marks; multifilament twine instead causes lesions that are similar to those made by nylon nets. The type of entanglement and thus the severity of the lesions depend on the type of the net. Large animals killed by a wide meshed net show net signs throughout their body. The net causes lacerations around the rostrum as well as breaks and can cause loss of teeth. The flippers or caudal lobe of smaller animals can be entangled in these nets and may show only minimum signs of entanglement. In general, however, diagnosis of death by entanglement can be considered certain only when the signs are clear and evident. If the lesions are deep and around the head or thorax, it is possible that the animal broke through several layers of the net before becoming completely entangled. Thin, linear lacerations of the epidermis are generally present on the fins, tail and caudal lobes (around or at the edges with tears in the skin) grooves are noted on the test and around the rostrum.

Floating carcasses that have been found have often been towed or moved or secured after death by ropes or lines around the tailstock or flippers; impressions and abrasions from these lines are usually quite clear. It is important to differentiate between physical evidence of *Post-mortem* events from recent trauma and as well as from scars of past events. Many delphinidae and ziphiidae also bear fresh or healed lesions caused by social interactions and these too must be differentiated from net marks. Tooth rakes, which are the most common form of social interactions, occasionally occur in a pattern similar to marks left by a multifilament net material.

Specimens which died following entanglement are usually in good physical condition with no evidence of emaciation. It is to be remembered that emaciated animals or specimens that have died of a chronic medical problem can also be caught in a net. An unusually thin blubber layer and atrophied neck or epaxial musculature (i.e. external depression posterior to the nuchal crest of the skull) sometimes associated with gelatinous edema of the subcutaneous tissues, pericardial fat, cavitary effusions and hepatic degeneration or atrophy are indicative of poor physical condition and may indicate the existence of chronic disease. In these cases, the debilitating chronic changes should be identified.

Other forms of damage or lesions, indicative but not diagnostic of entanglement in gill nets are penetrating wounds, the absence of extremities, subepidermal hemorrhage, fractures, lung edema with froth in the trachea. But it is to be remembered that these lesions can also be caused by other traumatic events. When these animals are found entangled in nets, fishermen often use harpoons or other instruments to hoist them on board and to free the nets. These wounds are generally evident around the head and the cervical region as these animals are hung by the tail. Other penetrating wounds can also be present.

Fishermen often mutilate the carcasses of small cetaceans to facilitate disentanglement. This is particularly true for large animals that are severely entangled. In these cases, flukes, flippers, or dorsal fin may be completely severed. Fishermen and observers working aboard fishing vessels have noted that it is often extremely difficult to remove the carcasses of dolphins, pilot whales, and beaked whales from their nets, while small-bodied animals can often be disentangled without mutilation. Fishermen will at times make a longitudinal slit along the ventral surface of the abdomen before discarding the carcass in the belief that the it will be less likely to float or to reach shore.

Signs that are considered characteristic or indicative of interaction with fishing gear include:

a) Lesions caused by contact with nets or during activities to remove the specimen from nets;

b) Lesions due to drowning or asphyxia;

c) Evidence of recent food in the stomach (fish present in the net);

d) The absence of other evident causes of death (bacterial infection, high parasite burdens, high levels of contaminants, epizootic diseases);

When skin lesions are being described, it is important to distinguish those caused by man from spontaneous ones such as damage caused by scavengers or opportunists or natural marks such as those due to social interactions or by particular pigmentations.

The animal should be examined carefully beginning with the head and proceeding towards the tail, first on one side and then on the other,

finishing with the tail and the fins. Any signs of human interaction should be indicated on the data sheet (Table 6.1) whenever any are noted or the fact that that part of the carcass was not examined should be marked as “not evaluated.” The exact position where lesions are found must be indicated. Generally, when these types of lesions are found on the ventral part of the animal, they are due to *Post-mortem* interactions, in fact as a result of fermentative and/or autolytic processes the dead animal tends to float belly up. Another useful parameter to establish if the interaction took place while the animal was alive is the presence of hemorrhage and hyperemia of subcutaneous tissues.

Whenever possible, the type of wound should be described utilizing the following terminology:

- Impressions are regular depressions in the skin left by a net or a rolled fishing line usually noted on extremities.
- Lacerations occur when the skin is cut exposing subcutaneous tissue. Generally both nets and fish lines leave regular linear lesions accompanied by impressions on the edges of extremities.
- Abrasions: the superficial layers of the skin have been scraped away with consequent bleeding and loss of substance sometimes involving wide areas of the body. They are generally consequent to stranding and to the use of ropes or proximity to vessels.
- Penetrating wounds from sharp instruments: these are generally circulating injuries which penetrate the underlying layers involving other organs and tissues besides the skin piercing the large cavities or stopping at the bones. They are often associated with bloody or purulent discharges and the edges are edematous.
- Scars from human interaction are usually similar to normal scars with regard to pigmentation but present the same characteristics described in the lesions above. It is important to note that these lesions can signify interaction. The evidence in this case is not sufficient to confirm the cause of stranding
- Other: in this case, describe the form of the lesion indicating the appearance of the edges, of this depth and its color.

If possible the lesion’s origin, or rather what provoked it (fishline, cord, nets, propellers, the prow of a boat) should be indicated. In particular, in the case of propellers, there will be deep lacerations with multiple, parallel, net, bleeding cuts. The cuts can be straight or circular. Other forms of anthropogenic trauma can be gunshot wounds, or wounds caused by knives, hooks, harpoons or floating bodies. Each lesion must be photographed and sampled for histopathology. The internal examination and collateral analyses will make it possible to confirm the diagnosis.

a) Diagnostic evidence of Fishing Gear

Detectable external signs of animals killed in purse seines are different with respect to those entangled in gill nets. Purse seines do not generally leave evident signs of skin abrasions, lacerations or net marks in particular on the rostrum. It is not generally necessary to mutilate the carcass to disentangle it from the net and as a result at most there can be some penetrating wounds caused by the use of harpoons by fishermen. Other signs noted at necropsy are hemorrhage of the subcutaneous tissue in particular in the cervical region and occasionally at the head, thorax and abdomen. Captured subjects can present a vast array of lesions that are not necessarily indicative/distinctive of nets.

Interaction with cords or lines may not be fatal, at least in the initial entanglement. Although their fate is unknown, animals that are caught are often released alive, many with hooks still embedded in some portion of their body which can lead to secondary pathologies.

Evidence of entanglement in ropes or lines is usually obvious. There are often deep abrasions in areas where the line was wrapped around the body or appendages. These abrasions are typically found around flippers, the caudal peduncle and at the insertion of the flukes. If an animal survived for a long time after the wound was inflicted, the abrasions can be severe, extending into connective tissue below the blubber, muscle and even the bone and can be accompanied by local or systemic infections. In some cases, the entangled animals can be found stranded with the gear still attached and all remnants should be labeled and stored as proof of the interaction.

If entanglement in a monofilament line is discovered or suspected, a thorough examination should be made of the oral cavity, esophagus, and forestomach during necropsy, for the presence of hooks. The line may have become wrapped around the larynx if fishing lures or bait have been ingested. Animals killed in connection to these instruments show the same types of gross internal evidence as those entangled in other types of fisheries.

Finally, it is important to distinguish accidental signs linked to the use of cords to tow the exemplar or placement of identifying tail tags which can mask or obscure existing impressions made by line or rope. Whenever possible, it is important to examine the carcass as soon as possible and to carefully record how and where tail tags and towropes were used to avoid making an incorrect diagnosis.

In brief, interaction with fishing gear can determine specific lesions such as:

- Loss of a fin or caudal lobes;
- Wounds around the rostrum, head, and fins caused by entanglement

in the mesh of the nets;
- Linear skin cuts;
- At times fishermen attempt to free the nets of living or dead animals using various pieces of equipment causing:
- Tears in the abdominal wall (knives);
- Penetrating puncture wounds (hooks and harpoons);
- Abrasions around the caudal peduncle (cords).
- Interaction with vessels, attempts to remove the animal or the carcass, fishing gear cause the following:
- Scratches and skin abrasions;
- Blunt trauma;
- Hematoma and bruising;
- Cords or net fragments on parts of the body.
- The type of external lesions found on stranded animals can indicate the type of fishing gear involved. For example, linear cuts of the head are indicative of entanglement in a trammel net while signs of a net, skin scratches, removal of fins and/or traumatic bruising often take place in connection to purse seines.
- Besides those provoked directly by fishing gear, fishermen and proximity to vessels, lesions can be linked to capture but not specific to it such as in the case of:
- Hemorrhage
- Skull fractures
- Ecchymosis and internal blood spots (sclera, glottis, epicardium, pleura, etc.)

These lesions can be attributed to the animal's attempt to escape or later when on board but they can also be determined by other spontaneous pathologies and if seen alone, although suspicious, are not necessarily pathognomonic for by-catch.

b) Lesions due to drowning or asphyxia

Entangled cetaceans sometimes show froth, pink fluid or blood in the bronchi. At times froth can be seen coming out of the blowhole of carcasses recuperated in nets. This symptom in human medicine is suggestive of drowning but it is not considered pathognomonic for it. The search for diatom growth and in general of marine flora in the lungs are important as these can indicate a death following immersion although these elements can also be linked to the final phases before death or immediately afterwards.

Signs that are compatible with a diagnosis of drowning or asphyxia are:
- Epicardial petechiae
- Froth in the bronchi, lungs and auditory bulla

- Watery pink liquid in the airways
- Partial atelectasis

These lesions are relevant when they are described in association with evident signs of by-catch as it is not yet clear if death was caused by drowning, asphyxia or other unknown events linked to capture.

The pathophysiology of drowning in cetaceans is not entirely clear nor is it clear if these animals have voluntary or involuntary control of breathing. In the former case, the breathing reflex is not demonstrated and thus water does not enter the air passages and death takes place as a result of axphyxia or the depletion of oxygen in the blood, the so-called “dry drowning.” In involuntary breathers hypercapnia activates some peripheral and central chemoreceptors which function to regulate respiratory activity. If the animal is under water it will inevitably inhale sea water (wet drowning). Finding sea water then in the caudodorsal portion of the lung parenchyma (rigor mortis causes the blowhole to open letting water enter the cranial parts even after death and affecting the diagnosis) suggests that water was terminally inhaled; this together with other signs indicative of by-catch can lead to the diagnosis of death by drowning. The presence of sea water in the lung parenchyma can be evaluated only under the microscope and the following should be searched for simultaneously:

- The presence of watery, pinkish liquid in the air passages;
- The presence of crystals or particulate matter and/or diatoms in the alveoli and in the bronchioles;
- The presence of alveolar and or bronchial edema;

Even if there is water in the lungs, in the absence of lesions compatible with by-catch it cannot be considered pathognomonic for accidental entrapment. At the same time, the absence of water in the lungs does not exclude the possibility of by-catch, as death may have been caused by dry drowning or suffocation.

c) The presence of food in the gastric chamber

Finding food in the gastric chamber during a *Post-mortem* examination is an important finding for the diagnosis of by-catch. Finding undigested or partially digested fish may be indicative of an animal that was in good health which died unexpectedly (although this may not be indicative of by-catch) would seem to indicate that the exemplar was not affected with any pathological processes. Interaction can be confirmed by recognizing the species found in the stomach and comparing them with those in that geographic area and in the fishing gear.

d) Lesions due to interaction with fishing gear

An external examination detecting and identifying lesions due to

interaction with fishing gear is often sufficient to emit a diagnostic suspicion but there may have been pathological conditions that may have predisposed for or been determined by-catch. The presence of evident but not specific signs linked to entanglement, such as evidence of a recently eaten meal and good body conditions are all compatible with by-catch but they cannot be used alone except as details which indicate and/or suggest a possible cause of death. At times animals which died of other causes have signs that are similar to those found in by-watch victims. Inter-species interactions can in effect determine penetrating wounds, subcutaneous bleeding with hematomas and fractures. The absence of extremities, an element indicative of human interaction, can also take place during the stranding itself.

Other possible causes of death or pre-existing debilitating conditions must therefore be excluded by means of a complete, thorough, accurate necropsy examination carried out by qualified, appropriately trained veterinary personnel. Only a careful examination of the features of those wounds and their localization can furnish sufficient details to elucidate their cause.

6.1.2 Damage by Scavengers

The degree and type of damage caused by scavengers varies from species to species and from situation to situation. These lesions vary from small superficial holes caused by amphipods to extensive external damage caused by shellfish and agnates. These benthic scavengers generally attack the area around the eyes, the mouth, the underarms and the genital zone. In some cases the damage is so extensive that it is impossible to evaluate if there are signs of entanglement. Small cetaceans can be bitten by sharks immediately after their death, in particular in warm waters. Signs of sea gull damage is initially limited to 1-2 cm skin scratches which are normally numerous and rather superficial. If the attack of sea gulls takes place when the carcass is floating in the water, the damage is limited to only one side or region. As the work of the scavenger/s proceeds other birds which arrive begin to dissect the carcass. Once the carcass reaches the coast the sea gulls continue to devour the meat in particular around the lower jaws to gain access to the lipid-rich mandibular fat pads. At this point it is difficult to distinguish the damage of the seal gulls from that of other scavengers.

6.1.3 Hemorrhages

The carcasses of many dolphins killed in fishing nets have signs of subcutaneous hemorrhage in particular at the dorsal and lateral cervical

regions, probably due to restraint imposed on the animal's head during its struggle in the net. These animals often exhibit broken bones and associated blood clots and macerated soft tissue. Some bones may be broken postmortem when the carcass is dropped on the deck or while it is being towed to the beach. Blood infiltration into the region of the broken bone and signs of hemorrhage in the surrounding area are often seen in these cases. Histopathological analysis of the wound edges and the search for fat embolism of the lungs can be evaluated under the microscope.

6.2 Physical evidence associated with other forms of anthropogenic trauma

As pointed out in chapter 3, there are other causes of death that can be directly linked to man and connected to fishing or navigating activities causing the traumatic events described or outlined. In these cases as well, a complete necropsy will help to uncover the predisposing factors that favored those events.

6.2.1 Gunshot wounds

Gunshot wounds vary in nature depending on the type and caliber of weapon and the proximity to the animal when the weapon was fired. Single bullet wounds are easy to distinguish from those caused by shot pellets by their size and number. Entry wounds may be blackened or singed if the weapon was fired at close range. The size of an entry wound is not a reliable indicator of the caliber of weapon used as the elasticity of the skin and the proximity of the weapon affect its diameter. An abrasive collar often extends around the entry point with even, smooth edges. Exit wounds may be of any size and are often characterized by jagged, irregular lacerations, eversion of the skin margins and marked removal of the tissue. Whenever the nature of the external lesion is unclear, a microscopic examination must be made to distinguish if it was made ante or *Post-mortem*; an evaluation of the internal organs must also be made.

The exact location and size of the wounds and all details about internal organs should be noted on the data sheets. The wound should then be marked with tags and photographed both externally and the internal areas affected. Before opening the wound site, it should be carefully probed with a blunt object to determine if the wound is penetrating and the approximate path of the projectile. There may be hemorrhagic tissue

or clots along the trajectory. The bullet may have broken bones during penetration and fragments may be perceived.

If the bullet is felt, it should be extracted with a gloved hand and not with metal forceps which can leave scratches on the bullet and alter future forensic analyses. Every bullet should be washed with water and wrapped in paper for forensic analysis.

It may be difficult to identify gunshot wounds if time has passed as the superficial lesions may have healed. In those cases an X-ray of the carcass can help to facilitate location of any bullets left in the body and their trajectory.

Unlike the wounds produced by scavengers, which are distributed over certain regions of the carcass, gunshot wounds are usually isolated on the carcass and surrounded by relatively undamaged skin

6.2.2 Vessel collisions

Marine mammals can also be killed by collisions with various kinds of boats of varied dimensions causing two kinds of grossly evident wounds: blunt trauma from the impact itself and lacerations from the propeller; either or both can be present depending on the nature of the collision.

These lesions are typically found on the dorsal surface often involving the dorsal fin. Evidence of blunt trauma may consist of hemorrhage, occasionally massive or diffuse around the point of impact. In severe cases, broken bones, ruptured organs, and torn underlying tissue may accompany the hemorrhage. As the hemorrhage may not be visible externally it is important to conduct a complete necropsy to verify the cause and mechanism of death and the presence of bruises. Searching for fat embolisms in lung tissue at the microscope using histochemical techniques (Sudan Black o Oil Red O stain on frozen tissue or the en-block after fixation technique with osmium tetroxide) can help to distinguish ante- or *Post-mortem* contusion injuries.

Lesions caused by propellers are usually obvious and distinctive, consisting of parallel, evenly spaced cuts on the body surface or appendages. They can be deep, sometimes penetrating completely through the dorsal fin. If the animal survives the initial collision, lacerations may begin to heal or can become infected. It is important to distinguish these cases from those in which the animal was killed outright from the collision and it is thus necessary to take photographs with measurement references and to take samples for histopathological analysis. As already mentioned above, these will make it possible to determine if the impact occurred ante-or post mortem as boats occasionally strike a floating cetacean carcass. In *Post-mortem* collisions, the propeller marks may be on the ventral or lateral surfaces.

6.2.3 Blast-Induced trauma

There are only scattered observations regarding the effects of blast-induced trauma on marine mammals. These lesions leave few grossly evident signs externally but determine both acoustic and percussive trauma in internal organs. The trauma is caused by rapid, massive pressure changes brought about by the shock wave from the explosion and the degree and extension of damage will vary depending on the size of the explosion and the distance from the blast. Principal lesions are localized in the ear and in the peripheral tissues. Fractures of the periotic bone tissues have been reported but as generally happens when injuries are caused by sound sources are difficult to detect with a routine necropsy. When blast injury is suspected it is wise to call in a specialist with experience in assessing the head and acoustic structures after this kind of trauma.

6.3 Forensic pathology techniques to confirm by-catch

Collateral diagnostic techniques can be used even in veterinary medicine to confirm a diagnosis. These are often methods coming from forensic medicine. With regard to marine mammals, cytology and the use of PAS staining on histologic preparations make it possible to detect the presence of diatoms in lung fluid. After fixation of tissues, a mixture of osmium tetroxide could be used to confirm traumatic events in the hard and soft tissues following a collision or interaction with military sonar.

In 2006 this same group of researchers proposed a new immunohistochemical technique to confirm the hypothesis of death due to accidental entanglement using a method which detects fibrinogen as an acute phase protein in the liver. Intracytoplasmic globules prevalently made up of glycoproteins - among which fibrinogen and alpha-1-antitrypsin - have been noted in animals killed in an acute manner with consequent acute hepatic congestion. These are acute phase proteins or rather proteins whose plasmatic concentration increases (or decreases) in elevated terms during acute inflammatory events. Moderate alterations have also been observed during ischemia and stress. Positive immunohistochemical staining for fibrinogen of intracytoplasmic globules were found in porpoises certainly victims of by-catch (found entangled in trammel nets). The idea of studying this and other similar molecules as markers of by-catch was thus proposed. Further studies on these techniques need to be carried out and if positive they can be adapted to be used in standard diagnosis protocols in marine mammals.

Mortality due to anthropic activities such as those linked to collisions

with vessels, gunshot wounds, and/or accidental entanglement in fishing nets is often established solely on the basis of external findings. The final diagnosis must in any case include not just external findings but must take into consideration all the other external and internal observations as well as the principal necropsy findings and the results from the *Post-mortem* laboratory examinations. This chapter has attempted to furnish veterinarians and biologists with useful instruments to carry out a complete postmortem examination. A data sheet is also provided in which findings concerning human interaction can be dated recorded, described and documented.

6.4 Post-mortem examination to evaluate human interaction

As all information and diagnoses have scientific value when carefully documented, use of a standard data form is recommended and provided here as it prompts the members of the necropsy team to check each category of physical evidence and to record opportune information.

Whenever possible, examinations should be performed on carcasses that are fresh and not after having been frozen. Freezing and thawing can in fact introduce artificial marks, cracks, or damage to appendages and lead to improper evaluation of microscopic data. There is also the danger of freezer breakdown. It is important then that the external examination be carried out as soon as possible after discovery even if the full necropsy is conducted only later.

6.4.1 General Information

All identifying data must be recorded: this includes the number given the exemplar, the species, sex, length, the date and place the external evaluation was carried out, the full name of the examiner, and if evident, the cause of death. It is critical that photographs and/or a video be filmed of the entire animal as well as all signs and unusual marks that must be detailed in the work sheet where it should be specified what photographic material corresponds to each of these. In the photographic material, besides the detail that is being photographed, the number of the exemplar, the place and date should always be visible. Care should be taken that the best lighting, exposure and composition be sought after while photographing/filming with the photographer proceeding in a pyramid fashion: from the general to the particular. If possible, there should be an audio narrative on the videotape describing the lesions being filmed. The condition of the carcass should be decided as outlined in chapter 3 bearing in mind that information can be useful up to code 3

and at times even from specimens in an advanced state of decomposition (code 4).

6.4.2 External examination

a) Body conditions

Following the criteria outlined in chapter 3, the body condition of the exemplar or at least an “emaciated” or “not emaciated” classification must be specified on the data sheet. The exemplar’s blubber thickness, where and how this was measured, the species and the season in which it is being evaluated should all be taken into consideration when the body condition is being analyzed. The apparent condition of a carcass can also change with decomposition. The body condition of animals in advanced states of decomposition (3 and above) should be classified as not determinable (ND). The nutritional status is useful in distinguishing between animals that died of chronic health problems and those that died of acute causes.

b) Net or Line Marks

The entire body and thus both sides of the carcass should be examined for marks that could have been made by lines or nets. As already mentioned above, lesions from monofilament gill nets appear as thin, distinct indentations on the skin of the animal that occasionally penetrate through the dermis. Multifilament net and lines often leave an impression of the braided material in the skin. Each lesion and mark should be described and photographed, including the cords or lines used to tow or secure the carcass.

c) Nets

Any object or net fragment attached to the carcass should be photographed and then removed and retained. There should be a note on the data sheet indicating where and under whose supervision that material is being conserved. If possible, it would be important to identify the fishery responsible for the entanglement.

d) Penetrating Wounds

The carcass should be examined for any penetrating wounds caused by gunshots, gaffs or other equipment. The size, depth, path and the localization of all wounds should be described. Obtain samples for histopathology to determine whether the wound occurred ante- or *Post-mortem*. The remains of a bullet or shot that are found during the internal examination within the vicinity of the gunshot wound should be collected. The entrance wound should be photographed to be able to evaluate the approximate distance from which the weapon was fired depending on its

characteristics.

e) Mutilations

All mutilations and thus any missing appendages or signs of cuts or slits into the body cavity must be described. Human removal of appendages can be distinguished from advanced decomposition, predation or scavenging by the clean, smooth surfaces left by knife cuts. Appendages or other body parts may also be severed or damaged from collisions with boat propellers which would leave a series of parallel cuts on the skin. In this case the distance between the cuts should be measured and the entire area where the wounds are found should be photographed. Ante- and *Post-mortem* wounds can be readily differentiated by histopatholgical analysis.

f) Bruising

Traumatic events generally cause hemorrhage with blood discharge from the vessels. These bruises may appear externally as subcutaneous hemorrhage which is purplish, red and/or swollen. The three dimensions of these wounds should be measured and the underlying tissues (blubber and muscles) should be examined during the internal examination to check for broken bones. In some cases, even massive blunt trauma may not be evident externally, so it is important to conduct a thorough, complete internal examination.

g) Animal scavengers

All areas on the external surface of the carcass where there are signs of scavenger damage should be described and photographed. This will help to give a general indication of the overall condition and appearance of the carcass. All circular holes, particularly if they appear to extend deep into the internal cavity of the carcass should be examined carefully to ensure that they are not gunshot wounds. Sea gulls cause the most common type of scavenger damage found on stranded small cetaceans. Gull pecks, typically appearing as multiple, shallow scrapes in the skin or appendages, are found on the areas of the carcass exposed on the beach or while afloat and preferentially around the lower jaws where there are lipid-rich mandibular fat pads.

6.4.3 Internal Examination

a) Sub-dermal Hemorrhaging

Blubber must be stripped from the carcass and the underlying muscles and tissues must be examined for any signs of inflammation or reddening. There are frequent signs of hemorrhage in the cervical region in exemplars which have been entangled and these must be described

and photographed. Focal hemorrhages are, instead, indicative of blunt traumas and any external trauma in overlying tissue should be matched, if possible, with sub-dermal hemorrhage. It is important to distinguish, if possible, ante- and *Post-mortem* trauma as the former may be linked to the cause of death. Ante-mortem hemorrhage is characterized by infiltration of blood and fluid from ruptured vessels into surrounding tissue and is often accompanied by swelling. There is no infiltration or swelling, but pockets of coagulated blood may be present in *Post-mortem* trauma.

b) Fractures

All broken bones and disarticulated joints must be described. Particular attention should be dedicated to the ribs and skull where fractures often occur, especially in small cetaceans. All broken bones must be carefully described as some may have been broken during *Post-mortem* handling. Signs of fracture healing such as fibrous connections between bone fragments, which might indicate that the trauma was not of a recent origin, should be sought. It is to be remembered that recent ant-mortem fractures are always associated with hemorrhage.

c) Lungs and Airways

The procedure to examine the respiratory system is similar to that used in ordinary necropsy procedures. Attention must be paid to the type, color and quantity of fluid present in the airways and any particulate deposited in the lungs. A sample should be taken to investigate the diatoms. Death by hypoxia is generally associated with edematous lungs that appear wet and heavy.

d) Stomach contents

A full stomach, containing partially digested prey, may be indicative of an animal that was in good health and died a traumatic death. The stomach should be extracted so that a careful examination of the contents can be made after the necropsy is completed. As explained in Chapter 2, the stomach complex of the cetacean consists of three chambers: the forestomach, the main stomach and the pyloric stomach. It is thus opportune to tie off the esophageal and duodenal ends with a string or cable before removing the stomach complex. As much as possible of the esophagus should be extracted as animals that die traumatically sometimes vomit and food may still be lodged there. The stomach complex and each individual chamber should be weighed and organic remains of prey as well as foreign bodies should be collected. The former should be frozen immediately. Cephalopod beaks should be placed in 70% ethanol and parasites should be fixed in absolute alcohol. The mucous membrane should be rinsed in water to dislodge

all remaining prey parts. A sieve or strainer should be used during the rinsing to collect small prey remains and foreign objects.

e) Pathological changes

All internal organs must be examined and the presence of parasites and evident gross abnormalities must be described. All of the ducts and lumens of the organs must be examined and the kind, quantity, and localization of parasites, if present, must be noted. Alterations in the volume, borders, surface, color and appearance of the organ both externally and at the cutting surface should be described as well as the dimensions and distribution of these alterations.

6.4.4 Histopathology Samples

Samples taken if possible from a fresh carcass (at maximum category 3) should be analyzed by an experienced pathologist, but samples from a frozen carcass can also be useful. Abnormal and adjacent normal tissues should both be sampled, as explained in chapter 3. In order to formulate a correct diagnosis even when the cause seems grossly evident, the principal organs including the brain, lung, heart, spleen, liver, kidney, adrenal gland, lymph nodes and urinary bladder should be sampled. Areas adjacent to hemorrhagic lesions, fractures, blunt trauma, infection and any regions of unusual pathology should also be sampled. Fat embolisms in the lung tissue can be searched for at the microscope using appropriate staining techniques (Sudan Black B staining or Oil Red O stain) or an en-bloc after fixation with osmium tetroxide.

6.4.5 Diagnosis

The cause of death can be diagnosed once the pathology report has been completed and a veterinary pathologist has been consulted. Several types of gross evidence are in any case considered diagnostic of traumatic death from human activities. Mutilation, for example, can occasionally occur on the beach and cannot necessarily be considered diagnostic of traumatic death at sea. Members of the necropsy team should be conservative in their evaluation and diagnosis. In the vast majority of cases it is not possible to assign a cause of death, but with due caution it is often possible to identify cases in which the animal died as a result of adverse interaction with humans.

Protocol for Examining Marine Mammals for Signs of Human Interaction

Exam Information *(fill in or circle most appropriate)*

1 Field #: _____ Species: _____

2 Examiner: _____ Recorder: _____

3 Date of exam:_____ Condition code (at exam): 1 2 3 4 5 CBD

4 Preservation: alive fresh frozen frozen/thawed Body condition: emaciated not emaciated CBD

5 Documentation: digital print slide video Image disposition:_____

6 Integument: normal abnormal decomp/scaven % Skin missing: <10% 10-25% 25-50% >50%

7

Explanation of terms:
YES = I have examined the area and found signs of human interaction
NO = I have examined the area did not find signs of human interaction
CBD = I have examined the area and could not determine whether there were signs of human interaction (i.e. the part was missing, degraded, or signs were ambiguous)
NE =I did not examine the area
NA = this animal doesn't normally have that part (i.e. seals have no dorsal, dolphins have no rear flippers)

	WHOLE BODY EXAM	YES	NO	CBD	NE	NA	Image taken (Y or N)
8	Appendages removed / Mutilation *(with instrument)*						
9	Pelt removed / Mutilation *(with instrument)*						
10	Body sliced / Mutilation *(with instrument)*						
11	Gear/debris present on animal *(includes tags)*						
12	Gear retained *(name & contact info in Comments)*						
13	External pathology *(pox, tattoo lesion, abscess , other)*						
14	Natural markings *(scars, tooth rakes, unusual pigmentation)*						
15	HI lesions *(fishery, gaff, gunshot, propeller, healed HI scar, brand)*						

16 Predation/scavenger damage *(circle all anatomical areas where damage hinders evaluation; numbers coincide with anatomical areas below)*: 17 18 19 20 21 22 23 24 25 26 27 28 29 30 NONE

						Type of Lesion					Origin of Lesion									
											Gear						Other			
	DETAILED EXAM OF ANATOMICAL AREAS	YES	NO	CBD	NE or NA	impression / laceration	penetrating wound	healed HI scar	abrasion	other / CBD	twine / line	net	other / CBD	monofilament	multifilament	CBD	propeller	gunshot	other / CBD	image taken?
17	rostrum/snout/palate																			
18	mandible/tongue																			
19	head																			
20	R front appendage																			
21	R body																			
22	dorsum/dorsal fin																			
23	L front appendage																			
24	L body																			
25	ventrum																			
26	peduncle																			
27	R rear appendage																			
28	L rear appendage																			
29	flukes/tail																			
30																				

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Field #:_____

	INTERNAL EXAM Date of exam:	YES	NO	Partial	CBD	Image taken	Detailed Info *(circle all that apply)*
31	Internal exam conducted						*Details in Comments section -use line number*
32	Bruising/blunt trauma						*Details in Comments section -use line number*
33	Skeleton examined						*Details in Comments section -use line number*
34	Broken bones present						*Associated tissue reaction: YES NO CBD*
35	GI tract examined *(circle contents)*						*intact prey partially digested hard parts only debris/gear empty other*
36	Lungs/bronchi examined						*Details in Comments section -use line number*
37	Lung/bronchi contents						*froth fluid air (color:)*
38	Other pathologies noted						*Details in Comments section -use line number*

39 **Comments** (*note line number from left margin before each comment*):

40 **Signs of Human Interaction Observed:** YES NO CBD (transfer to Level A Datasheet)

41 **Stranding Event History/Circumstances:**

42 **FINAL HUMAN INTERACTION EVALUATION:** If you circled YES above (#40), evaluate the external exam, necropsy, carcass condition and circumstances surrounding the stranding event to answer the question below.

How likely is it that the documented human interaction contributed to the stranding?

0: Uncertain (CBD) 1: Improbable 2: Suspect 3:Probable

43 **Justification:**

Data Sheet developed by CCSN & VAQS (12/27/2005) with funding from the John H. Prescott Grant Program

Tab. 6.1: Protocol for examining marine mammals for signs of human interaction; Data sheet developed by CCSN & VAQS (12/27/2005) with funding from the John Prescott Grant Program.

7

FIRST RESPONSE FOR CETACEANS STRANDED ALIVE

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This chapter focuses on the procedures and protocol to be followed by a vet physician of a rapid response unit when a living exemplar is sighted on the beach, offshore, or entangled in nets and requiring medical attention. In particular, it is concerned with those steps that should be taken when a beached animal is sighted and the factors that need to be taken into consideration when its fate is being decided.

As already explained, first response units are called upon to actively participate in the phases of an emergency situation centering around a stranded cetacean, that is a living beached animal, as well as in those atypical events in which multiple exemplars or a single animal of large dimension are/is stranded. Qualified persons must intervene in a timely and rapid manner to provide technical support, assistance and consultation services to local institutions and organizations.

7.1 Procedures for cetaceans in difficulty at sea

These indications are applicable in the following cases:

a. When cetaceans accidentally entangled by fishing gear (by-catch) are sighted;

b. When cetaceans at sea apparently in difficulty are sighted;

c. When cetaceans are sighted in shallow waters or in water characterized by numerous anthropic activities (such as docks or ports);

These conditions, in particular those outlined in the first point, are generally characterized by numerous risks and dangers for those involved in rescue operations as well as for the animal itself which may require direct interaction/assistance. In these cases expert personnel trained for similar emergencies belonging to or working for organizations such as the Civil Protection Agency, the Fire Department, or the Army should be called in to collaborate with vets. Trained personnel is in any case absolutely necessary whenever the animal's length exceeds 5 meters.

7.1.1 Animals in difficulty close to the shoreline or near ports.

If the stranded animal/s is/are near the shore the following features, situations and conditions should, if possible, be determined before beginning rescue operations:

- The species;
- Its approximate dimensions;
- Possible identifying marks;
- Its breathing rate;
- The presence of other animals of the same species in the vicinity;
- The presence of behavioral or physical abnormalities.

Generally speaking, in the absence of wounds or of physical, ethological or behavioral disabilities, the subject should be left in the water and monitored continuously while it is located in a dangerous area. If the decision is made to intervene because, for example, of adverse weather conditions that can prove dangerous for the exemplar, it is best, once the response unit has expressed its evaluation, to aid it to return to sea. If weather conditions are calm, boats (being careful about the problem of the propellers) conducted by trained personnel and chains of persons (being careful about safety distances) can attempt to push the animal towards the sea making noise by splashing their feet in the water or banging together metal objects and keeping themselves between the shoreline and the animal.

Using a metal tube on the boat could lead to a positive result but may cause the stranded cetacean stress. These attempts cannot, however, last for long periods: if the animal is debilitated, no effort will produce results and even if it does, it may not necessarily be in the right direction. Other possibilities are those of helping the animal to regain its equilibrium or moving it on tarpaulins or stretchers. It is important to consider in these cases the safety of the personnel involved in the rescue operations and the animal's welfare carefully. If the animal is debilitated and tends insistently to move towards the sea, it is best to transport it towards the shoreline in order to help it become stabilized and to prepare to carry out the necessary clinical investigations.

7.1.2 Liberating an entangled animal

Freeing animals from nets, cords or other fishing gear is/can be a particularly delicate procedure which may require capturing it. These are, needless to say, extremely dangerous operations that require the presence of expert underwater divers, and if the animal is located in waters deeper than a meter, underwater equipment may be opportune for breathing evaluation.

It is to be remembered that even if the animal is hindered by the nets or cords and appears easy to approach, it can easily drag rescue workers underwater by unexpected movements.

Cetaceans between 4 and 6 meters can tranquilly be approached by small (even inflatable) boats to ensure soft support in case of falls and once opened can avoid the risk of breaking cables. As a safety precaution, it is best to carry out all rescue operations during daylight; it is thus not advisable to begin work near nightfall. In this case, it is best to monitor the animal during the night and to begin the operations early the next morning.

7.1.3 Approaching and Monitoring Entangled Animals

As any rescue attempts are extremely dangerous, it is important to proceed with utmost caution informing all involved on what to do and how to do it. Personnel on board should be equipped with life vests and helmets. Videos and pictures could help in planning the intervention with people involved. Greatest dangers are connected to the equipment, the animal's movements, ropes which can become entangled around the boat or the operators or the buoys which can be tugged or attacked. Rescuers in a boat should not use fishing boots which, in cases of falls, could become filled with water and become dangerous to rescuers. The entangled cetacean should be approached from behind or to the side to avoid alarmed responses. Rescuers should observe the animal for a long as necessary and be ready to alter the plan if advisable. During this phase, binoculars can be used to evaluate exactly how the animal is entangled. Cords or net fragments that may become caught in a boat motor must be monitored. Once positioned along the length of the body, the exemplar should be examined – while always keeping a careful eye on the tail - in order to evaluate how to proceed. Movements should be as few and quiet as possible and silence or near silence should be maintained with rescuers communicating by gestures of the body and head.

One of the rescuers tied to a rope to the side of the boat and with a face mask can be let down from the boat in order to evaluate the situation from that point of view. Ropes and nets could be cut using a

blade hook-shaped at the end of a long stick, in order to grapple any object maintaining safe distances

7.1.4 Capture or release

In view of the risks involved, it may be necessary to capture the subject in order to liberate it whenever its dimensions make this a feasible option. Large entangled animals should in any case be approached with caution even when they seem docile. The manner to approach these animals varies considerably, but rescuers should be aware how potentially dangerous the animal can be if it is completely free to move about. The alternatives are outlined in other reference manuals.

In those cases exemplars do not show alterations in swimming or preying activities, it is best to monitor the situation until it seems safe to approach. If the exemplar is clearly in danger and conditions seem safe for rescuers to work, it may be possible to head it into the direction of a sea arm with a low sandy floor. It may, alternatively, be possible to close off an area using floating, closed-bottomed wide mesh nets, in which case veterinarians may utilize sedatives to facilitate disentanglement or to capture the animal to later free it. Use of sedatives should, nevertheless, be avoided unless authorized agencies give approval and are collaborative.

It is important to remember that this approach may not work in all species and in particular in pelagic fish and that a prolonged physical constriction can cause further stress with onset of myopathy or shock. If night is approaching or the sea is becoming rough, it is best to monitor the subject from afar and to wait for better conditions.

Using sedatives in open sea on free-ranging subjects is not recommended, as they could cause the cetacean's death. In the time that it takes for sedation to take effect, an attempt to flee could be made and the sedated animal could drown or die of other complications.

7.1.5 Releasing Captured Cetaceans

Before cutting net fragments away from a cetacean, it is important to analyze how the subject became entangled in the first place. Some cords can be liberated simply by cutting them with a knife at one end. If cords are attached to floating buoys which are tied around the cetacean only once, the cord can be cut at the end without the floaters as close as possible to the buoys.

The aim is that of making that part of the cord float so it can be recuperated so as not to become a danger for other animals. If no part of the cord is attached to a floater, one can be anchored to it before cutting it so the fragment is not lost. The buoy can then be tied at every

loop, node or bulge of the cord. The cord or the line can be cut when the animal is on the surface of the water. If it is impossible to cut it entirely, it can in any case be cut as close as possible to the cetacean to avoid shredding.

Working near to an animal that is entangled in a net is generally complicated. In many cases a large part of the net can be removed leaving only those fragments that do not impede the animal's movements. Once the major portion of the gear has been removed the cetacean can often move about freely and may even be able to rid itself from the rest. It is important to cautiously cut the nets while keeping a careful eye on the animal's movements. The freed fragments must be attached to floaters.

If the caudal lobes are caught, it is best to remove the material that is above the tail which is easier to reach while making sure that the rest is attached to a cord or a buoy. The quicker the material is removed the less risk for the rescuer. Once the material above the tail is cut, the rest will slither away as the animal moves about.

7.1.6 Precautions

As animals may flounder violently when nets are being slackened or cut, whatever movements it could make must be calculated and rescuers must be prepared to move quickly and out of danger. It is best not to enter the water to free cetaceans from nets or cables or to analyze how it is entangled. Operations to free the cetacean should be carried out from within the boat.

Once the animal has been disentangled, it should be monitored for a few days to be sure that it has recuperated entirely. Once completed, the various phases of the operation should be reviewed and evaluated to verify and to learn from mistakes/success.

7.2 Medical and Behavioral Evaluation

Living beached cetaceans should be cared for by qualified veterinarians who have experience in evaluating, treating and rehabilitating living beached cetaceans. As there are other excellent manuals guiding individuals involved in activities to rescue stranded cetaceans and intending to furnish guidelines on carrying out a clinical examination, this chapter does not intend to substitute more extensive, complete texts on this subject. For a full explanation and interpretation of signs and symptoms and collateral investigations to carry out, please refer to: CRC Handbook of Marine Mammal Medicine, Second edition, 2001, Eds. L.A. Dierauf and F.M.D. Gulland.

7.2.1 Collecting Data and Recording the Event

The event must be reported to the appropriate Data Bank (which, depending on the place, can be a national or international organization) where cetacean strandings are documented and stored. Geographic information, and biological and logistic details concerning the stranding should be recorded and data sheets filled out. Once the event has been recorded, a unique identification number, which should be utilized at all subsequent contacts, will be assigned to it. Information relative to the following data must be collected:

- Weather conditions in the 15 days leading up to the stranding including data regarding abnormal tides, temperatures, wind currents and storms;
- The stranding risk for that particular area and information about the number of strandings recorded on that piece of coastline should be obtained from the pertinent data Bank;
- Other unexpected or stranding events of different marine species in the 15 days leading up to the stranding;
- The interventions carried out before the response unit arrived on the scene and the observations made by the Health Service veterinarians and individuals connected to the Stranding Network;
- Any possible ordinances emitted by the U.S. Collaborative Perinatal Project (CPP) concerning any anthropic activities connected to the event;
- The presence of industrial sites, ports, or oil platforms in the vicinity of that water basin;
- The subject's species, biometric measurements, age, estimated weight, and the presence on the animal's body of signs of anthropic activities

7.2.2 Approaching the Animal

The first step is that of observing the animal and its behavior and planning the best approach to be adopted. The stranded animal should be approached with great caution either from the side or straight on. Loud noises, brusque movements, and bright lights should all be avoided and the animal should be given time to become accustomed to the presence of the human operator. Even if the animal is not aggressive, it may in any case accidently bite or strike with its tail anyone in the vicinity in particular at the height of the knee. All attempts to approach the animal should be made cautiously and particular attention should be paid to the mouth and tail.

As the animal may be agitated (e.g. in the case of a mother separated from her calf or of a member of the group separated from the others) the

members of the rescue team must have a plan and a response prepared for every reaction. Once the exemplar has been circled all members of the team must act contemporaneously taking care to simultaneously block the rostrum, the body and the tail.

7.2.3 Clinical Examination

The procedure for carrying out a clinical examination is made up of various parts and does not differ greatly from the objective examination carried out on land mammals. The procedure to carry out a complete examination is outlined below.

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. Once this has been done, the veterinarian should go on to examine skin lesions and mucous membranes.

b. Floating

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 (space-occupying mass, thoracic or lung fluids, pneumonia etc.). Another parameter to evaluate is equilibrium; if, for example, at rest the animal is rotated with respect to the longitudinal axis and if that alteration is due to a postural aberration or by a willful postural deviation which would be confirmed while the animal is swimming.

c. Behavior

Behavior 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 and the reflexes associated to them should be evaluated and the animal's breathing rate should be observed bearing in mind that a mild arrhythmia could be considered physiological. The physical examination is, of course, limited by the anatomy of the cetacean which makes internal organs difficult to reach and making it, for example, impossible to palpate the lymph nodes. Auscultation of the heart may not be feasible and may only be possible in smaller cetaceans in which case the thorax and abdomen should also be examined. The stethoscope should be wrapped in a plastic bag.

The blowhole should also be examined, and this can be done using a torch. The rapid movements connected to opening and closing the blowhole during breathing make it difficult to make a thorough examination and it is wise to photograph or film the orifice during normal breathing to permit a more complete examination. Note any pathological discharge coming from the blowhole and, eventually, sample it as reported below.

e. Collecting Blood 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.

An 18-21 gauge needle about 2.5 to 9 cm long is recommended in these cases depending on the size of the animal. Blood samples can be taken at sites linked to the large central ateriovenous complex destined to thermoregulation on the dorsal fin and the central veins of the lobes of the caudal fin. The pectoral fins and the caudal peduncle have also been used for this purpose, but there is greater risk of thrombophlebitis if samples are collected repeatedly in those areas. The area should be disinfected with alcohol and betadine at least three times before the

needle is inserted as these procedures can easily lead to secondary hyatrogenic infections with formation of abscesses where the needle is inserted.

Ten ml of blood should be collected without coagulant, 10 ml with heparin, and 5 with ethylenediaminetetraacetic acid (EDTA) or whatever amount possible depending upon the species and the situation. It could be useful also to prepare a slide for microscopic observation to be stained later. If the unit has a commercial kit at its disposal, it is possible to carry out blood analyses rapidly or laboratory instrumentation can be used for a portable hematology-biochemistry workup.

If the blood samples are to be kept for retrospective investigations they should be centrifuged, and the sample collected without coagulant should be separated before sending it off using a courier service. It is wise to have simple blood tubes to separate the blood components. Samples sent to the laboratory must be labeled with the exemplar's identification number and clinical and life history information.

f. Collection from the Blowhole

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. It is important to clean and disinfect the blowhole maintaing it out of the water to avoid any contamination. 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. If lung diseases are suspected, a bronchoscopy and a bronchoalveolar lavage should be carried out, which must however be conducted under sedation.

g. Other samples

Other samples that should be collected are those of urine, feces and milk. In the case of both males and females a urine sample could be taken using catheterization, but take care because this procedure is very stressful for the animal. Using appropriate catheters feces should be collected from the rectum until the descending colon. The quantity collected should be sufficient to carry out culture tests and cytological examinations. If that procedure is unsuccessful, the next step is washing with saline solution. Milk can be collected in lactating females after massaging the external area and using a catheter syringe (a procedure described in detail in reference texts). It is important to remember that all procedures must be carried out after the operator has washed his/her hands to avoid transmitting pathogens to the exemplar.

7.2.4 Triage

Before deciding on the measures to be taken with regard to a live cetacean stranded on the shore, various elements and factors including the availability of means of transportation, weather conditions, features of the stranding site, and the conditions of the animal itself must be taken into consideration. International guidelines and conventions, national regulations and numerous organizations recommend that all efforts be directed to releasing the animal rather than attempting prolonged rehabilitation which can prove to 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. The safety of the persons involved in the rescue operations must in any case be safeguarded. Here below are key factors needing to be considered.

a. Environmental conditions

During the preliminary phase, those in charge must prepare to work in safe conditions in accordance with national/international guidelines and with adequate logistic and instrumental support from volunteer organizations and law enforcement agencies. If safety conditions cannot be guaranteed as outlined by the protocols, the animal has only a limited number of opportunities available. For many stranded cetaceans a rapid, well organized release is de facto the only alternative to euthanasia or to natural death.

b. The animal's condition

It is important to know how long the exemplar has been stranded, the species involved, and the subject's physiological features, as all these details may influence the outcome of rescue attempts. Knowledge about these parameters may help qualified experts to select the animals that have the greatest chances of successfully being recuperated. In cases of mass strandings, priority should be given to the animals with the greatest chances of being successfully returned to the ocean.

Generally speaking, sub-adult cetaceans which are independent of their mothers and young adults of small dimensions are good candidates in that they are easy to move and to transport, respond well to diagnostic and therapeutic procedures, and are easily managed. Coastline species certainly have more chances with respect to pelagic ones (Common Dolphins – *Delphinus delphi*; Striped Dolphins – *Stenella coeruleoalba*). A disoriented, healthy animal which became stranded for social reasons in a mass event has a better prognosis with respect to a sick, debilitated one. Dependent calves and orphans are not the best candidates for being returned to the sea. In view of their weight and anatomy, after a long period on the beach these cetaceans show alterations in cardiovascular

functions which may lead to hypoxia or ischemia and ultimately to organ failure.

In cases of abnormal or mass strandings, rescuers should use even greater caution in releasing single individuals to avoid future strandings of the same subject or to avoid transmitting infective agents to wild animals possibly responsible for the event. The following aspects are important to decide the proper course to follow.

c. Medical Triage

The medical conditions of the exemplar must be verified by the unit's veterinarian. The criteria and the modality of the evaluation are explained exhaustively in reference texts. Here below are the principal parameters to consider during clinical examinations and the triage process.

i) Malnutrition and Dehydration

Examining the muscles behind the blowhole and the dorsal fin, which are well rounded in a healthy exemplar, can be the easiest way to assess the animal's physical condition. A severe depression in the back behind the blowhole has been found to be associated with severe emaciation in some exemplars . It is important to check the mouth for teeth problems or pathological processes in the oral cavity which may be indicative of debilitated health conditions.

In view of a series of factors listed below, tropism of the muscular masses is nonetheless a complicated factor and cannot be evaluated simply on the basis of external body conditions:

- it is more complicated to evaluate this aspect when the exemplar is out of water than in the water particularly in large animals due to the compression exercised by the body mass which lifts up the epiaxial muscles;
- the muscle mass can vary from species to species also depending on the time of year;
- animals in good body condition can, nonetheless, be unhealthy as they can be affected by acute pathologies or traumas
- All animals with cachexia are not qualified for being released.

Stranded animals are often dehydrated. This is difficult to verify but can be detected by a spongy consistency or by the loss of tone to pressure on the animal's side.

ii) Wounds and Traumas

Superficial trauma occurring during the stranding may not be significant despite heavy bleeding. These wounds are usually abrasions on the beak, melon, flippers and/or tail fluke. Obvious wounds penetrating deep into the muscle layer or exposing bone will affect the animal's prognosis, as

will persistent flexion of the trunk, which may be associated with muscle damage or spinal injuries. Other injuries may be important but difficult to detect, such as fractures and dislocations of the pectoral flippers or muscle damage that may have taken place during or after stranding.

iii) Deterioration in skin conditions

This occurs as soon as cetaceans leave the water as the skin dries out quickly and begins to wrinkle and eventually to peel, a process that is all the more rapid on sunny and/or windy days. Excessive skin loss is a negative sign, as it is associated with fluid loss and a risk of secondary infection. If the process appears extensive throughout the body, the animal should not be released.

iv) Reflexes and muscle tone

Assessment of key reflexes and muscle tone can provide an indication of the level of consciousness in animals showing little movement, vocalization, etc.. The aspects that should be assessed include:

- Palpebral reflex - the eye should blink when the corner of the eye is touched;
- Pupillary reflex - the pupil should become smaller for at least 20 seconds when it is exposed to direct sources of light;
- Response to threat reflex: the eyes should close and blink if a hand is suddenly moved towards an open eye;
- Oculvestibular reflex: when the animal is rocked gently from side to side there should be a compensatory movement of the eye bulb;
- Blowhole reflex – which is normally held closed should tighten if its edge is touched;
- Jaw tone – the animal should resist attempts to open its mouth;
- Tongue and flipper tone - gentle tugging on the tongue or flipper should be met with strong resistance;
- Corneal reflex – if a small amount of clean, natural temperature water is sprayed in an open eye, the eye should close. This operation should be carried out only in those cases there is no response to any of the other reflexes

v) Hemorrhage of the orifices

Loss of blood from the blowhole, the mouth and/or anus are indications of an unfavorable prognosis if noted in the absence of superficial wounds occurring during the stranding

vi) Breathing abnormalities

Breathing difficulties may become evident in cases of respiratory pathologies and stress. The cetacean's breathing rate is species specific

and can be evaluated watching the opening and closing of the blowhole which is covered by a muscular flap of tissue. In its relaxed state the animal keeps the blowhole shut but opens it when it breathes. Inhaling and exhaling are in one explosive action, and immediately afterwards the flap relaxes and the blowhole is closed.

The animal's rate of breathing should be closely monitored as this is useful indicator of a cetacean's state of health. An increase in the breathing rate indicates that the animal is stressed. Unless considered essential for veterinary purposes, any activity which causes an increase in the animal's breathing rate should be stopped immediately. A useful rule of thumb for all dolphins is: two to five breaths per minute is normal, above six per minute is indicative of the animal being stressed or compromised in some way, and above ten breaths per minute indicates severe stress. Other signs indicative of shock are a delay of more than 4 seconds between expiration and inspiration (in small cetaceans) and prolonged apnea. Breathing pathologies can cause breathing variations such as shallow breathing, bad breath, or drainage at the blowhole. The animal may cough but this sound must be differentiated from others produced by some dolphins when in stressful conditions.

vii) Temperature Alterations

It is to be remembered that cetaceans are used to swimming in cold ocean waters and that heat loss in water is much quicker than in the air at the same temperature. The circulatory system of cetaceans is able to make adjustments to conserve or dissipate body heat and to maintain the animal's body temperature. When they are stranded, cetaceans risk hypothermia. Emaciated, weak animals sometimes can show hypothermia, particularly in cold water. An appropriate thermometer should be positioned at least 30 cm (20 cm in animals under 50 kg) into the rectum. Only experienced veterinarians should carry out this operation. A normal temperature is between 36 and 37.5°. If the temperature is over 40° this is indicative of a critical situation. When its temperature is over 42° the animal is considered terminal. If the temperature begins to go down this may be a sign of recovery linked to efforts to keep the animal warm.

7.3 Release categories

The decision to release a marine mammal is based on numerous factors including health and behavioral considerations, its ecological status and, if the animal is deemed releasable, on a well-thought-out release plan. All information concerning the animal's life history, medical records and

observations made during the stranding event must be reviewed carefully and stored. Once all variables have been reviewed and analyzed, a decision must be made and the exemplar in question declared releasable, potentially releasable, or not releasable. Appropriate government and inter-government agencies should be involved in this review process. The destiny of a stranded animal that has already undergone a clinical examination depends not only on medical considerations but also on ethological and logistic factors and on national regulations governing wild fauna. Generally speaking, European Conventions and the regulations and guidelines of the member states of the Agreement of the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic Area (ACCOBAMS) recommend that all measures should be taken in view of releasing a stranded exemplar. During the review process, reference should also be made to and decisions conform with the national and European/international regulations concerning competent agencies, use of drugs, the facilities where exemplars are held and recuperated and, if necessary, the procedure for euthanasia.

7.3.1 Life History Evaluation

The commission examining the question must take into consideration all of the data and parameters outlined in the life history including information collected during the rehabilitation period. On the basis of these data, the commission may decide to modify how the case is being managed and the final decision concerning release. Obviously measures must be taken during the rehabilitation stage to prevent disease transmission to other animals or to human caretakers and all of these data must be transmitted to other facilities if the animal is to be sent to another structure.

Life history data regarding the stranded animal can thus define how the animal is to be managed during rehabilitation and the release plan. Important information that must be considered regards:

a) precedent strandings: cetaceans which have already been stranded, were released, and found stranded again are to be considered releasable on condition. These animals must be carefully evaluated and further diagnostic examinations must be carried out. The other alternative is giving them a not releasable classification and transferring them to a temporary facility or deciding on euthanasia.

b) mother-child couple. Strandings of this kind could be due to injury to the mother, to the calf, or to both. If the calf dies or is euthanized, the mother can potentially be considered releasable and can be helped to rejoin her group or others of her species. If it is the mother which dies, the calf probably should not be released as it is still dependent and not able to feed on it own.

c) involvement in an epidemic event with other marine mammals or an atypical epidemic stranding. If the stranding is correlated logistically or time-wise to an area being affected by polluting substances with signs of dead fish, algal growth, etc., the cetacean can be considered releasable on condition. The agencies can request other documentation, tests, and post-release monitoring.

d) stranding site. Areas linked to strandings or to release operations which require careful evaluation are those characterized by anthropic activities or dangerous environmental conditions;

e) exposition to or wounded by other wild or domesticated animals: even if there are no evident clinical signs, cetaceans with a history of being exposed to domesticated or wild animals during rehabilitation may have acquired pathogens that may be potentially dangerous to the wild population. In this case, the animal is potentially releasable but consultation with authorized agencies is required.

f) transfer to another site. The animal may have been exposed to various pathogens during the rehabilitation stage. All documents, tests, documents linked to that period must be requested.

g) signs of interaction with humans or forensic evaluations;

h) part of a mass stranding. Behavioral aspects often play a fundamental role during mass strandings and healthy animals may require aid for immediate release. Some exemplars can be sent for rehabilitation and then released on condition in the case that some pathological signs were found in dead animals at the stranding site during the event.

7.3.2 Evaluation of biological and developmental aspects

A neonate cetacean goes from a state of total dependence on its mother to a state of partial dependence when it learns to capture fish and squid. The age that occurs is variable depending on considerations such as state of health and environmental factors. A cetacean can be considered releasable when it has reached an adequate state of development and of independence as far as feeding is concerned or it can be released together with its mother. Lactating animals cannot be released without their mothers, and neonates or very young animals in the weaning stage which are stranded alone or whose mothers have died should not be considered releasable. Some young exemplars with some knowledge of feeding in the wild can be considered releasable after they have been carefully evaluated and a well-thought-out release plan has been prepared. An emergency release may be attempted when the head veterinarian considers it possible because there are others of the species in the area or in view. The young cetacean's ability to hunt and to feed independently from its mother is critical for it to be returned to the wild. Being well nourished and the presence of

adequate fat reserves are important considerations. It is important that there are experts in ethological aspects and life history of cetaceans in the examining commission as they will be able to answer some of the important questions which needed to be answer and which are: does the species to which the exemplar belongs live in social groups or as solitary individuals in the wild; has the exemplar developed a predatory capacity adequate to be able to survive in the wild; has the exemplar developed the social skills necessary to become integrated successfully in wild groups; what are the migration routes and the displacement distances that are usual for this species; does the exemplar have the capacity to recognize potential predators? The answers to these questions will determine the chances that the animal will have to successfully make it on its own if it is released.

The reproductive status of the exemplar cannot be considered the only criteria for classification unless a female is stranded with her neonate or she gives birth during rehabilitation. A single pregnant female should be released as soon as possible and as soon as the medical and behavioral examinations have been completed. A mother-child couple should always be considered conditionally releasable. Time spent in a rehabilitation facility should be kept as short as possible in this case.

Finally, geriatric patients should potentially be considered releasable. The conditions of these exemplars should in any case be analyzed by an evaluating commission as it is difficult to establish the age of an adult exemplar. Suppositions can be made about age on the basis of physical characteristics. These animals may have subclinical pathological conditions that may have contributed to the stranding or their condition may no longer be compatible with life in the wild both from an ecological as well as behavioral point of view.

7.3.3 Evaluation of ecological and behavioral aspects

The initial evaluation of the exemplar's behavior is an important finding that should be taken into due consideration. At this stage the exemplar's ecological state, and its medical and behavior data can be used to extrapolate hypotheses about how it could behave in the wild under normal ecological pressures. This analysis should go beyond the normal evaluation carried out in an artificial environment as it should consider the subject as already in the wild in normal life situations. This evaluation is based on a multidisciplinary approach involving experts who must work together in a complementary manner to evaluate the exemplar's chances of surviving in the wild. Experts who know the species' habitat, social behavior pattern, feeding habits, potential predators and risks relative to humans can make an articulated hypotheses on the exemplar's possibilities. A basic conditioning for wild cetaceans may facilitate the

medical procedures and their management during rehabilitation, but it is extremely important to limit contact with human beings. The training can, moreover, be useful to check its feeding and its position within the group besides reducing stress during the physical examination or while biological material is being collected. Conditioning should last for a limited amount of time during rehabilitation and be eliminated well before release in order to eliminate any association between a food prize and humans.

In order for animals to be considered eligible for release, some basic criteria such as the following must exist: being able to dive, to swim, and to breathe normally, the absence of abnormal behavior or visual/ hearing dysfunctions which could compromise the animal's chances for survival in a wild environment or which may be indicative of a relevant pathology. The rehabilitated animal must be able to recognize, capture, and consume prey before it is released. As a behavior alteration can be indicative of a pathological condition, this evaluation must be made by an experienced veterinarian. The ethological evaluation must be carried by an expert who is able to recognize if there is a dependent state or attraction towards humans and to analyze the exemplar's activities/ behavior in a natural environment; the following should be taken into considerations when release is being decided.

a) Breathing, swimming and diving - any abnormal behavior during breathing, which can be evaluated by counting the breaths taken during a five minute period, should be noted. Evaluation of swimming and diving should confirm that the cetacean can move about efficaciously and is without any abnormalities such as impairment in diving, asymmetrical motor pattern or other potentially debilitating conditions. Cetaceans may not be able to demonstrate the full range of their motor abilities in small pools and, if possible, a larger pool should be used. Cetaceans which continue to show abnormalities in breathing, swimming or diving should be considered releasable on condition or not releasable and the appropriate agency should be consulted. The exemplar's organic condition after, if needed, collateral investigations have been carried out should be reported so the release status of the animal can be considered.

b) Abnormal behavior. The initial evaluation should include confirmation that the exemplar does not present swimming abnormalities. These abnormalities include but are not limited to regurgitation, pushing with the head, postural abnormalities such as buckling or repeated bending, reduced movement, alterations in swimming or breathing, excessive interest in interaction with humans. These animals may have an underlying pathology or a permanent lesion or behavior deviations that may make survival in the wild impossible. These animals should be considered potentially releasable or not releasable and the case should

be discussed with the authorized agencies.

c) Auditory and visual skills. The ecological and ethological evaluation of the exemplar should, moreover, include visual and auditory skills. Any auditory dysfunction with regard to the production and reception of typical sounds and signals present in nature may reflect a pathology or a permanent lesion or a degeneration linked to senility. An auditory dysfunction should be suspected if the exemplar has difficulty localizing a prey, there are defects in echolocation, or the response to new sounds is minimal. Hydrophones should be used to record and analyze vocalizations in order to evaluate the normality of these emissions. As these dysfunctions would limit the exemplar's chances of survival in nature, the case should be discussed with authorized agencies which may suggest that other diagnostic tests be carried out.

d) Capturing prey. The cetacean that has been rehabilitated must be able to nourish itself (capable of hunting and capturing prey) before it is released. Ingesting solid food must be verified as a part of the medical evaluation (it must be verified that the animal is able to swallow and has no obstruction of the gastrointestinal tract). Live prey which is part of the exemplar's natural diet in the wild should be used when this aspect is being assessed; other species may be used for this test although it is to be remembered that many cetaceans do not consume animals that are not considered prey. When an exemplar belonging to a social species is being evaluated, hunting behavior within the social group should also be considered. Junior or young adult cetaceans thought to have had a limited experience in hunting before the stranding event should be evaluated with particular care. Generally speaking, in fact, this aspect is learned from others of the same species and thus at the time of the stranding these individuals may not have been capable of maintaining themselves alone. Animals with amputated extremities that are released may be impaired in their capacity to hunt but there are reports of animals belonging to this category living successfully in the wild.

e) Avoiding predators. Evaluating the ability to avoid predators is not an easy task. Only indirect proof can be collected. For example evidence of being attacked by a shark could indicate that the animal was unable to avoid the predator. This is particularly important in young specimens just separated from their mothers.

f) Social factors. The cetacean's survival may depend to a large extent on its position in the social organization and its relation to other members of the species. Members of a species involved in a mass stranding should not be rehabilitated alone or in unnatural groups. The composition of these groups should be considered when these animals are being recuperated. It is not to be taken for granted that two cetaceans captured together are part of a functioning social unit or that

any two subjects can constitute one. It is important to understand group dynamics and behavior patterns within that particular social species. Cetaceans belonging to solitary species do not necessarily have to be released together. Possible social implications linked to the strandings need to be taken into consideration. Alterations of a medical character linked to teeth problems, amputation of extremities, abnormal sound perception or production are all indirectly involved with socialization and thus require special consideration.

7.3.4 Medical Evaluation

Even if this manual focuses on evaluating and preparing rehabilitated cetacean for release, it is also concerned with the entire rehabilitation period and in particular the animal's state of health before it is released. The medical evaluation includes information relative to diagnostic tests, to treatments which have been prescribed by the veterinary and to test and treatment results. The veterinary should also carry out a physical examination both at the time of the stranding and before determining the conditions for release. He/she should review the animal's life history including how the stranding took place, the results of the diagnostic tests, the medical records and the data collected by the rehabilitation facility.

The aim behind many of these efforts is that of uncovering diseases that can affect marine mammal populations living in the wild or zoonotic pathologies that can create preoccupation concerning public health or the safety of persons involved in rescue operations. Rehabilitation facilities may harbor pathogens which the animal might have never encountered in the wild or new strains with a high level of antibiotic resistance. In the case of infections caused by this kind of pathogen, animals that become ill could become carriers transmitting these pathogens to conspecifics or to human operators. These pathogens and in particular viruses, bacterium and some protozoas can grow and divide quickly in their new hosts and become susceptible to selective pressure causing them to evolve modifying their transmission rates, virulence and pathogenicity. These agents can thus become highly pathogenic and be transmitted to new individuals in the native species. The veterinarian should use his/her entire range of diagnostic instruments to evaluate the exemplar's state of health as soon as possible. Blood and urine electrolytes, complete biochemical and hematological parameters including hematocrit, blood urea nitrogen (BUN), creatinine, enzymes, serology, microbiological cultures (bacterium and fungi from the blowhole, swabs taken at the rectum and of any lesions), cytology, urine and feces should all be analyzed. Polymerase chain reaction (PCR) should be used to evaluate the presence of pathogens and microarrays and toxicological techniques can also be employed. Diagnostic imaging techniques such as X-rays,

ultrasound, bronchoscopy may be necessary. Tests to uncover emerging diseases may be required as a part of surveillance measures to identify potential epidemic risks or to establish evolving disease patterns. Other investigations, as for example tests measuring algal biotoxins, may be required if these animals are part of an atypical mass stranding.

The veterinarian should interpret the biochemical and hematological tests and the other investigations in the light of the physical examination, the animal's age and reproductive status and other life history factors such as how the stranding took place, recent environmental events, pathological problems characterizing resident species. It should be better to conclude antibiotic treatments should two weeks before the animal is released in view of the pharmacokinetics of the active ingredients to avoid spreading residues in the ocean environment which could increase the problem of antibiotic resistance.

7.3.5 Selecting the site for the cetacean to be released

The rehabilitated cetacean should ideally be released into its natural environment and within its genetic and social unit. It is important to free coastal animals such as Bottlenose Dolphins along their particular coastline track; this is less important for animals with a wider range of action such as Pilot whales. Freeing a mammal in an area that it knows increases its chances of survival as it implies being able to hunt and to enter into social interaction successfully. If possible, the period of year that the animal was stranded and the migration routes utilized by its social unit should also be taken into consideration.

In many cases these are not known. A photo-identification may be able to provide useful information about the typical area frequented by that exemplar's unit. When these data are not known, the exemplar should be liberated in a site near to where it was stranded which is regularly frequented by conspecifics, if possible of the same genetic stock. Pelagic animals should, likewise, be released in open waters where conspecifics are known to be found. With regard to mass strandings, it would be important to maintain the same social unit which probably comprises a unique group whose members were probably stranded together as a result of social factors. As many behaviors are learned, young mammals should be left with adults or in the presence of conspecifics and dependent calves should be left with their mothers. It is, of course, important to verify if the habitat has become degraded, if food is available, or if there are obvious dangers before releasing the animals. The animals should not be released in anthropized areas characterized by high concentrations of fishing or maritime activities.

7.3.6 Individual Identification

Released dolphins should have at several forms of identifications including a photo-identification, subcutaneous microchip, a marking on the dorsal fin, and freeze branding (used in the United States but not a validated method in Italy). Photo-identification for delphinids should include a description of the exemplar's characteristics such as its dorsal fin, body, head, pectoral fins and tail. All other marking systems of subjects of different species need to be evaluated with the competent authorities.

7.3.7 Post-release monitoring of cetaceans

There are few data available regarding released cetaceans. Monitoring these animals after their release could provide information which could prove useful to establish new criteria for rehabilitation and release. This activity should be planned in conjunction and agreement with appropriate authorities. Monitoring techniques include visibly observing the released animal from land, a vessel, or an airplane or monitoring it by radio or satellite. Monitoring a pelagic species is, of course, a rather costly, demanding activity. If resources are scanty, this should be considered at the time of the stranding and should affect the final decision.

7.3.8 Releasable cetaceans

A cetacean is suitable for release if its health and behavior states are optimal and if there is a release plan. The following aspects of the exemplar must be classified suitable if it is to be considered releasable:

a. Life History

The animal has not been stranded more than once over a brief period of time and was not part of a mass death event in which pathological conditions involving environmental risks such as algal boom, toxic waste, acoustic pollution, and/or infective pathologies are suspected

b. Stage of development/Life History

· The animal has reached an adequate stage of development and is old enough to be independent from a feeding and social point of view;

· The exemplar is not a female with neonate;

· The exemplar is not a geriatric patient and does not present conditions linked to an elderly age;

· The cetacean has not been exposed to animals kept in captivity or to domesticated animals during its rehabilitation.

c. Behavioral evaluations

· The cetacean breathes normally, and swims and dives in an optimal manner;

· The exemplar does not show behavioral alterations or visual/auditory

defects;

· The exemplar shows adequate capacity to feed;

·If the stranding took place due to predators, incapacity to procure food or direct interaction with conspecifics needs to be evaluated.

d. Clinical Alterations

· The cetacean has been declared fit by the attending veterinarian (who feels that the animal will probably survive release and poses no risk to wild populations);

· Clinical examinations were carried out during rehabilitation;

· Laboratory tests including hematological and biochemical diagnostic tests such as Na, K, Cl, Ca, P, Fe, bicarbonate, alkaline phosphatase, ALT, AST, GGT, BUN creatinine, uric acid, CPK were carried out; a blood sample was taken; a microbiological analysis was carried out to detect aerobic bacteria from swabs of the rectum, the blowhole and any lesions;

7.3.9 Potentially releasable cetacean

If the cetacean does not meet the requirements of an immediate release, it can be released once it is deemed fit by the attending veterinarian or by the evaluation commission. The evaluation and the decision to release the exemplar must take place within a reasonable amount of time to avoid conditioning it to humans or taking an undomesticated being out of its natural environment in view of the fact that most national/international norms and regulations recommend that every effort should be taken with the intention of releasing the animal into the wild as soon as possible. If it is impossible to release the exemplar or another stranding may be possible, the decision may be made to carry out further rehabilitation, to release it to a controlled basin, or euthanasia.

a. Life History

· The cetacean is stranded in association with weather and/or geographic conditions linked to an abnormal mass stranding event;

· The cetacean is stranded in association with alarming environmental events such as abnormal algal bloom, waste discharge, acoustic pollution;

· The animal was stranded once before or on more than one occasion;

· It is a single stranding of an exemplar of a gregarious species.

b. Life History development

· The cetacean is dependent from a nutritional point of view, as far as it is known from its life history or recent observations with regard to its capacity to capture food,

· It has just recently been weaned;

· It is a female with a neonate;

· It is a compromised geriatric patient with evident problems linked to its age.

c. Behavioral evaluations

· Aberrant behavior including abnormal breathing, swimming and/or diving patterns, visual and auditory dysfunctions have been noted;

· It is difficult to establish its capacity to hunt autonomously or it does not demonstrate to possess full autonomy;

· The animal appears to be markedly conditioned by age or health related conditions;

· The lesions caused by predators should probably be considered secondary to other pathological conditions;

· The cetacean belongs to a social species and was stranded as a result of aggression on the part of conspecifics.

d. Clinical evaluation

The attending veterinarian establishes that the animal's state of health is uncertain as far as a successful release outcome is concerned. The veterinarian emits the diagnosis of "potentially releasable" after carrying out a complete physical examination and analyzing all the hematological, biochemical, microbiological investigations, including ultrasound or radiographic examinations and any other assessments deemed necessary. The animal's response to any treatments prescribed should also be recorded.

7.3.10 Not releasable cetacean

The animal's condition are far from those considered minimal for release and it does not seem to have a reasonable chance to successfully survive if freed or could be a health risk for other marine animals.

a. Life History

· The exemplar has become accustomed to conditions in captivity and counterconditioning techniques have proven to be unsuccessful;

· The cetacean has already been stranded once or more times;

b. Development/Life History

· The cetacean is socially dependent as far as feeding is concerned;

· The cetacean is a geriatric patient and shows behavioral and/or medical abnormalities.

c. Behavioral alterations

· Abnormalities in breathing, swimming diving and or in other functions which may compromise the exemplar's chances of survival or may be determined by a pathology that can be dangerous to free living cetaceans are evident;

· Visual and auditory alterations which can compromise life in the wild or may be determined by a pathology that can be dangerous to free living cetaceans are present;

· It is not capable of capturing prey on its own;

· It is not able to avoid predators.

d. Clinical evaluation

The attending veterinarian establishes that the health conditions of the cetacean preclude releasing it into the wild. In these cases the clinical conditions of the exemplar do not guarantee basic functions that would ensure an adequate quality of life in the wild or could be dangerous to free living cetaceans. The veterinarian underlines the condition in view of the physical examination and the hematological, biochemical, microbiological examinations including if possible ultrasound or radiographic examinations and any other further assessments that were prescribed to verify the animal's state of health. The veterinarian presents his/her evaluation to the other members of the commission outlining the reasons for his/her diagnosis and proposing euthanasia because of the exemplar's scanty chances of survival in the wild and or the potential risk of compromising the health of free ranging mammals or of transmitting contagious diseases.

Conditions sustaining this decision are thus:

· Impairment of sensory systems (visual and auditory);

· Impaired motor capacity;

· Collateral tests resulting abnormal or suspicious as far as potentially transmissible diseases are concerned;

· Geriatric patients suspected of having chronic debilitating diseases making survival in the wild impossible.

7.3.11 Reporting

The veterinarian in charge of evaluating the stranded cetacean will draft a full report outlining all of the information pertaining to the physical examination and tests taken and will express an opinion that has been endorsed by the other members of the committee. That opinion, dated and signed, will be sent to the competent authorities.

7.4 Transportation

Moving a cetacean implies maneuvering it towards deeper water or loading it onto a vehicle. All of these procedures are potentially dangerous both for the animal and the persons involved and can be carried out only in the presence of expert, trained, experienced personnel.

7.4.1 Moving

It may become necessary to move an animal if it is exposed to light and/or the sun or if it is lying on rocky ground or is being pushed around by ocean waves. Small cetaceans can be moved using waterproof

tarpaulins. The animal should be prepared by positioning the pectoral fins flat against the body which is made to rotate on its side. The modality of positioning it during transportation is outlined in reference manuals. Six persons can transport an average Bottlenose Dolphin (Tursiops truncatus) on a stretcher but a large cetacean may require up to 15 persons.

If the animal it too heavy to lift, it can be pulled, but this is never to be attempted without a sheet beneath the animal. It is never to grabbed by the pectoral fins, the tail, or the dorsal fin as this could be dangerous both for the animal and the transporters. Cords to pull the animal are never to be used without protection; mammals must never be dragged over rocks or boulders but can be pulled over flat, smooth surfaces once all obstacles have been removed.

Rolling the animal over is not recommended as this could cause fractures or injury to the pectoral or dorsal fins. A crane or other equipment can be used to move large animals or to load them onto other vehicles for transport.

a. Equipment

Special stretchers that can be lined with padding and with holes for its thoracic fins are available for dolphins. Heavy equipment can be used to move large cetaceans utilizing special pontoons. If the mammal is too large to be safely moved, it should be made as comfortable as possible while awaiting the tide to refloat it.

Waterproof air mattresses (not to be inflated excessively) can be used to provide additional support under a cetacean when it is being moved over rocky/shingle beaches to reduce the level of trauma or wounds as animals are being moved to a more comfortable substrate or into water.

Made up of two inflatable parts connected by a canvas sling, pontoons are designed to aid in the attempt to refloat large cetaceans or they can be useful in supporting these animals in an upright position while beached. There are specially designed ones that can be used to lift whales weighing about two tons but they have also been used for larger mammals. They must be used only by personnel that has been trained in fitting and using them. They can be used to keep a beached animal afloat in knee-deep water or to move it to deeper water until it is finally released.

b. Safety for operators

The health and safety of all those involved in these operations is of utmost importance. Appropriate safety measures must be taken/ be provided during moving and transporting operations. All persons participating in lifting/moving operations need appropriate training.

The path to be taken must be planned carefully and the animal lifted as gently as possible. Rescue operations must be coordinated and all efforts and details, including how to manage an exemplar on a slippery beach should be planned and calculated; all boats in the area should be evacuated while the operation is being carried out. When a crane or other equipment is being used, no one should stand under or nearby the load. Operators should be provided protective clothing (preferably dry or survival suits) and should not remain in the water too long because of the risk of hypothermia.

7.4.2 Transportation

Over the past 30 years new techniques and transportation systems have been developed to satisfy the specific physiologic needs of these animals and these have markedly improved the results. Transportation can be safe and successful only if all details and appropriate technological support, which depend, of course, on the mammal to be moved, have been planned. There are, moreover, national and international laws and regulations that must be respected while transporting a marine mammal.

As it is necessary to constantly monitor breathing, posture, thermoregulation and behavior parameters during journeys between one facility and another, it is important to have qualified persons in the vehicle and an escort, if possible, could accelerate the journey.

The water in which cetaceans spend their lives furnishes support to their bodies by distributing pressure throughout, permitting them to breathe normally. Cetaceans are adapted to cooler sea temperatures and as heat dissipates more quickly (about 25 times) in water, they often overheat when they stranded. It is important then to carefully monitor the stranded cetacean's temperature.

Cetaceans in captivity should then be transported using a special tarpaulin or a stretcher and suspended in a filled with water to mimic the absence of weight in water.

Temperature control is very important: in water thermoregulation in cetaceans is maintained only through peripheral vessels in the pectoral, caudal and dorsal fins without blubber. As a result, cetaceans are unable to dissipate excessive heat when they are out of water. The animal can be assisted in a variety of ways. Suspending the animal in a container/tank filled with water makes it possible to check and to modify the temperature depending on the animal's needs. Even the loading compartment or the hold of an airplane is normally furnished with an air-conditioning system that can be regulated as needed. If possible these animals should be transported during the night thus avoiding traffic on the streets and the hottest hours of the day. They should never be moved under the direct sun which could cause burns. If there are no other alternatives wet towels

can be placed directly on the animal to prevent burns and dehydration of the skin.

Cetaceans must always be transported with its head in the direction of where it is being moved, never backwards with respect to the conveyance to avoid disorienting or confusing the animal.

The numerous transportation failures in the past on an international level have generally been caused by the lack of suitable equipment. Organizing transport of a live marine mammal entails employing qualified personnel and specialized equipment. If there are an any unforeseen events along the way an emergency plan must be ready that ensures that the animal is always in satisfactory condition. In trips using ground transportation all details concerning the route and possible problems (the presence of bridges or structures that may have height or weight limits, traffic jams, maintenance road work, weather conditions) must be well planned out.

All persons involved in transporting the cetacean must be aware of the various organizational aspects, the jobs assigned to each, and all safety measures needing to be taken. The number of persons needed depends to a large extent on the animal's dimensions but also on logistic conditions at the stranding site. Generally speaking there should be a transportation coordinator who is in responsible for organizing logistic aspects, assigning jobs, and of training and preparing the persons involved. A veterinarian experienced in working with cetaceans and capable of evaluating the health status of the animal throughout ever phase of the journey must also be present. He/she must be prepared to solve any problems linked to emergencies arising unexpectedly, must be aware of all hygienic and safety protocols that must be followed in these cases (disinfecting the vehicle once the transportation has been carried out, filling in the data sheet during the trip, stocking up on specific drugs needed and in the case of an emergency) and capable of giving clear, precise directions to technical support persons.

7.4.3 Possible complications during transportation

As being transported generates a high level of stress in these marine mammals, the following health problems have arisen during this type of situation in the past:

-Higher susceptibility to infections (especially of the respiratory system);
- Gastritis/gastric ulcers and anemia;
- Hyperthemia;
- Dehydration;
- Neurogenic shock;
- Post-transportation anorexia.

If supervision and conditions during the transportation are inadequate,

the following complications can take place:

- Muscular contractions and/or rigidity;
- Eye lesions;
- Bruises and abrasions and anemia;
- Ischemic skin necrosis;
- Asphyxiation by drowning.

These can be avoided by using the new transportation techniques which make it possible to modify the temperature and allow the animal to make some movements and thus avoiding problems of muscular rigidity at the end of the journey which would worsen the animal's state of health.

Abrasions and necrosis by compression can be avoided if a stretcher made out of non- abrasive material is used and the animal is positioned correctly above it in the loading compartment.

If the trip is quick and comfortable, assisted by qualified personnel and well planned out, antibiotics and steroids used to prevent respiratory infections secondary to transportation can be avoided.

A careful examination of the animal's state of health including not only a physical examination but also hematological tests and urine analysis must be made and the animal's behavior must be observed. The animal should be transported only if judged able to sustain the operation and the veterinarian is convinced that it will not worsen its health conditions.

Animals should be kept fasting for 24 hours to minimize the amount of feces/urine/vomit the animal could emit in the loading compartment. If this is not possible, containers with spare water as well as empty containers in which contaminated material can be spilled must be available.

7.4.4 Transport Procedure

a. Positioning on stretchers or transport stretchers.

At the stranding site or in the rehabilitation pool, the cetacean should be positioned on a stretcher or other equipment that is large enough for the subject in question made of soft non abrasive material normally in a flexible nylon or in a resistant washable cotton material that can easily be disinfected and which does not deteriorate when in contact with salt water, chlorine or other disinfectants. These stretchers can be lined with appropriate material (sheepskin, or suede leather for example) to make them even more comfortable especially if the trip is long and they should be made to measure the animal's size (its length, the diameter of the thorax including the dorsal pin, the distance between the pectoral fins and between the attachment points of the pectoral fins). This will ensure that the animal is comfortable in the stretcher and that its weight will be evenly distributed. There should be at least two opening for the pectoral fins so that the animal can extend them freely in a natural position. The

borders of the opening should be carefully examined as these might cause chafing or compression injuries at the attachment point of the fins (especially at the axillary region). The caudal fin should be left free to move so the stretcher should be able to support the body up to the peduncle. It is important throughout the transportation operations that lesions of the skin, which is highly vascularized and extremely delicate in these animals, are not provoked. The stretcher should be equipped with rings or holes in which metal/aluminum bars are threaded for lifting. These bars must be extremely resistant and able to sustain even the weight of an animal that has suddenly becomes agitated. The edges of the bars must be blunted and smooth so the animal and the operators do not become injured.

Strong cords, chains or belts, lifting straps and tow-ropes so that the bars can be connected to a forklift truck or other kinds of lifting systems must be organized. Two tie-rods (anterior and posterior) are necessary for a small cetacean while a large animal will need at least 4 or as many as 12 sometimes with a double safety system and intermediate tie rods.

Generally a crane or a forklift truck is necessary to lift a heavy cetacean safely not only because of the animal's weight but also because it could make sudden movements during the operation. All material and the number of persons needed during these operations must be well thought out and planned and discussed with the technicians operating the crane or forklift truck. Hydraulic cranes are preferable with respect to mechanic ones as they are easier to operate and their movements tend to be gentler.

For safety reasons, the animal should be lifted only when the loading procedure is ready.

b. Wet Transport

Animals can be transported using full water transport, i.e. in tanks within which they are partially or totally floated in water (generally water covers 2/3 of the animal's body) suspended on a sling with cell foam cushioning and support underneath and on either side. Reinforced fiberglass or plywood holding tanks with metal bars lined with sponge or polyvinyl chloride (PVC) now exist. There are openings for the pectoral fins so these can remain free to move about but without touching the extremities of the tank. Wheels can be placed on the bottom of the tank and removed when the vehicle is moving at which time the tank needs to be anchored. There are three possible ways to lower the stretcher into the tank and each basically adapts its length and width to the needs of each case. These systems help to make transportation more comfortable and make it possible to intervene if it becomes necessary to treat the animal during the journey. Once the animal has been positioned, water

should be added that covers most of the animal. If possible normal water should be used as salt water could potentially ruin parts of the vehicle. If the decision is made to use salt water, it is best to use sea water or that from pools in which the cetacean was given hospitality. Once immersed, the animal should be placed on its abdomen and not suspended on the stretcher which serves only to limit its movements or to lift it in case of emergencies or during the loading and unloading processes.

Placing the cetacean in water ensures that the animal is kept moist and its temperature cool and being in a floating condition means that it is able to breathe freely and without effort throughout the journey. The water temperature should be kept as close as possible to its source or to keep it even lower so it falls in the animal's normal comfort range (about 15°) At times ice must be added to the transport water, particularly when it is very warm. Ideally the temperature of the vehicle should be between 14-20°C.

c. Dry Transport

Transport over land poses much stress and should be undertaken only with proper preparation; is in any case appropriate only for short journeys or in cases of emergencies. Transporting animals that are longer than 5 meters is unwise because of the physiological stress that it causes due to its weight which literally crushes it and impedes it from breathing. Properly padded stretchers and cooling to avoid hyperthermia need to be prepared and moist, heavy foam rubber or the equivalent should be used in the bottom of the transport vehicle with care being taken that the pectoral fins are flat against the sides of the animal. Before positioning the animal on a sling it should be rubbed with ointment to protect the skin from drying and from rubbing against the sling. The ointment should be made of equal parts of vaseline and lanolin to which a lotion which promotes healing such as Mitosyl (vitamin A and D with sinc oxide) has been mixed. The lotion should not be applied to the surface of the fins which must be kept humid and cool as this would impede the animal from dissipating heat. As the risk of hyperthermia increases during transport, the animal must be kept covered in moist towels and the blowholes and other exposed areas kept protected from drying out. Animals must be supervised throughout the journey and misters and sprayers should be used to keep them moist and ice may be taken to pack around the animal.

d. Overland transportation

The type of vehicle that is used depends on the season, its availability and the species needing to be transported. The tank should be positioned as close as possible to the cab as that is the most stable area and least

subject to oscillations. The load should be distributed symmetrically along the longitudinal axis. It is of utmost importance that exhaust fumes do not enter the cab contaminating the air. The most practical vehicles to load and unload are those with side openings, furnished with refrigerator cars and anchoring systems with washable surfaces that are easy to disinfect and equipped with a lighting system to facilitate the loading unloading operations and to monitor the animal during the journey. It is better to travel during night hours. During rest stops the vehicle should be positioned in the shade and direct sunlight should be avoided. If more than one vehicle is involved in the transportation operation, cell phones should always be turned on and functioning in case of emergencies, breakdowns, or loss of water from the tanks. The truck should never go faster than 80 km an hour and the diver should avoid abruptly changing directions or taking steep roads.

e. Monitoring during transportation

Extremely important aspects which must be carefully considered are the following:

- The temperature of the animal, the melon, the fins and the rectal temperature (only if necessary), this can be done simply by placing the palm of the hand on the areas listed and if there are any doubts a rectal temperature should be taken;
- Hydration of the skin in exposed areas;
- Check areas exposed to friction and chafing such as the attachment points of the fins, the genital area, the peduncle, the throat; these should be checked frequently to avoid skin lesions;
- Dorsal fin: to monitor the animal's temperature; when it is hot it tends to curve backwards, it should always be kept cool;
- The quality of water in the tank;
- Signs of pain, discomfort, the animal's behavior, appetite;
- Breathing: frequency, quality and presence of secretions;
- The water level, the stretcher's state of tension; check that water is not entering the blowhole and that the stretcher is not too tense;
- Eyes: check that they are not rubbing against the stretcher, that the secretion is normal, that they are not kept closed because of pain;
- Pectoral fins: check that they are free and covered with water;
- Air temperature. Avoid brusque temperature changes;
- Water temperature. Avoid brusque temperature changes by adding too much ice;
- Animal's position. The animal should be repositioned frequently and should be massaged in the areas where the pressure is greatest to favor blood circulation and to avoid ischemia, preventing the animal from touching the tank walls;

- Vocalizations. If emitted with a certain frequency they may indicate pain or discomfort;
- How the tank is fastened. Check if the tank is anchored well verifying the tension of the belts.

f. Unloading the animal at its destination

Once the animal has arrived at its destination the unloading operations should take place as quickly as possible under the direction of the transportation coordinator as near as possible to the pool where it is to be moved and where there is ample maneuvering space. Before lifting the animal, the belts or cords should be positioned for lifting on the bars. The lifting bars should be kept at a constant distance to prevent them from opening if the animal becomes agitated or if they close crushing the animal, in particular the dorsal fin. At times it is necessary to empty the tanks to gain access to the animal. If there are no pumps, which in any case are not the best solution because they are quite noisy, the water can be emptied using a rubber hose with a negative pressure on the other end. It is unwise to build transportation tanks without holes for emptying water.

The basin where the cetacean will be housed should not be very deep and there should be operators and equipment ready to intervene in case of necessity (divers, nets, oxygen etc.). Before immersing the animal in water it should be supported on the board of the pool to check if it is responding to stimuli. After a long journey it is also wise to carry out some stretching exercises, lifting repeatedly its caudal fin and massaging some areas before placing it in water. It is important to wait for the animal to take a few breaths and then the cranial portion can be slowly lowered, accompanying it, into the water.

The animal must be monitored for at least 24 hours; its breathing frequency and behavior should be checked and it should be offered food. Blood should be collected and a clinical examination should be carried out after a few days' time.

All of the equipment utilized and the internal parts of the means of transportation should be washed and disinfected.

7.5 Euthanasia

Main euthanasic approach reported in the literature are described here below in the attempt to provide operators who find themselves facing this difficult decision with a wide overview. It is possible, however, that a situation not described may arise and the references at the end may provide further information

Here below then is an outline of the major euthanizing procedures utilized and considered applicable for the animal's best interest.

7.5.1 Firearm

A skilled shooter is able to use a firearm to cause an instantaneous or nearly instantaneous unconsciousness and a rapid, ethical death in small animals. In some cases a gunshot is the only practical method to carry out euthanasia. It should be used however only by highly qualified personnel trained in utilizing firearms, with a gun permit, and complying with all firearms regulations. The safety of the personnel, the public and any animals nearby must be considered. If the decision is made to use a firearm to euthanize an animal in captivity, it should be aimed at the head with the projectile penetrating the brain causing immediate loss of consciousness. This must be done bearing in mind the various positions of the brain and the shape of the skull and the sinuses. The optimal targets of firearms have been described in the various species. The preferred target in wild and free animals is the head. The most appropriate firearm should be chosen depending on the situation and with the aim of penetrating and destroying brain tissue without exiting on the other side of the head. Shooting aiming at the heart or the neck does not cause loss of consciousness and does not satisfy the definition of euthanasia made by experts.

A horizontal shot aimed just above (dorsally) the center of the line between the eye and ear can be lethal. Alternatively, a shot aimed through the blowhole at a 45° angle directed down and back (ventro-posteriorly) to an area behind the pectoral flippers has been shown to be effective. The preferred shot is the lateral approach, particularly in larger sized cetaceans (longer than 4 meters) since the skull is closest to this body surface. The ventro-posterior shot can be used, for example, in small sized dolphins (less than 4 meters long) shooting preferably when the blow hole is open, but not placing the muzzle of the firearm in it as pressure during exhalation can cause firearm explosion. The firing range should be 40 to 100 cm from the head. Shooting cetaceans through the thorax will likely result in persistent consciousness and a slow death. Generally speaking, bullets with a minimum of .30 or 7.62 mm caliber (.303 is recommended), totally jacketed and solid (FMJ and/or RN) and with a minimum weight of 9.8 grams is recommended. Use of high-powered rifles may not be efficacious could become dangerous when the animal exceeds 9 meters, such as mysticetes and odontocetes (in particular *Physeter microcephalus*), in view of the anatomic conditions of the skull and of the melon which protect the brain.

With regard to Sperm whales and the particular anatomy of their head, the Department of Conservation in New Zealand has reported

the development of a specialized round and firearm for the humane euthanasia of sperm whales using a 14.5 x 114 mm anti-aircraft, 61 gram, 12 L14 leadloy bore-riding bullet with a flat tip. A firearm was also extensively modified to effectively use this round. The result was an 11.8 kg firearm with a 2.4 meter recoil which must be operated standing sideways.

Advantages

- There is immediate loss of consciousness if the bullet destroys the greater part of the brain;
- In view of the need to minimize the stress due to manipulation and contact with humans, the firearm can in some cases be the most practical and logical way to euthanize wild and free species.
- Disadvantages:
- It can prove dangerous for the personnel;
- It is aesthetically displeasing;
- In field conditions in may be difficult to hit a moving target;
- When the target is the head, brain tissue can become unusable for evaluation of rabies and chronic debilitation

Recommendations

In situations in which other options are unavailable, the skilled use of a firearm can be a conditionally acceptable method of euthanasia. Before proceeding, animals accustomed to the presence of humans should be treated in a calm, reassuring manner to reduce their stress. Animals belonging to wild species should be euthanized by a firearm following as little human contact as possible.

7.5.2 Injectable drugs

Use of non-inhaled agents is a rapid, reliable method to carry out euthanasia and it is a preferable one when it can be carried out without causing pain or fear in the animal. In view of the stress that large marine mammals experience during the restraining measures required for injection administration and the risk that handlers run in incurring personal injury, sedation prior to euthanasia is essential. When carrying out an intramuscular injection is considered risky for the veterinarian, a intraperitoneal or intracardiac injection of a non-irritating agent not containing neuro-muscular agents can be administered to an anesthetized or comatose animal. This is not considered an acceptable procedure in a vigilant or sedated animal in view of the difficulty of carrying out this type of injection and the animal's unpredictability.

Subcutaneous, intrathoracic, intrapulmonary, intrarenal, intrasplenic, and intrathecal routes irritate tissues, have a prolonged onset of action, and are considered unacceptable for administering euthanasia agents.

a.Derivatives of barbituric acid

Barbiturates act by depressing the medullary respiratory and vasomotor centers to a degree that results in unconsciousness and respiratory and cardiac arrest causing a rapid and targeted action.

As fatal doses of barbituric acid administered intravenously induce deep anesthesia they are widely accepted euthanasia agents. The onset of its reaction is quick, thus minimizing the pain linked to the injection is minimal or transitory.

Barbiturates that are strong, long-lasting, stable and economic are preferable. Pentobarbital sodium salt fulfils all of these requirements and is the most utilized agent although other barbiturates such as Secobarbital are also acceptable.

Their advantages are:

- The principal advantage of barbiturates is their rapid action, which depends on dosage, concentration, route and rate of administration;
- Babiturates induce euthanasia gently with a minimum of discomfort for the animal;
- Barbiturates are less costly than many other euthanizing agents.
- Their disadvantages are:
- Licensed or expert personnel are necessary to obtain the best results;
- The animal must be restrained;
- A death rattle may be heard which may be unpleasant/displeasing for those present;
- These drugs tend to remain in the carcass causing sedation or even death to animals feeding on it.

Recommendations

The advantages of barbiturates in small animals are notably greater with resepect to their disadvantages. Intravenous injection of a barbituric acid derivative is the preferred method for euthanasia of dogs, cats, other small animals and horses. When an intravenous injection can be considered stressful, an intraperitoneal injection can be administered. An intracardiac injections should be used only in animals that are heavily sedated, unconscious or anesthetized.

b.Combinations of pentobarbital with other agents

Many euthanasia agents contain barbituric acid derivatives (usually pentobarbital sodium) associated to a local anesthetic agent that metabolize to pentobarbitol. Some additives have slow cardiotoxic effects, a pharmacological effect not considered consequential. These pharmacological associations are listed by the Drug Enforcement Agency (DEA) as Schedule III drugs and are thus easier to obtain, store,

and administer with respect to Schedule II drugs such as pentobarbital sodium. The pharmacological properties and drug information of these products combining pentobarbital with agents such as lidocaine or phenytoin are identical to pure barbituric acid derivatives. The combination of pentobarbital with neuromuscular blocking agents is not considered an acceptable method of euthanasia.

c.T-61

T-61 is an injectable mixture of a local anesthetic, a hypnotic agent, and curariform drug (N-2- (m-methoxyphenyl)- 2-ethylbutyl-l-gamma-hydroxybutyramide 20%), 4,4'-methylene bis-cyclohexyltri-methyl ammonium iodide (5%) and tetracaine HCl (0.5%) in aqueous solution with formamide). The drug furnishes a combination of general anesthetic, curariform and local anesthetic effects. This drug is no longer produced or sold in the United States, but it is available in Canada and in countries including Italy. It should only be used intravenously because of potential differences in absorption when administered by any other route. The dose extrapolated from small animals is 0.3 ml/kg IV. In cases of large sized animals or in locating venous access, an intrathoracic injection can be administered.

d. Etorphine

This drug is an ultra-potent opioid and one of the preferred agents for euthanasia of marine mammals at an international level (e.g. etorphine hydrochloride – M99 – in association with acepromazine, immobiolon; carfentanil) although its use is not always possible and poses some risks to personnel handling the drug. Only the emergency kit should be used when this drug is chosen as the euthanasia measure and the carcass must be burned to avoid risks to handlers and other animals. The dose of etorphine for immobilization can range from 0.5 µg/kg to 5 µg/kg, but euthanasia dosages for most marine mammals have not been determined.

e. Other drugs

The combination of potassium chloride with succinylcholine chloride using an intravascular or intraperitoneal route of administration or with magnesium salt has been utilized to induce euthanasia. This combination leads to muscular rigidity, spasms, vocalizations, and gasping; in the end there is complete respiratory paralysis while the animal is still conscious causing death by hypoxia. Alternative methods of euthanasia are recommended. If other options are unavailable the drug, if possible in association with sedation with barituric acid derivatives, can be considered more ethical with respect to death by suffocation in an animal

stranded for hours or days.

7.5.3 Natural death

Taking place since the beginning of time, strandings are for the most part natural events often not involving humans in any way and with death taking place in a natural and spontaneous manner. This option can be foreseen and taken into consideration in those cases that it is impossible to take appropriate safety measures for the handlers or there is not an adequate knowledge of or means for appropriate euthanasia measures. It may be more humane to leave the animal to die on its own rather than applying any substandard method of euthanasia especially in larger whales like sperm or baleen whales. The gravitational weight on the internal organs will likely induce a more humane death than repeated rounds of projectiles fired inaccurately. In these cases, if possible, it is opportune to use sedatives to accelerate normal processes and produce unconsciousness. If possible, curiosity seekers should be kept as far as possible away from the scene and loud noises and any other disturbing elements should be avoided.

7.5.4 Diagnosis of death

It is imperative that death be verified and this is not a simple venture with large sized animals. Generally speaking the following parameters are evaluated:

1. Is there any muscular activity? If there is any doubt about the exemplar being conscious, proceed to point 2
2. Is there any kind of response to evaluation of reflexes Pupillary light reflex, the palpebral reflex the corneal reflex closure of the lids on irritation of the cornea, the menace reflex). If there is, proceed to point 3
3. Does the animal have a perceptible heartbeat or capillary refill < three seconds? Or is the difference between the skin and eye temperature >4°. If it is, the animal is probably alive.

If there is any doubt about confirmation of death, a secondary, physical means of euthanasia should be performed to ensure death. Physical methods include bilateral thoracotomy, exsanguination, and gunshot through the heart or brain of course always accompanied by the appropriate safety measures.

Handbook for Cetaceans' Strandings

Fig. 2.1:Skin and blubber *Tursiops truncates*

Fig. 2.2: Caudal fin, cross-section; notice the supporting tissue more fibrous than the blubber of the rest of the body and blood vessels with countercurrent system *Stenella coeruleoalba*

Fig. 2.3:Mammary slits in a female *Tursiops truncatus*

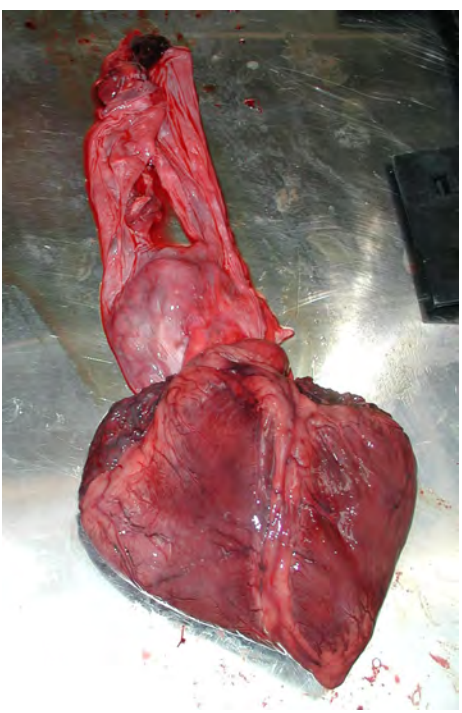

Fig. 2.4 Heart, external view
Stenella coeruleoalba

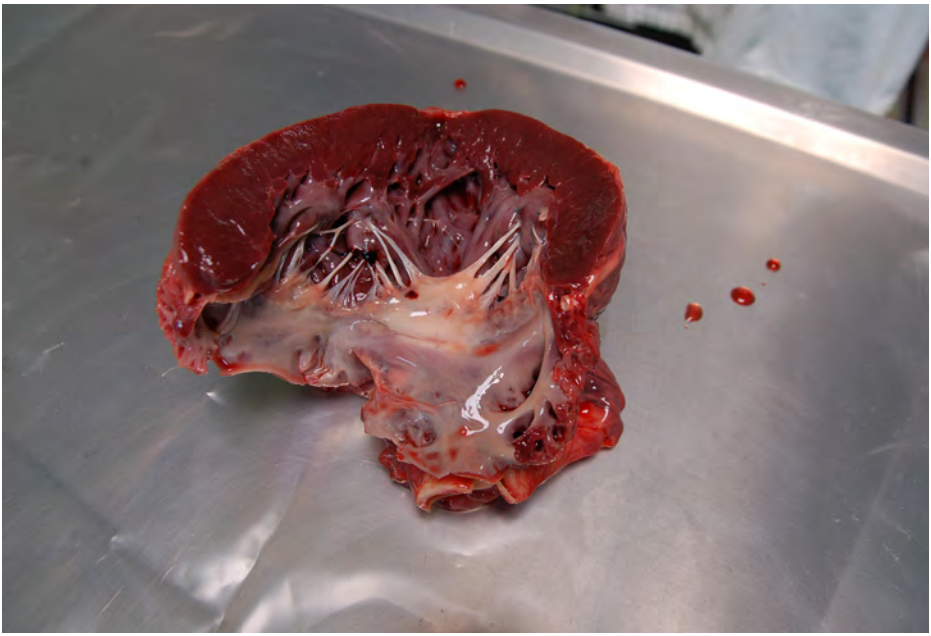

Fig. 2.5: Heart, cross-section of the left ventricle *Stenella coeruleoalba*

Fig. 2.6: Spleen *Tursiops truncatus*

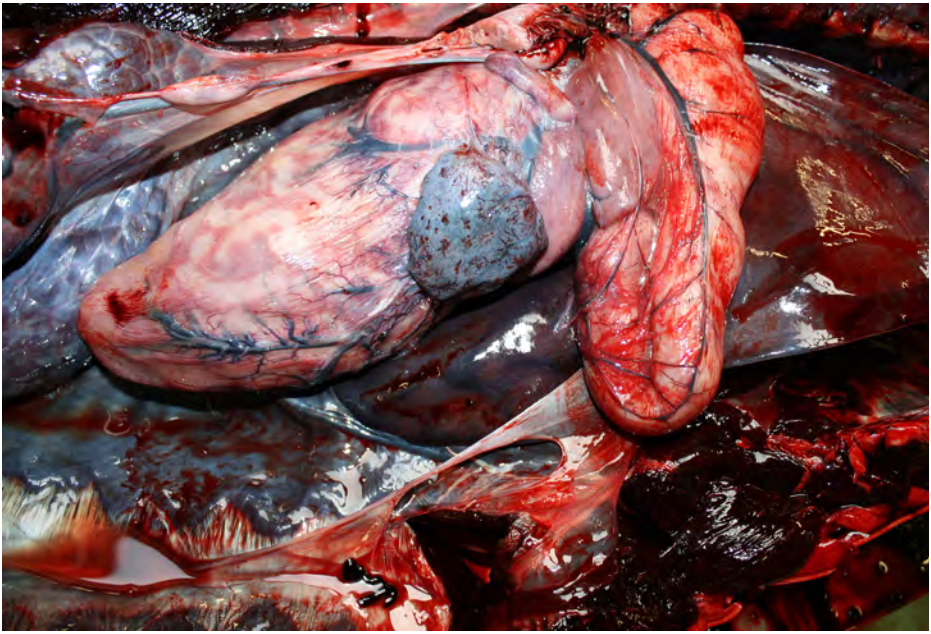

Fig. 2.7:Spleen in the abdominal cavity, right lateral view *Tursiops truncatus*

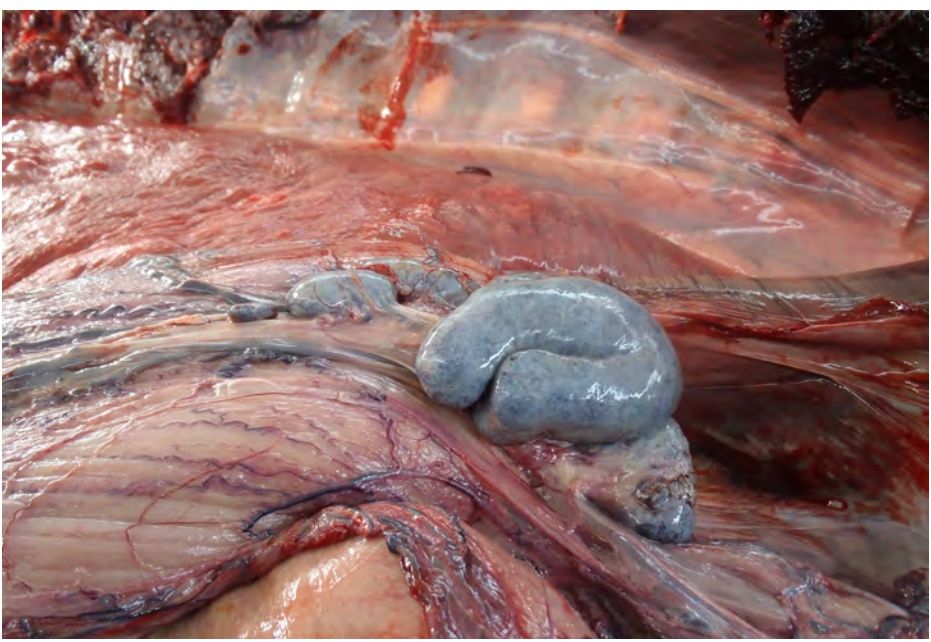

Fig. 2.8: Accessory spleens *Grampus griseus*

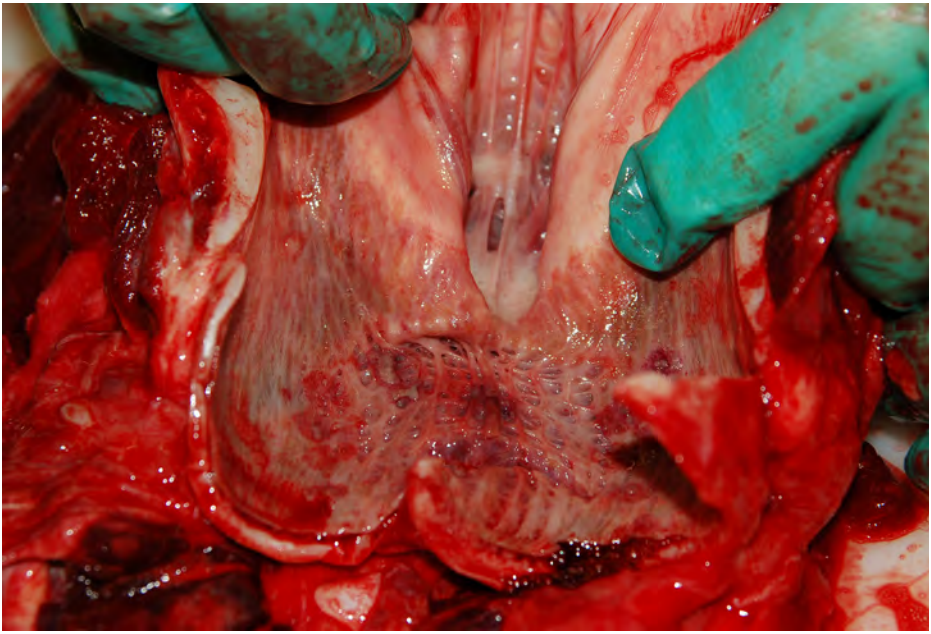

Fig. 2.9: Laryngeal tonsils *Tursiops truncatus*

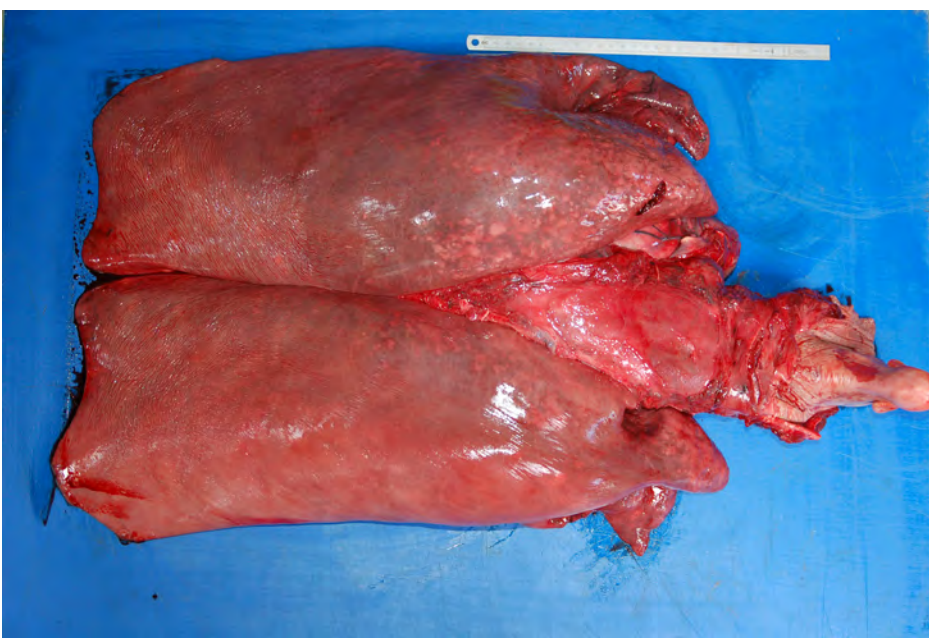

Fig. 2.10: Lung, dorsal view *Tursiops truncatus*

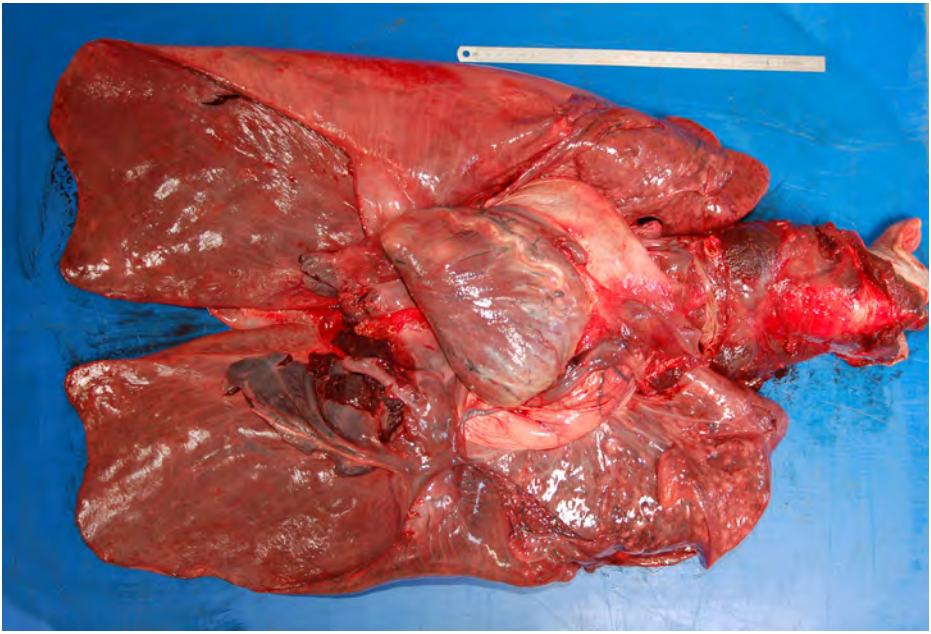

Fig. 2.11: Lung, ventral view with heart *Tursiops truncatus*

Fig. 2.12: Lung, cross-section *Tursiops truncatus*

Fig. 2.13:Teeth *Stenella coeruleoalba*

Fig. 2.14: Teeth *Tursiops truncatus*

Fig. 2.15: Teeth *Grampus griseus*

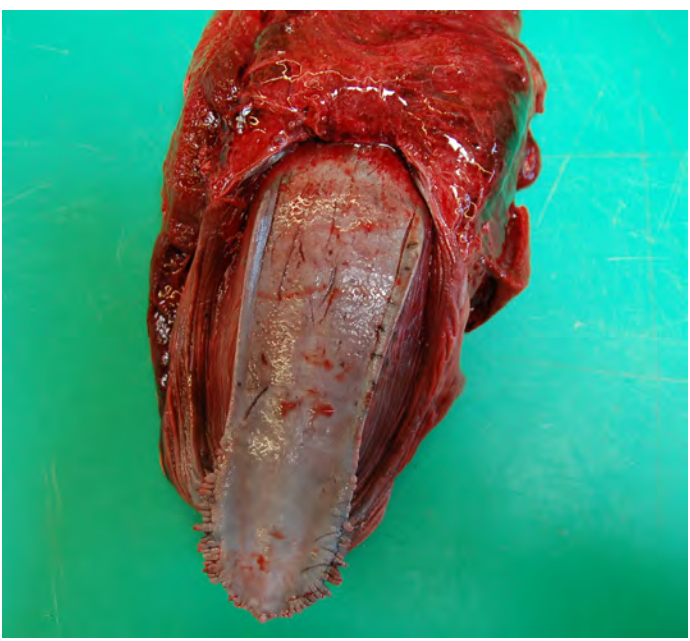

Fig. 2.16: Tongue in a young animal *Tursiops truncatus*

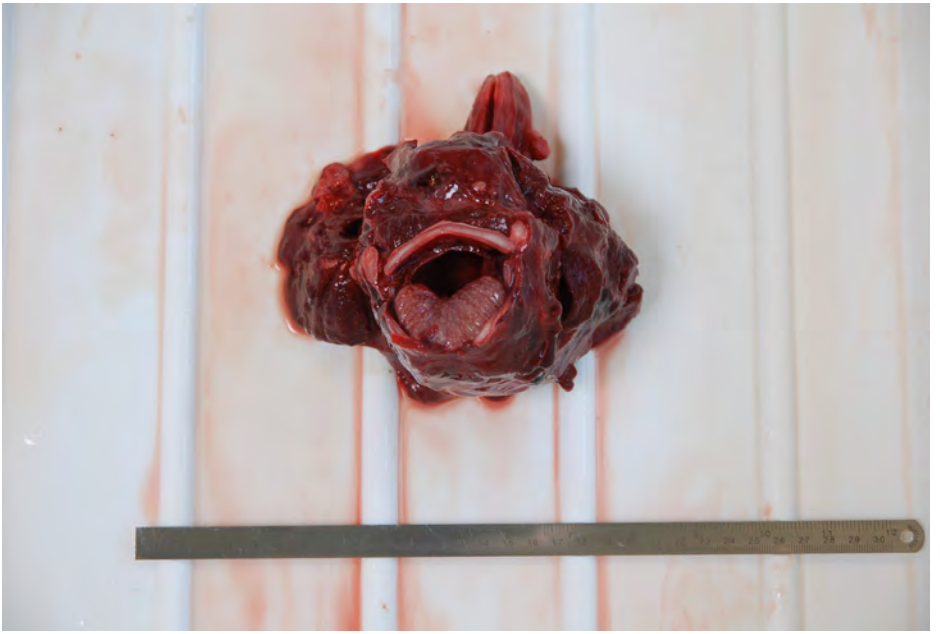

Fig. 2:17: Pharynx *Tursiops truncatus*

Fig. 2.18: Baleen *Balaenoptera physalus*

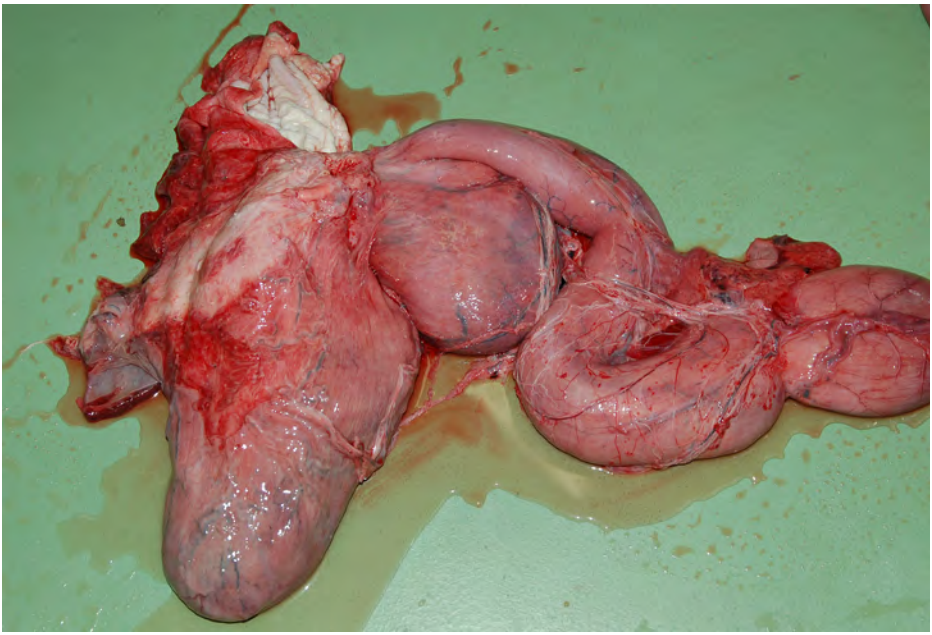

Fig 2.19: Gastric chambers *Tursiops truncatus*

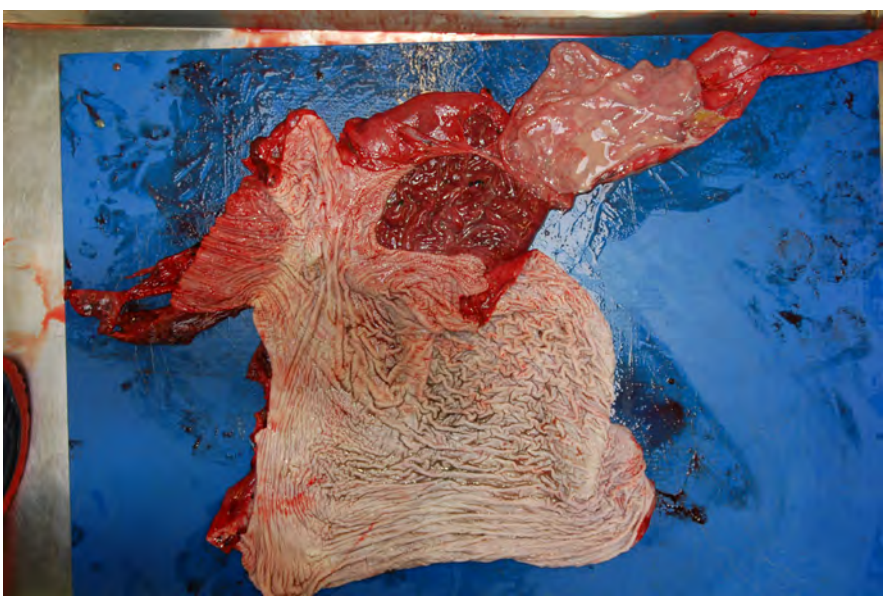

Fig. 2.20: Mucosa of gastric chambers *Grampus griseus*

Fig. 2.21: Intestine *Tursiops truncatus*

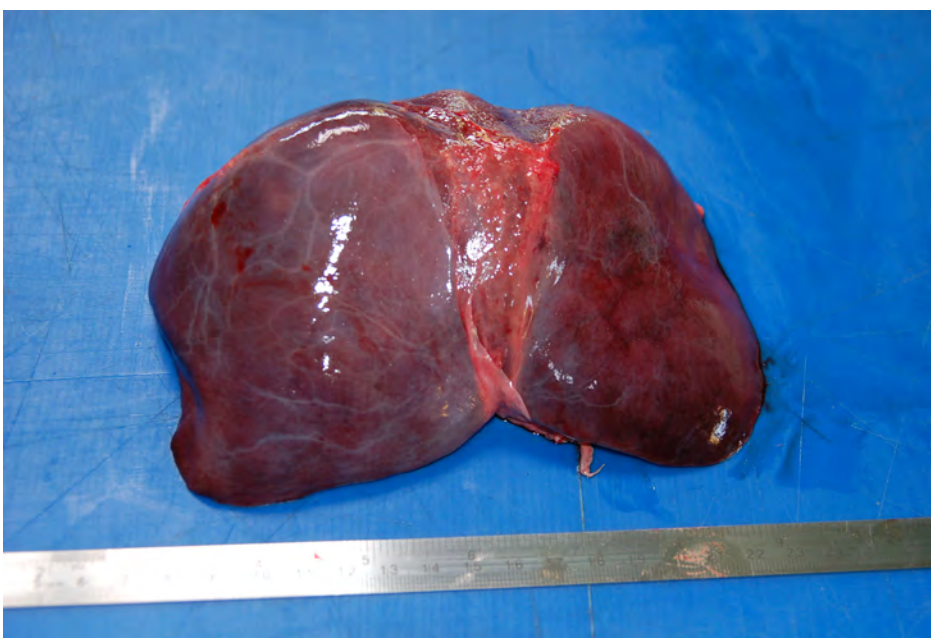

Fig. 2.22: Liver, diaphragmatic view in a young subject *Tursiops truncatus*

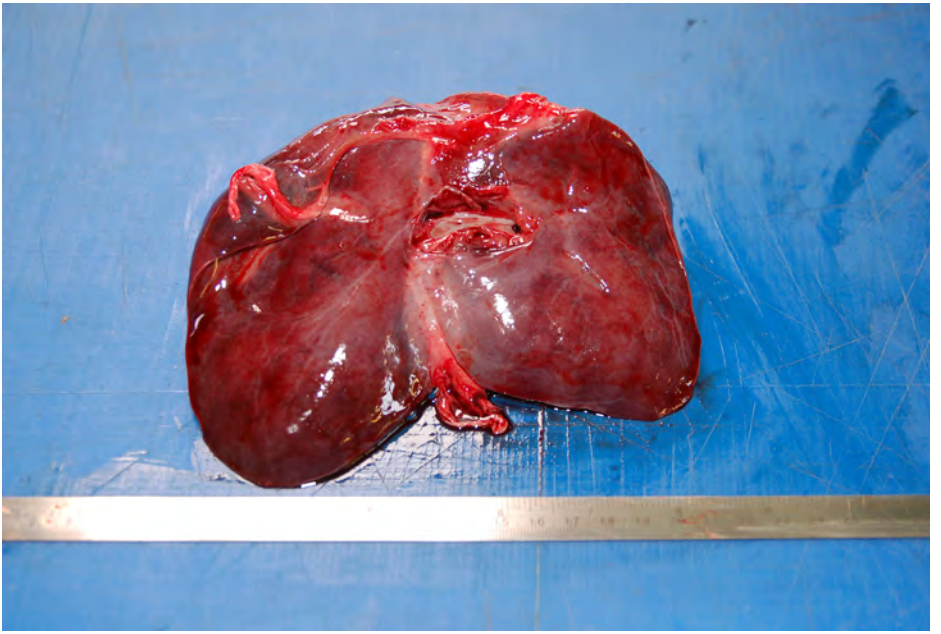

Fig. 2.23: Liver, visceral view in a young subject *Tursiops truncatus*

Fig. 2.24: Male urinary tract *Tursiops truncatus*

Fig. 2.25: Kidney, *reniculi Tursiops truncatus*

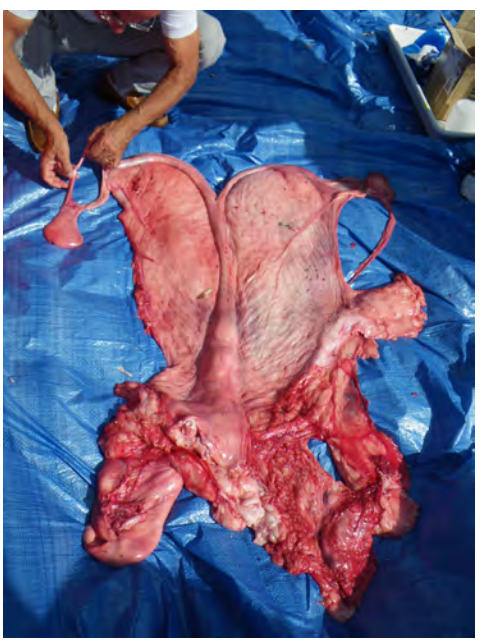

Fig. 2.26: Female reproductive system *Balaenoptera physalus*

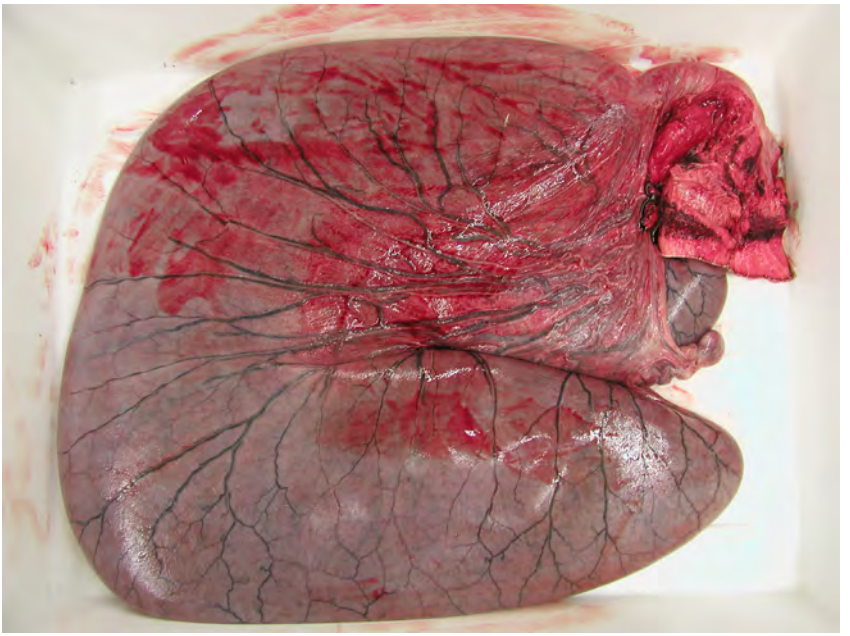

Fig. 2.27: Placenta *Tursiops truncatus*

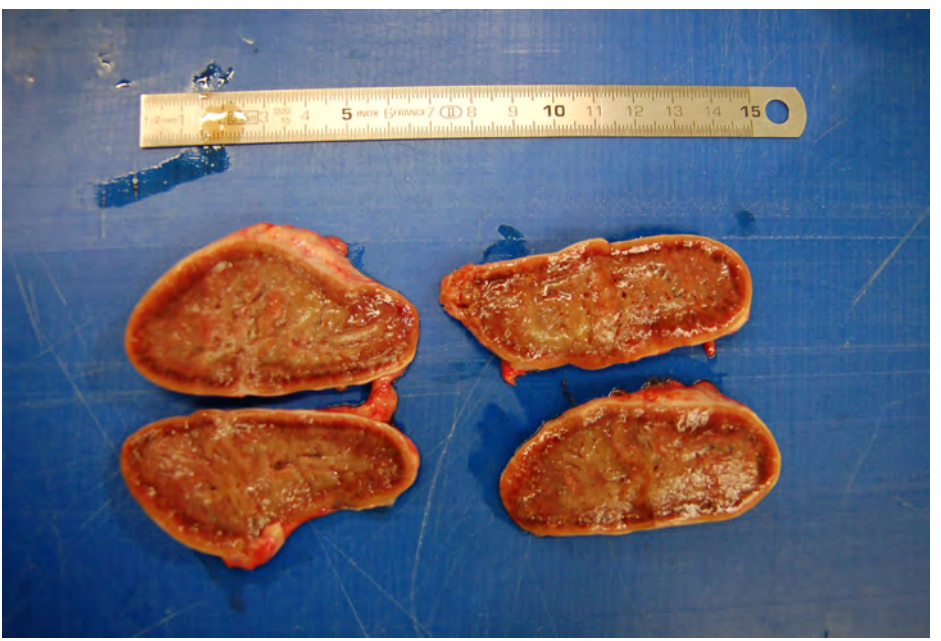

Fig. 2.28: Adrenal glands, cross section *Grampus griseus*

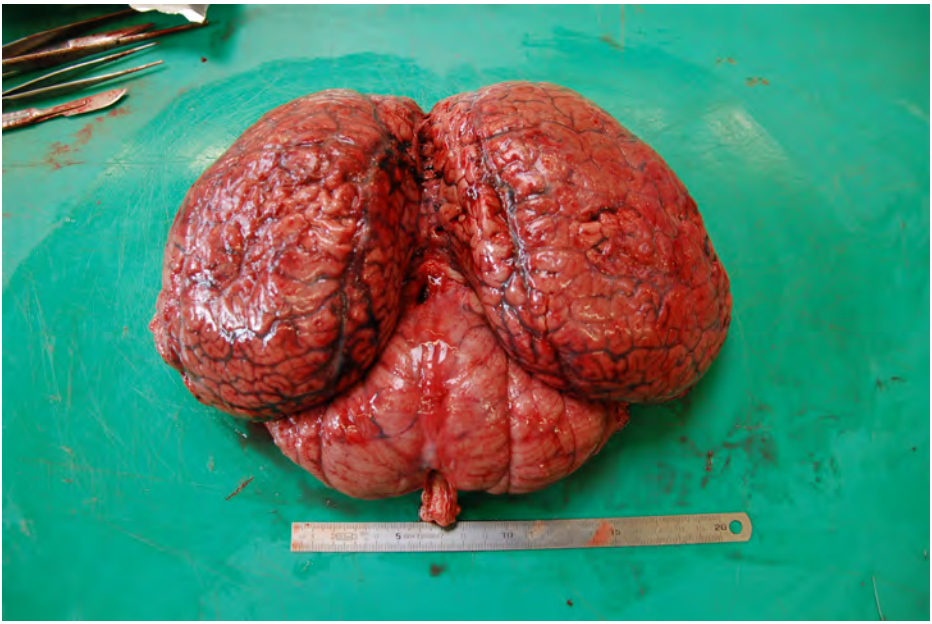

Fig. 2.29: Brain, dorsal view *Tursiops truncatus*

Fig. 2.30: Brain, ventral view *Tursiops truncatus*

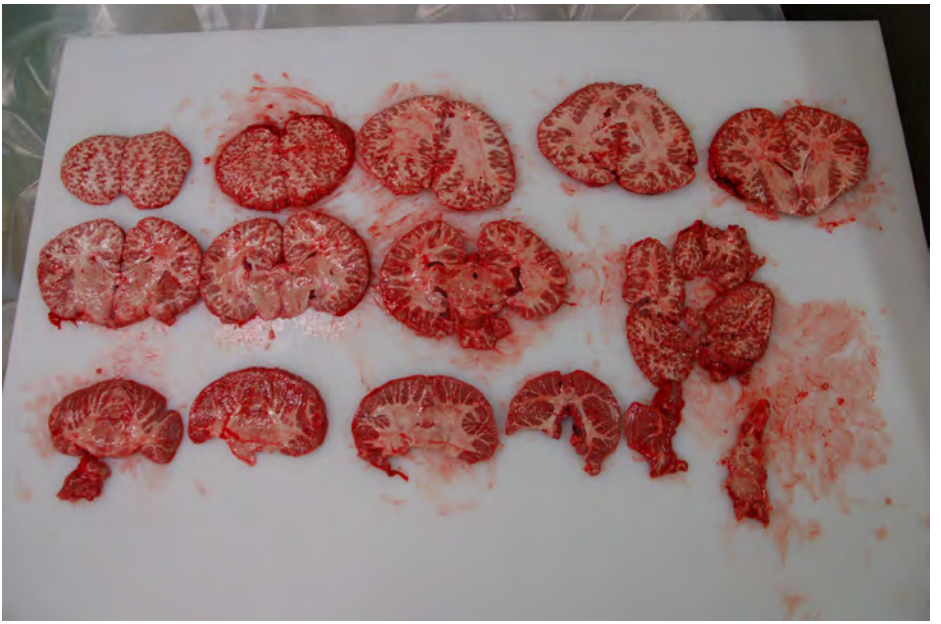

Fig. 2.31: Brain, cross-sections *Tursiops truncatus*

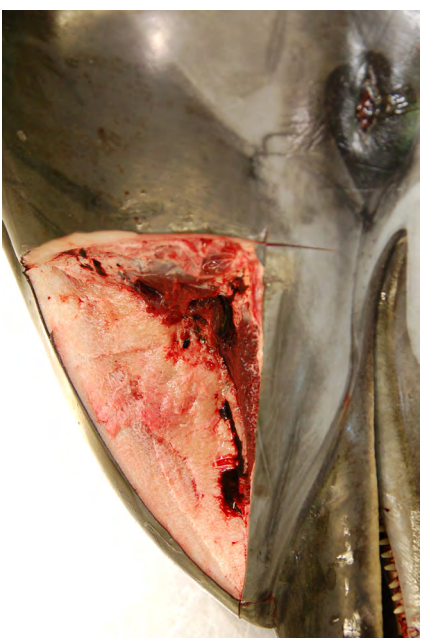

Fig. 2.32: Melon, cross-section *Stenella coeruleoalba*

Fig.3.1: Carcass; code of conservation 1 *Stenella coeruleoalba*

Fig. 3.2: Carcass; code of conservation 2 *Tursiops truncatus*

Fig. 3.3:Carcass; code of conservation 3 *Tursiops truncatus*

Fig. 3.4:Carcass; code of conservation 5 *Stenella coeruleoalba*

Fig. 3.5: Carcass; evaluation of the body condition: a reduced body profile could be assessed observing any atrophy of the epi-axial muscles on the dorsal area *Tursiops truncatus*

Fig. 3.6: Teeth; newborn. Vibrissae are evident on the picture; they will be lost with adulthood *Tursiops truncatus*

Fig. 3.7:Genital and mammary slits in a female striped dolphin *Stenella coeruleoalba*

Fig. 3.8: Genital split with the penis in a male bottlenose dolphin *Tursiops truncatus*

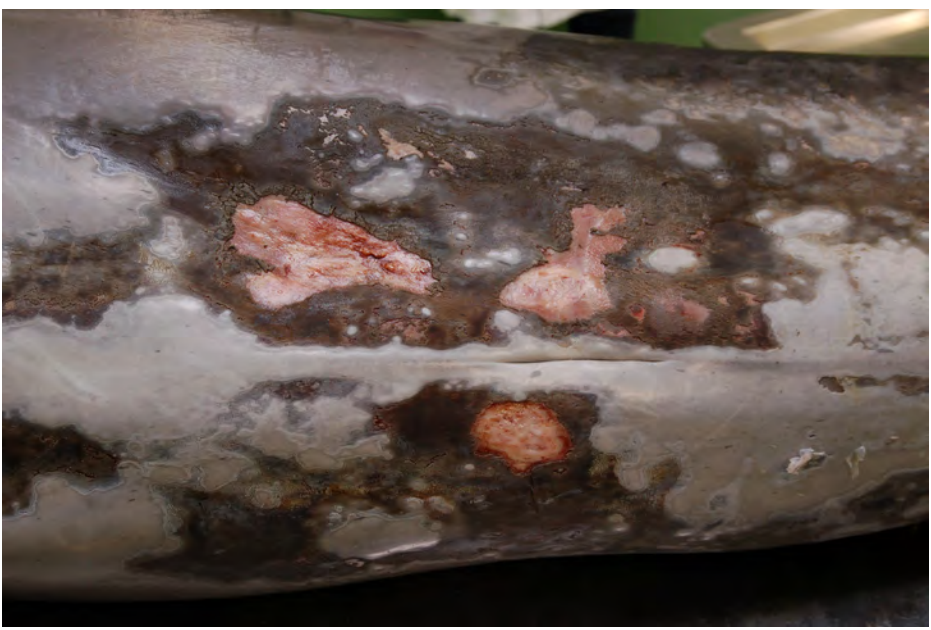

Fig. 3.9:Skin; ulcerative dermatitis due to mycotic infection *Tursiops tursatus*

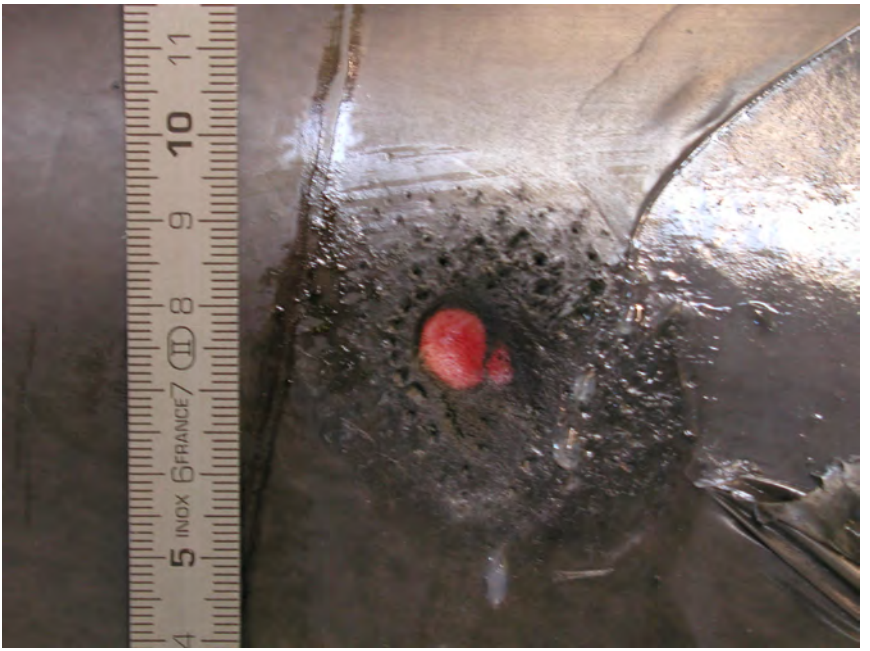

Fig. 3.10: Skin; perforating injures likely due to lamprey (*Petromyzon marinus*) *Grampus griseus*

Fig. 3.11 Skin, tail; ulcerative lesion on the right lobe *Grampus griseus*

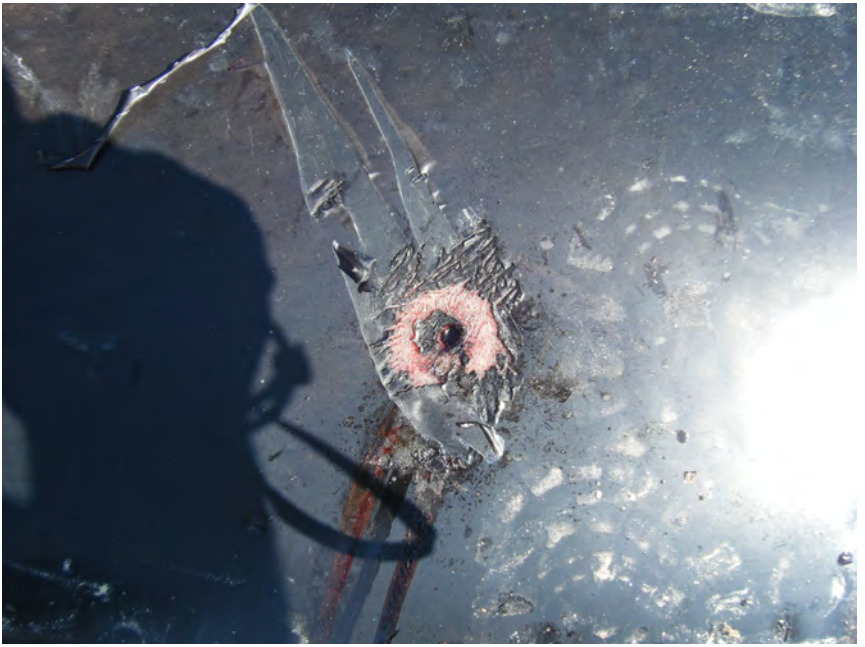

Fig. 3.12: Skin; contact range gunshot entry wound *Tursiops truncatus*

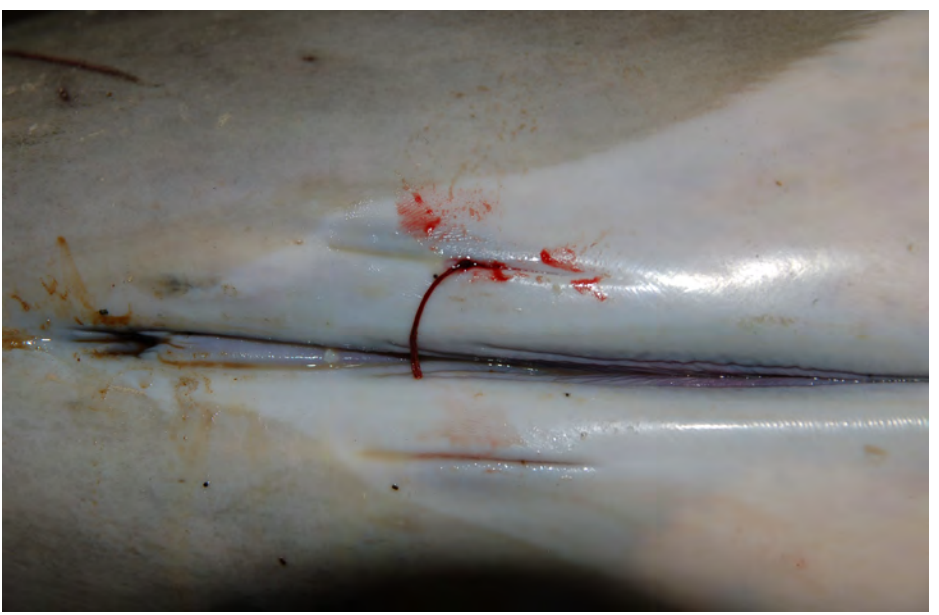

Fig. 3.13: Skin; *Pennella spp.* within the mammary slit *Stenella coeruleoalba*

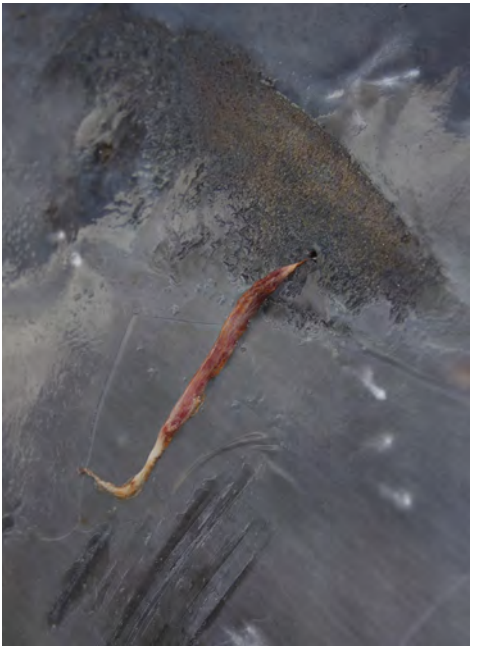

Fig. 3.14:Skin *Pennella spp*. anchored within the skin; this could be considered a para-physiological condition *Balaenoptera physalus*

Fig. 3.15: Skin; several *Pennella spp*. individuals within the skin *Stenella coeruleoalba*

Fig. 3.16: Oral cavity; gengival hyperplasia, age-related change *Tursiops truncatus*

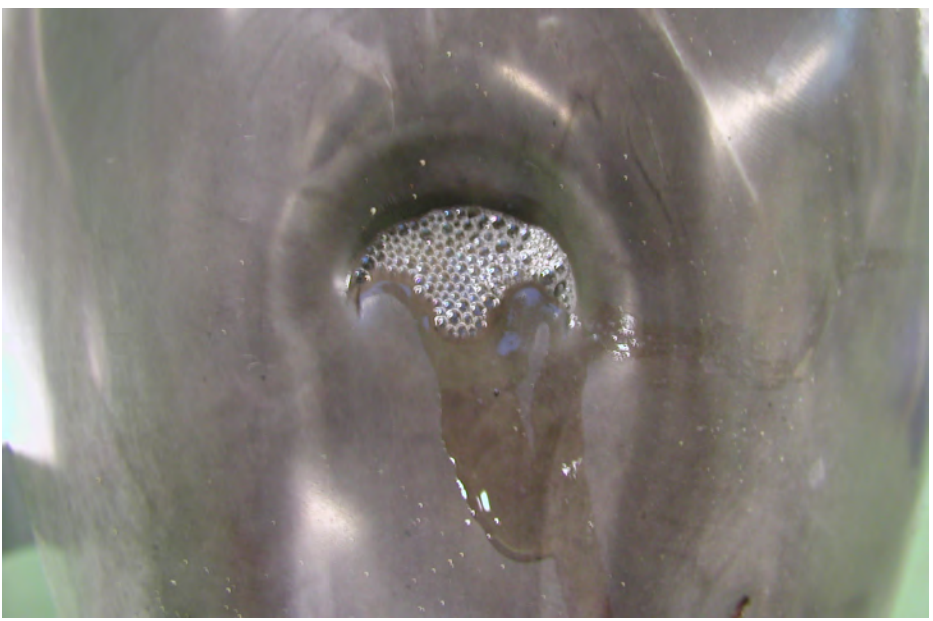

Fig. 3:17: Blowhole; foamy fluid (edema) coming out from upper airways *Tursiops truncatus*

Fig. 3.18: Blowhole; external parasites (Synciamus spp.) externally to the nose *Tursiops truncatus*

Fig. 3:19: Genital split, greenish watery diarrhea coming out from the anus *Grampus griseus*

Fig. 3.20: Skin and blubber incisions to remove external layers (ventral view) ventrale *Tursiops truncatus*

Fig. 3.21 Skin and blubber incisions to remove external layers (lateral view)*Tursiops truncatus*

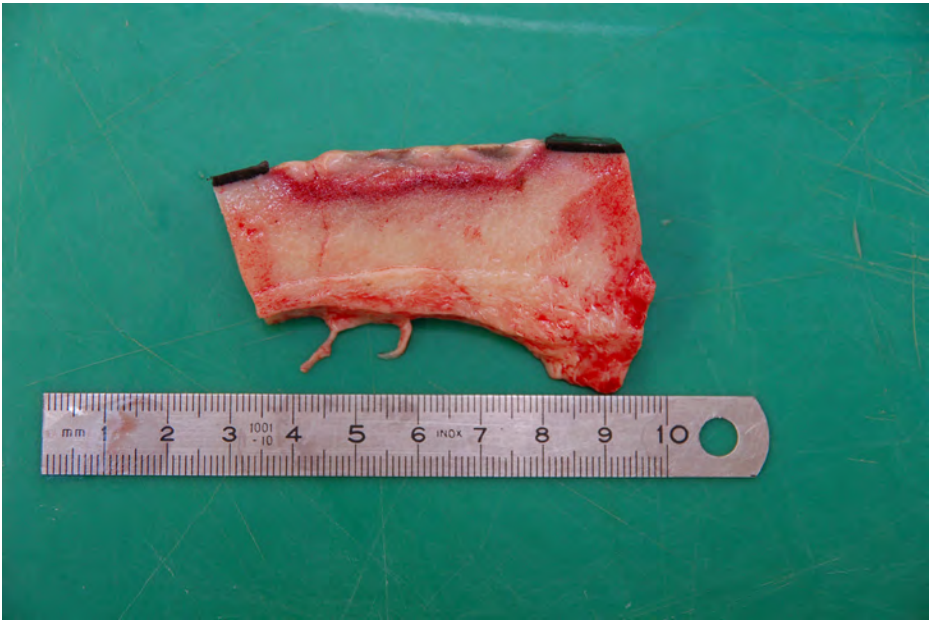

Fig. 3.22: Skin; ulcerative lesion of the skin surface *Tursiops truncatus*

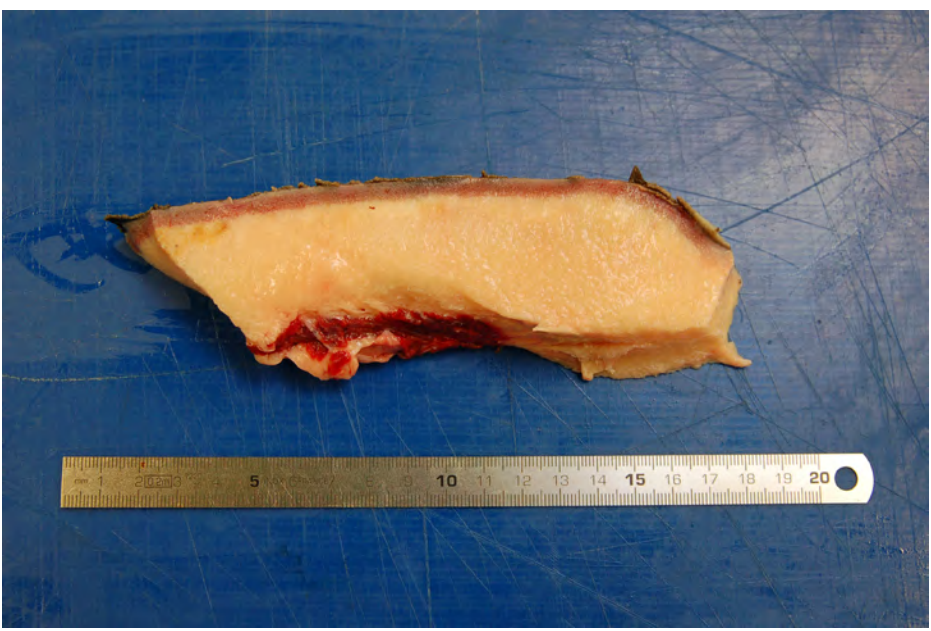

Fig. 3.23: Skin, transversal cut; multiple orange areas on the superficial layer of the blubber and diffuse hyeremia of the derma due to mycotic panniculitis *Cryptosporidium spp. Tursiops truncatus*

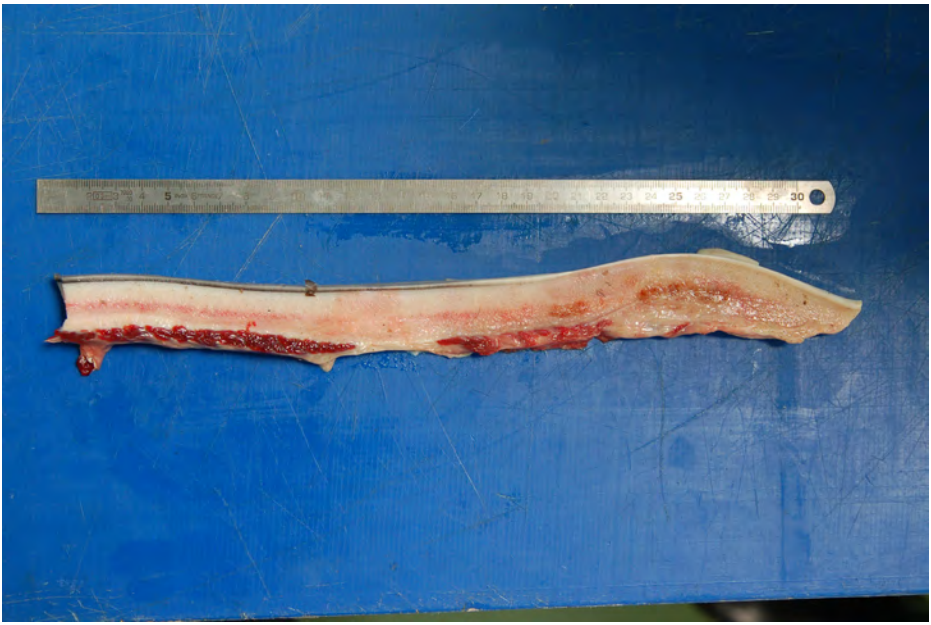

Fig. 3.24: Skin, transversal cut; multiple orange areas in the deep layers of the blubber; granulomatous panniculitis likely caused by vitE/Se deficiency *Stenella coeruleoalba*

Fig. 3.25: Skin; *Phyllobothrium spp* in the blubber thickness *Ziphius cavirostris*

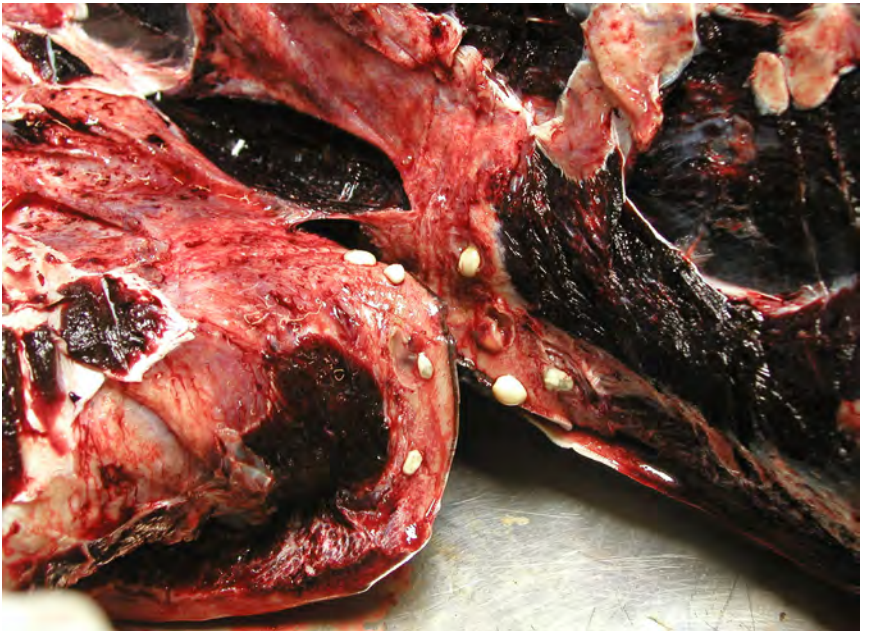

Fig. 3.26: Skin, transversal cut; *Phyllobothrium spp*. in the blubber thickness, *Stenella coeruleoalba*

Fig. 3.27: Subcutaneous tissue; gelatinous edema *Stenella coeruleoalba*

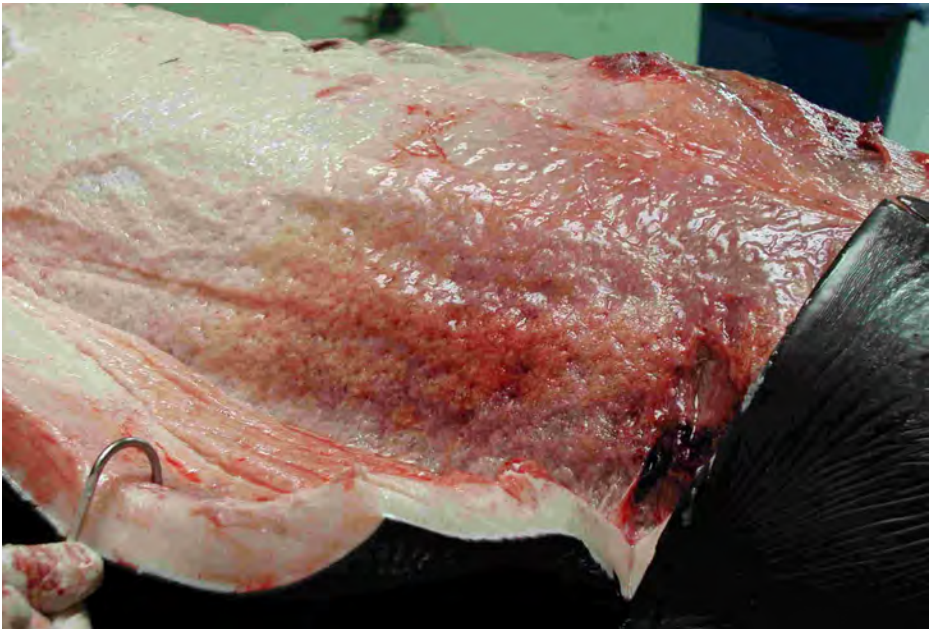

Fig. 3.28: Subcutaneous tissue; necrotizing and hemorrhagic fascitis *Tursiops truncatus*

Fig. 3.29: Subcutaneous tissue; yellowish discoloration due to systemic hycterus *Grampus griseus*

Fig. 3.30: Thoracic and abdominal cavities; opening procedures of the chest *Stenella coeruleoalba*

Fig. 3.31: Thorax; procedures for removing internal organs from the tongue; larynx is evident *Tursiops truncatus*

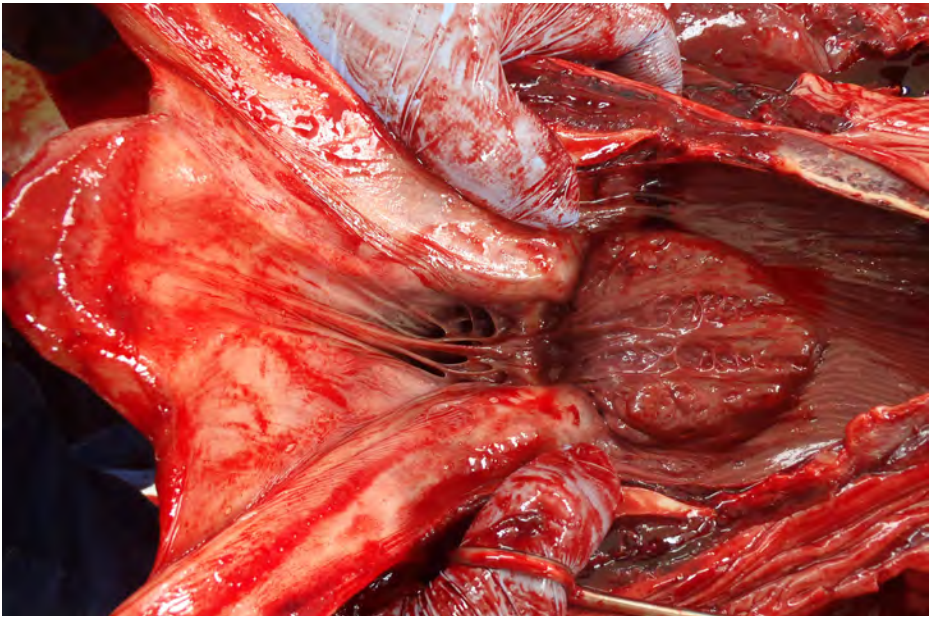

Fig. 3.32: Larynx; hyperplasia of laringeal tonsils *Grampus griseus*

Fig. 3.33 Epiglottis; foamy pinkish fluid (edema) within the lumen *Grampus griseus*

fFig. 3.34:Thyroid; thyroideal cyst *Tursiops truncatus*

Fig. 3.35:Tracheal division; foam within the lumen (edema) *Tursiops truncatus*

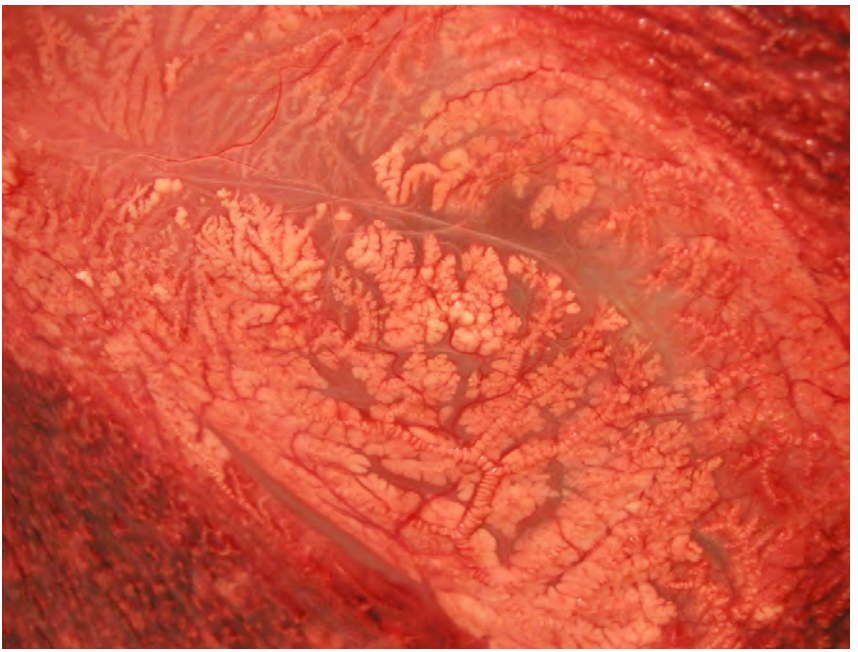

Fig. 3.36:Lungs; alveolar hypoplasia, physiological change in small odontocetes *Stenella coeruleoalba*

Fig. 3.37: Lungs; whitish nodules scattered in the pulmonary tissue likely due to parasites *Tursiops truncatus*

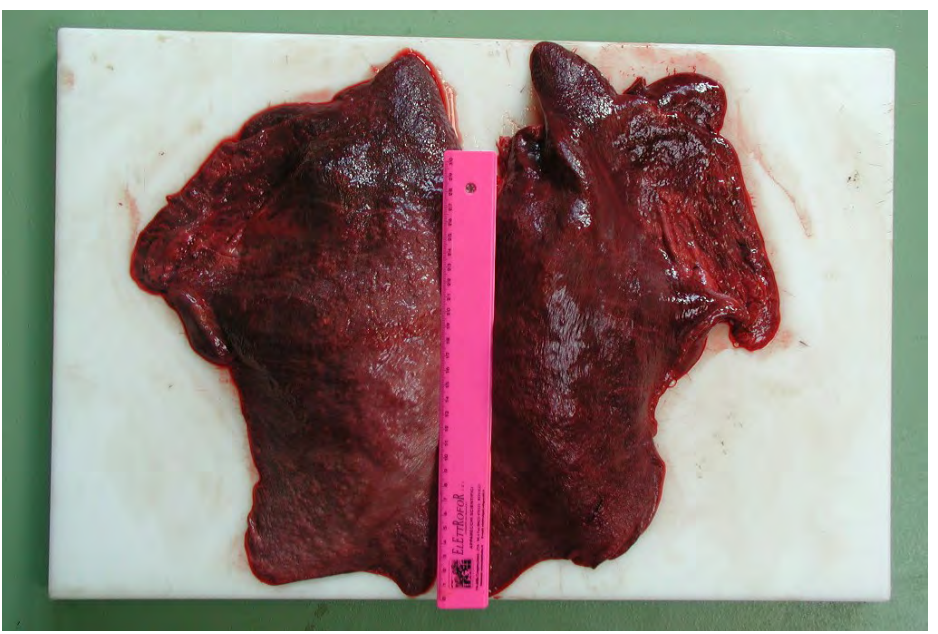

Fig. 3.38: Lungs; hyperemia *Tursiops truncatus*

Fig. 3.39:Lungs; monolateral lymphangectasia due to focal pneumonia *Tursiops truncatus*

Fig. 3.40:Lungs; evidence of parasite within the bronchial lumen *Stenella coeruleoalba*

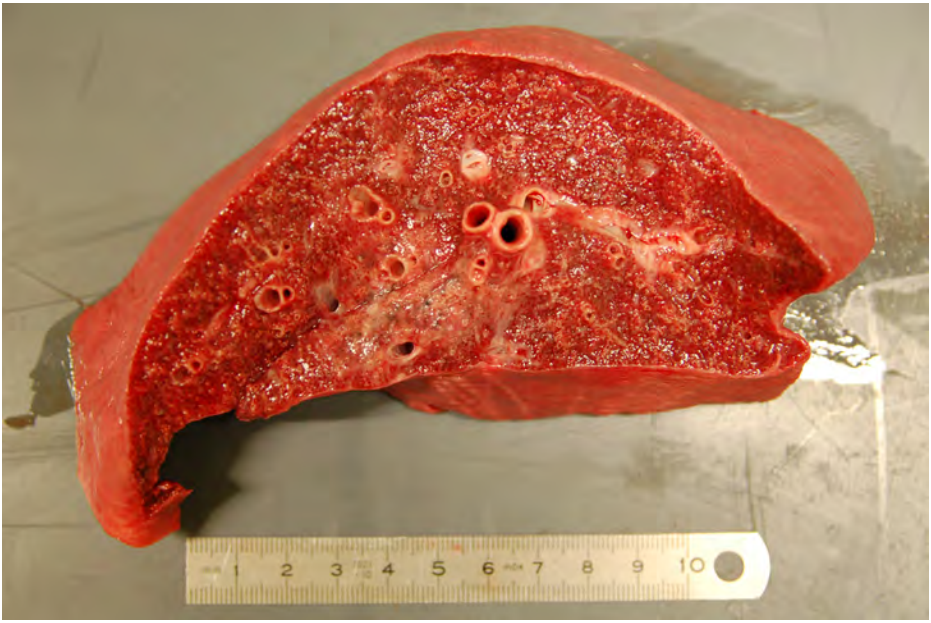

Fig. 3.41: Lungs; scar with fibrotic tissue *Tursiops truncatus*

Fig. 3.42:Lungs; focal mycotic pneumonia *Tursiops truncatus*

Fig. 3.43:Lungs; parasitic nodule associated to peripheral inflammatory reaction *Tursiops truncatus*

Fig. 3.44: Lungs; abscess due to *Staphylococcus aureus Tursiops truncatus*

Fig. 3.45: Spleen; orange discoloration due to hemolysis *Tursiops truncatus*

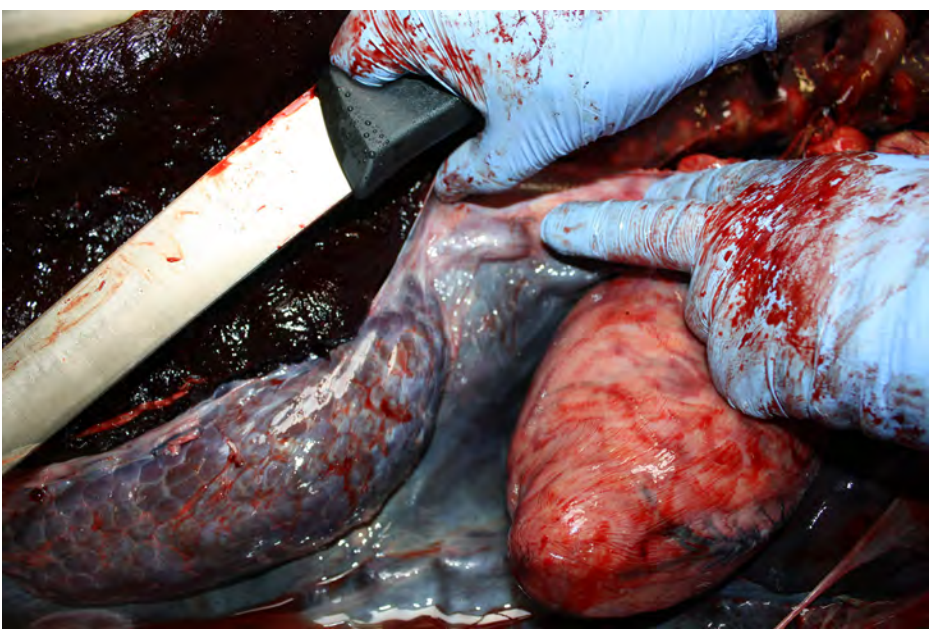

Fig. 3.46: Adrenal glands; normal topography compared to kidneys *Stenella coeruleoalba*

Fig. 3.47: Adrenal glands; Adrenal glands chronic inflammation *Stenella coeruleoalba*

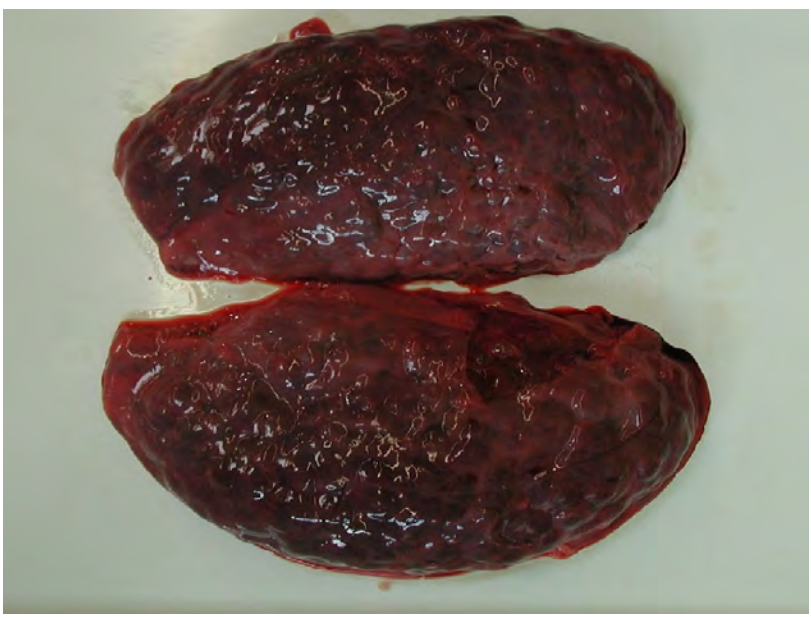

Fig. 3.48:Kidneys; diffuse hyperemia *Tursiops truncatus*

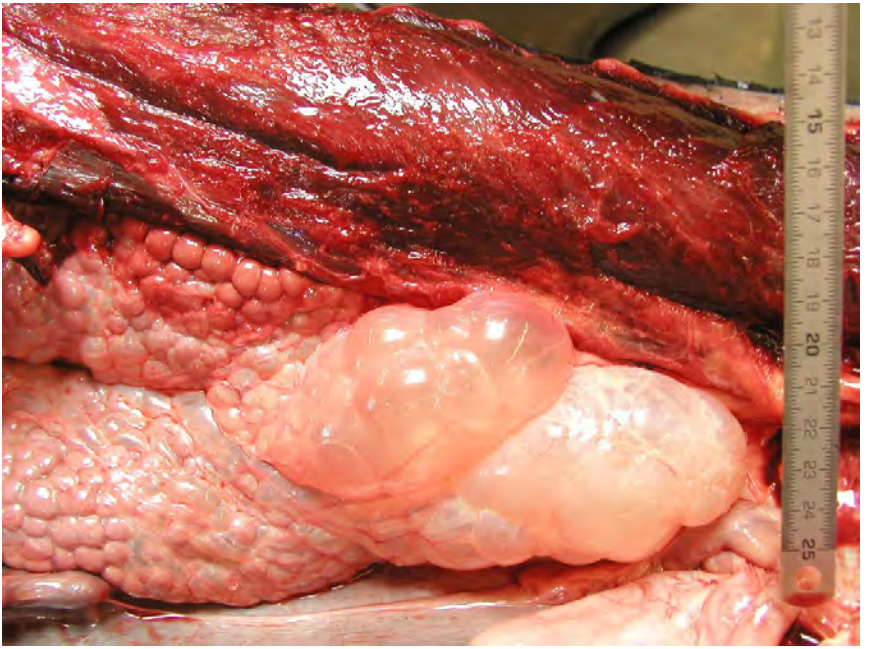

Fig. 3.49: Kidneys; peri-ureteral cysts associated to renal glicogenosis *Grampus griseus*

Fig. 3.50: Renal artery; intra-luminal thrombi due to parasitic infestation *Crassicauda grampicola, Balaenoptera physalus*

Fig. 3.51: Kidney; parasites (likely *Crassicauda spp.*) within the renal vein *Balaenoptera physalus*

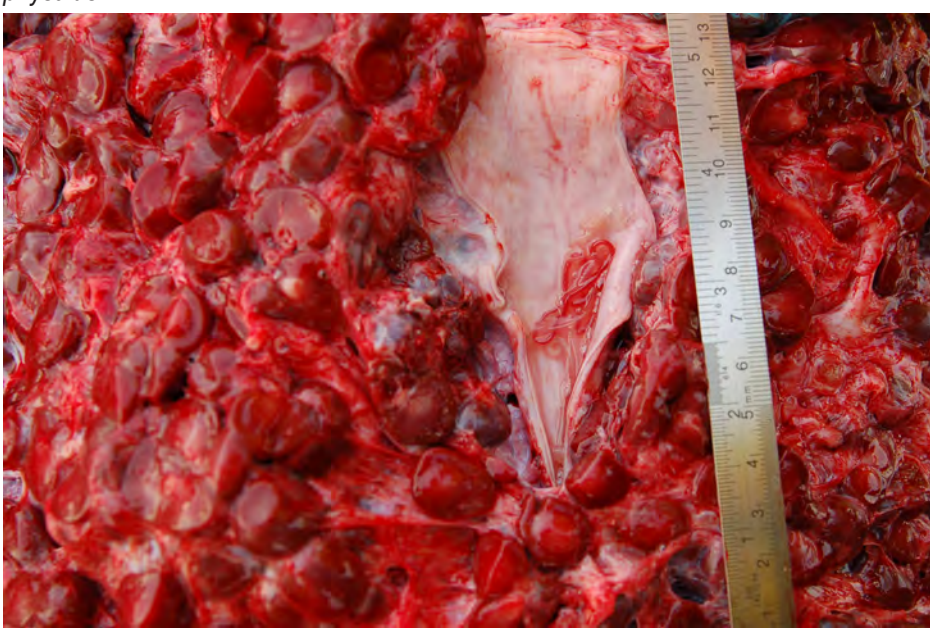

Fig. 3.52: Ureters; parasites (likely *Crassicauda spp.*) within the organ *Balaenoptera physalus*

Fig. 3.53: Liver; hepatic degeneration (glicogenosis) *Grampus griseus*

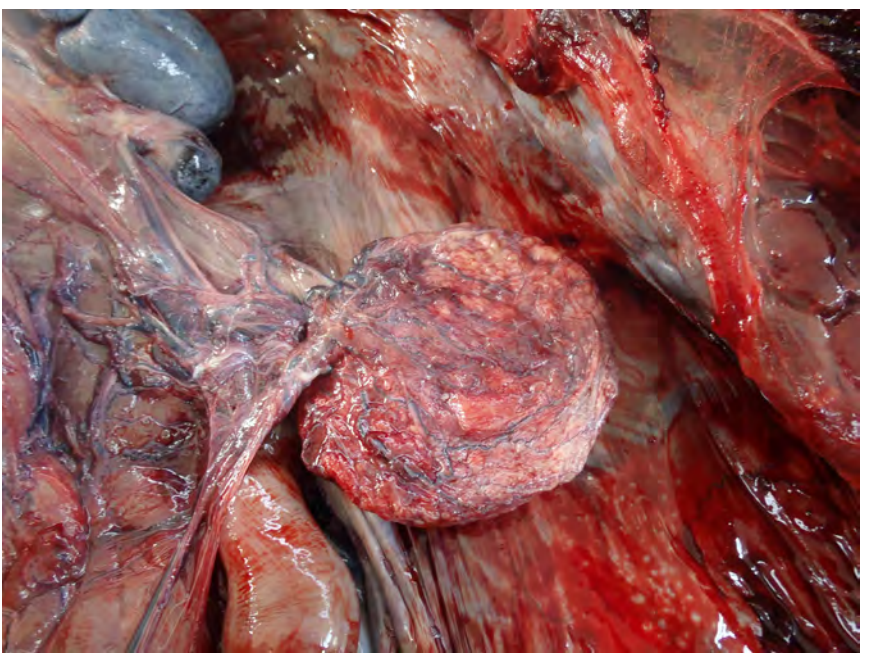

Fig. 3.54: Pancreas; cystic change attached to the mesentery *Grampus griseus*

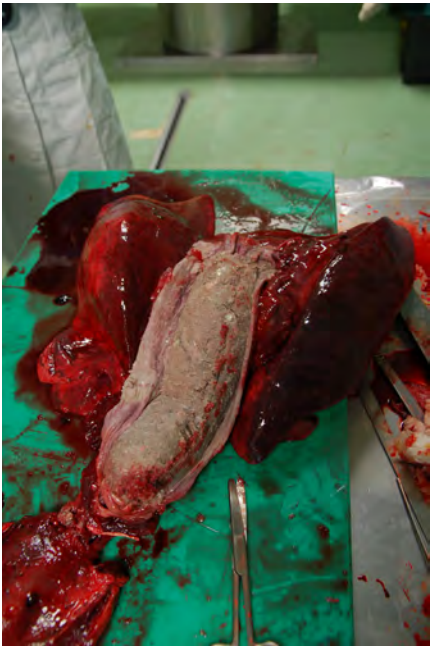

Fig. 3.55: Esophagus; obstruction of the organ *Stenella coeruleoalba*

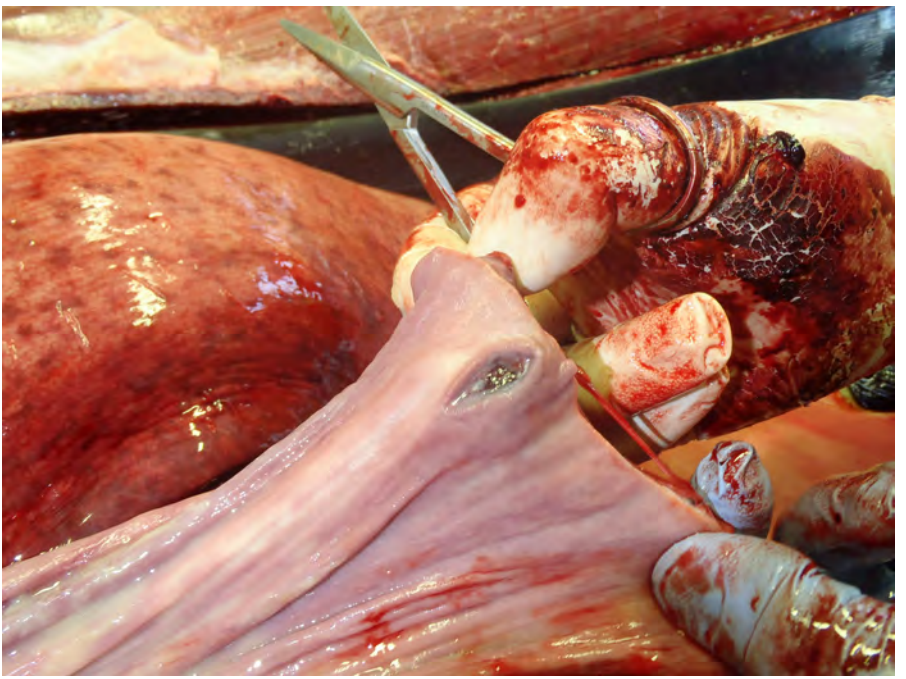

Fig. 3.56: Esophagus; esophageal ulcer *Stenella coeruleoalba*

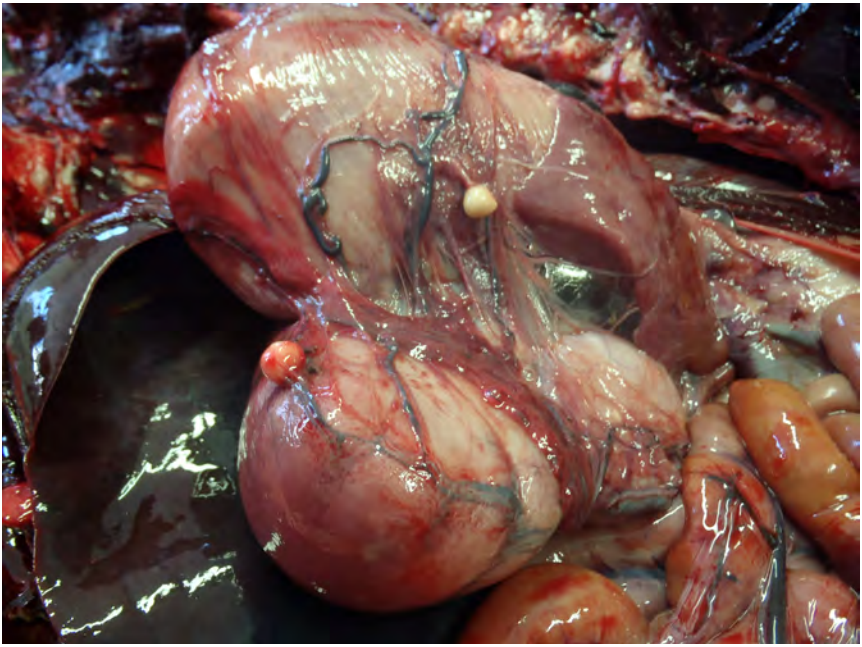

Fig. 3.57: Gastric chambers; ectopic parasites location in the organs serosa *Stenella coeruleoalba*

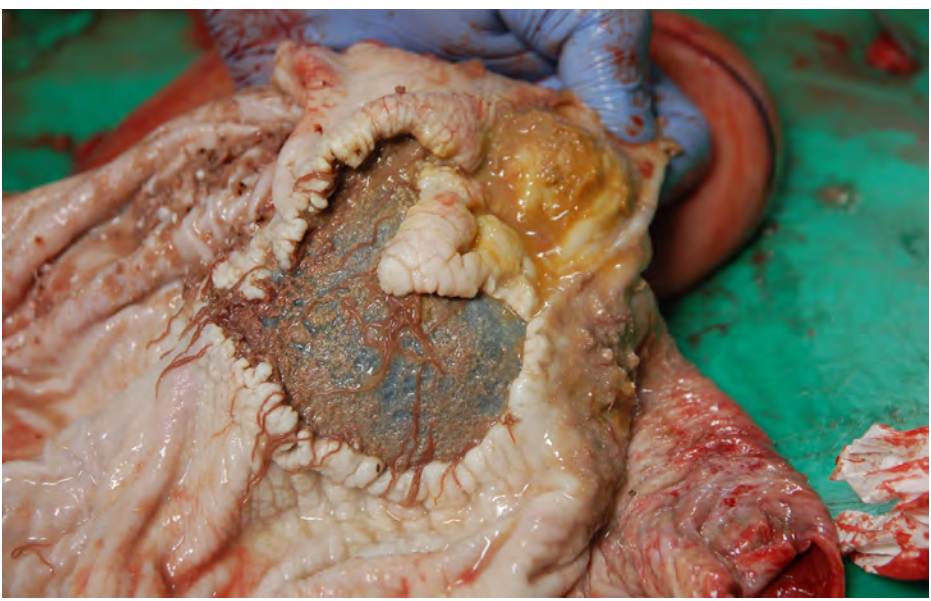

Fig. 3.58:Forestomach; severe ulcer between first and second gastric chammbers associated to larval *Anisakis spp*. *Stenella coeruleoalba*

Fig. 3.59: Forestomach; massive chronic infestation due to *Anisakis spp*. *Stenella coeruleoalba*

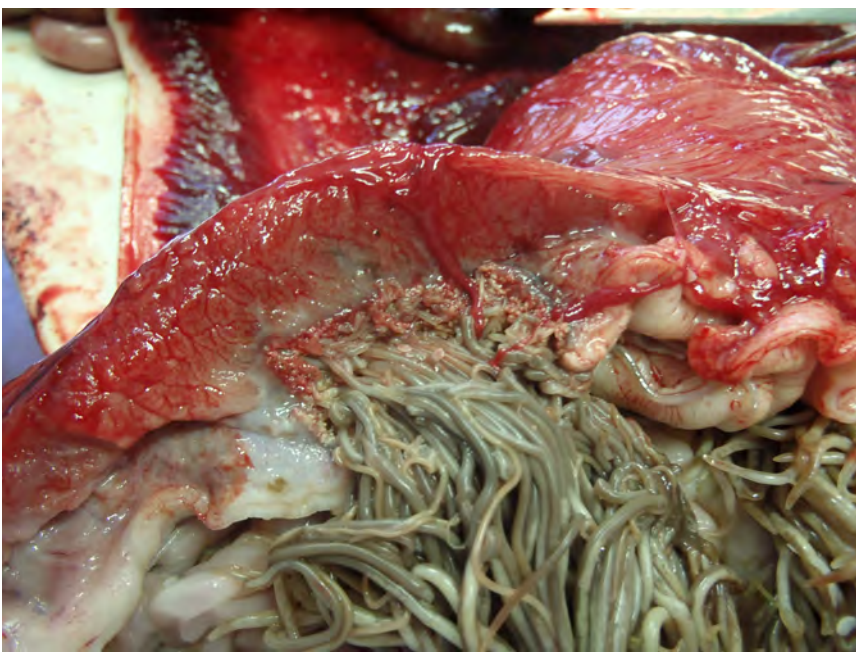

Fig. 3.60: Forestomach; nematodes (likely *Anisakis spp.*) attached to the gastric wall *Stenella coeruleoalba*

Fig. 3.61: Forestomach; plastic bag in the gastric lumen*Stenella coeruleoalba*

Fig. 3.62: Forestomach; 5 kg of fishing gear in the gastric lumen of a badly preserved carcass *Tursiops truncatus*

Fig. 3.63: Forestomach; severe gastric impaction *Stenella coeruleoalba*

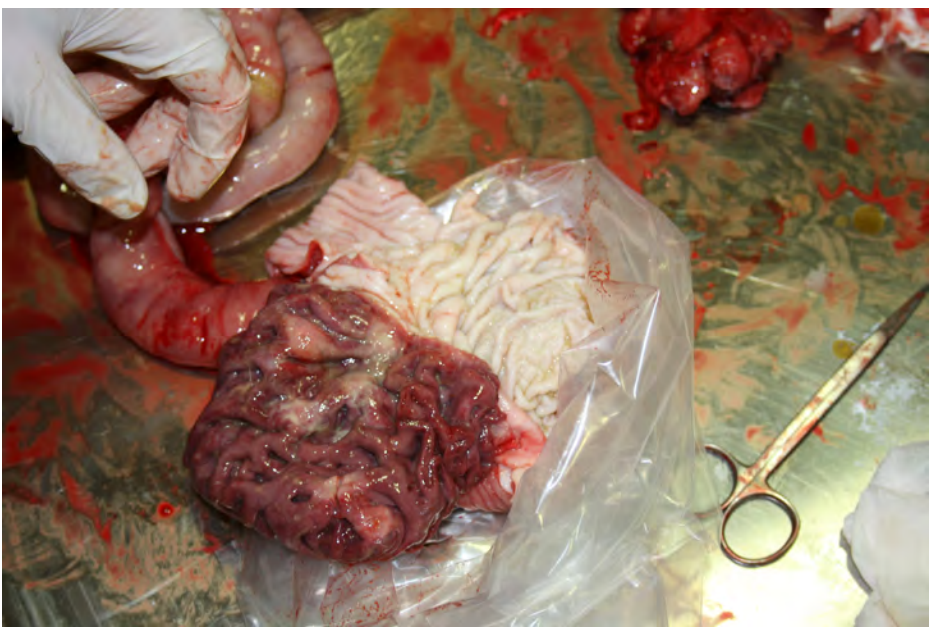

Fig. 3.64: Second gastrica chamber; whitish nodules associated to *Pholeter gastrophilus, Stenella coeruleoalba*

Fig. 3.65: Second gastrica chamber; acute diffuse gastritis with stenosis of the the passage between first and second stomach due to a parasitic nodules determined by *Pholeter gastrophilus* (typical exit pores on the surface of the nodule) *Stenella coeruleoalba*

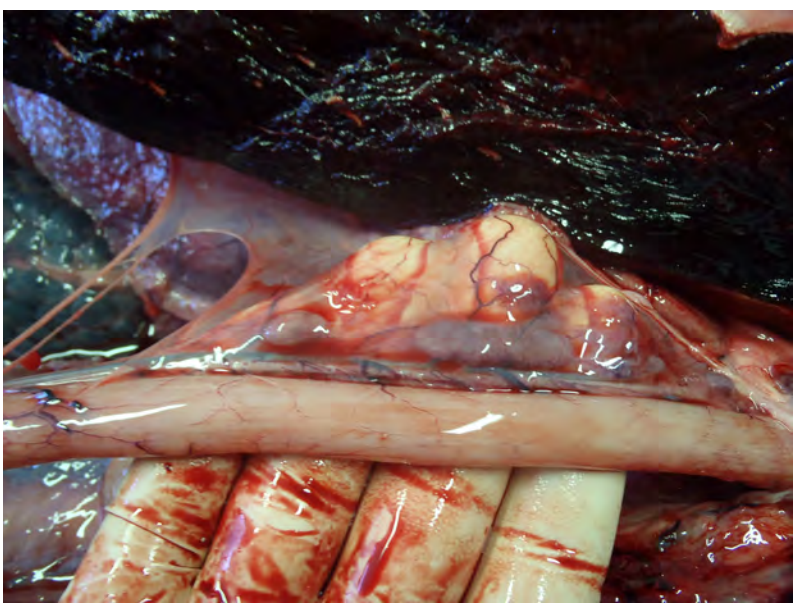

Fig. 3.66:Rectal lymph node; acute lymphadenopathy *Stenella coeruleoalba*

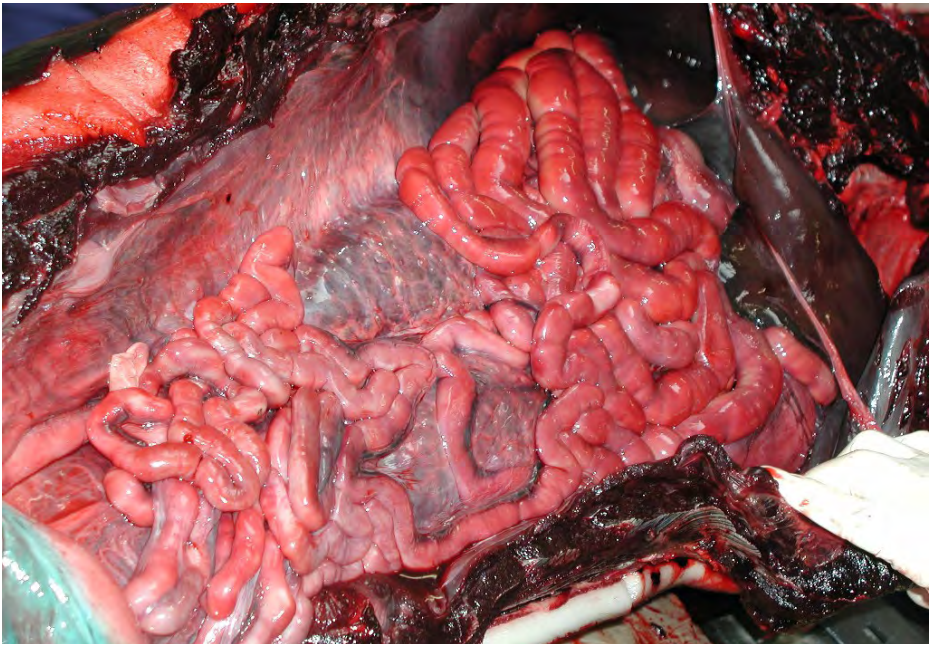

Fig. 3.67:Intestine; diffuse enteritis *Stenella coeruleoalba*

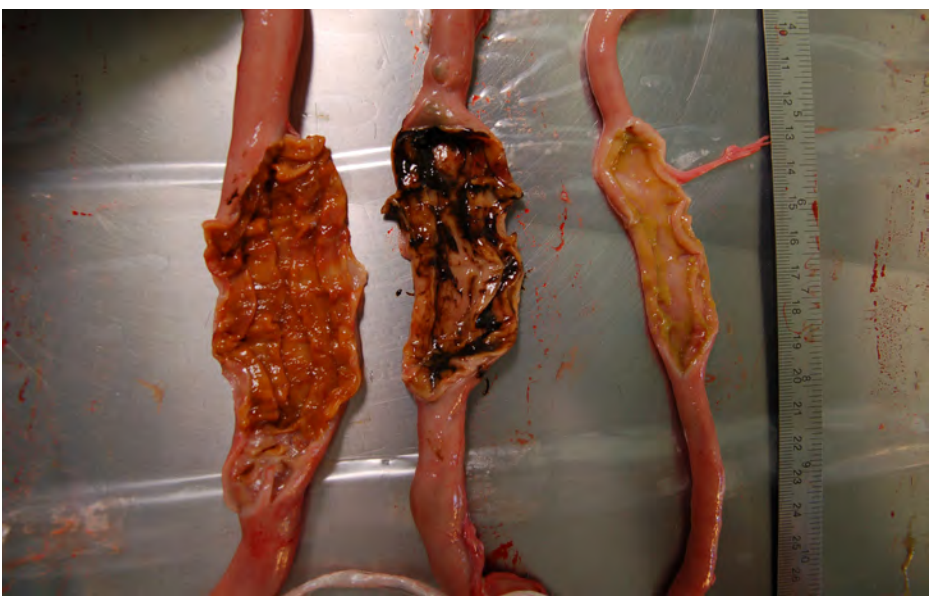

Fig. 3.68: Intestine; chronic segmental enteritis associated to digested blood *Tursiops truncatus*

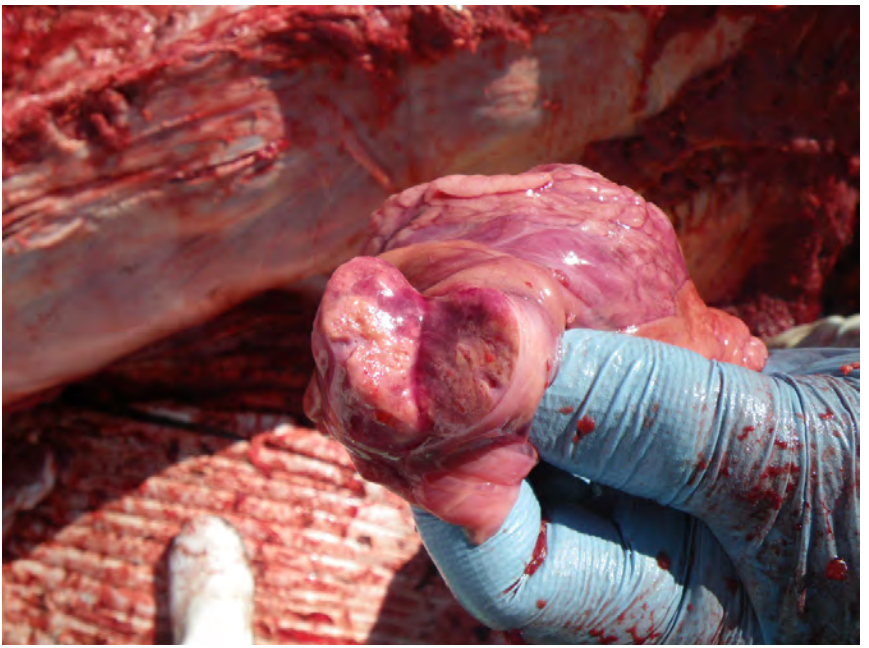

Fig. 3.69: Mesenteric lymph node; lymphadenopathy associated to Morbillivirus infection *Balaenoptera physalus*

Fig. 3.70: Mesenteric lymph node; reactive lymphadenopathy with fibrosis *Tursiops truncatus*

Fig. 3.71: Mesenteric lymph node; chronic reactive lymphadenopathy *Tursiops truncatus*

Fig. 3.72: Mesenteric lymph node; eosinophilic lymphadenitis *Stenella coeruleoalba*

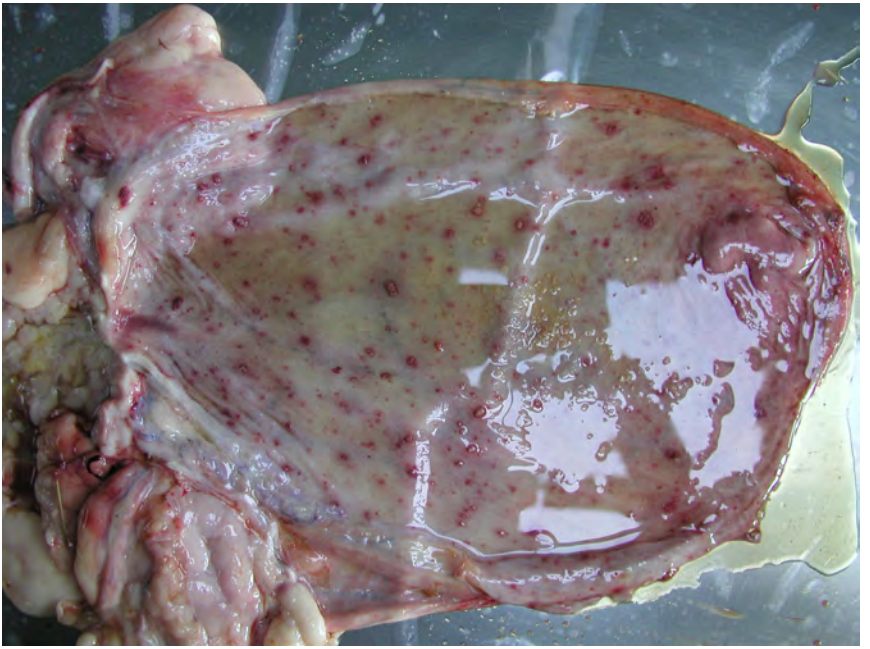

Fig. 3.73: Bladder; acute, disseminated, severe ulcerative cystitis with sand (calculi) *Tursiops truncatus*

Fig. 3.74: Bladder; chronic cystitis *Grampus griseus*

Fig. 3.75: Vagina; vaginal stones *Delphinus delphis*

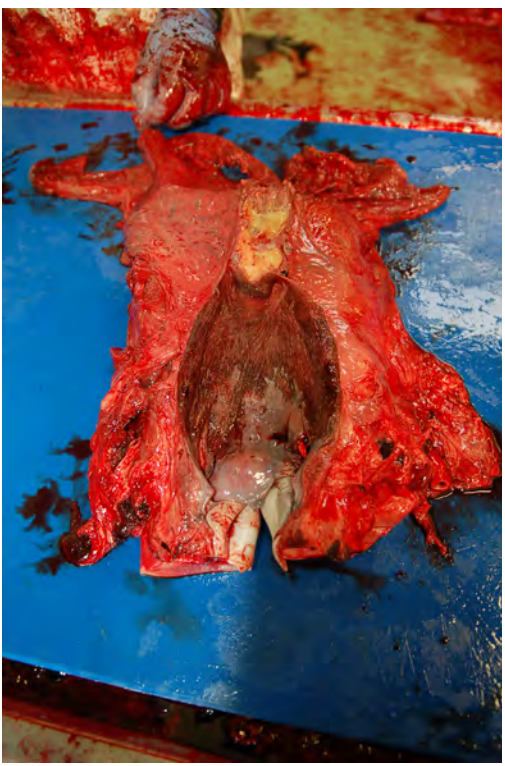

Fig. 3.76 Vagina; severe chronic ulcerative necrotic vaginitis *Grampus griseus*

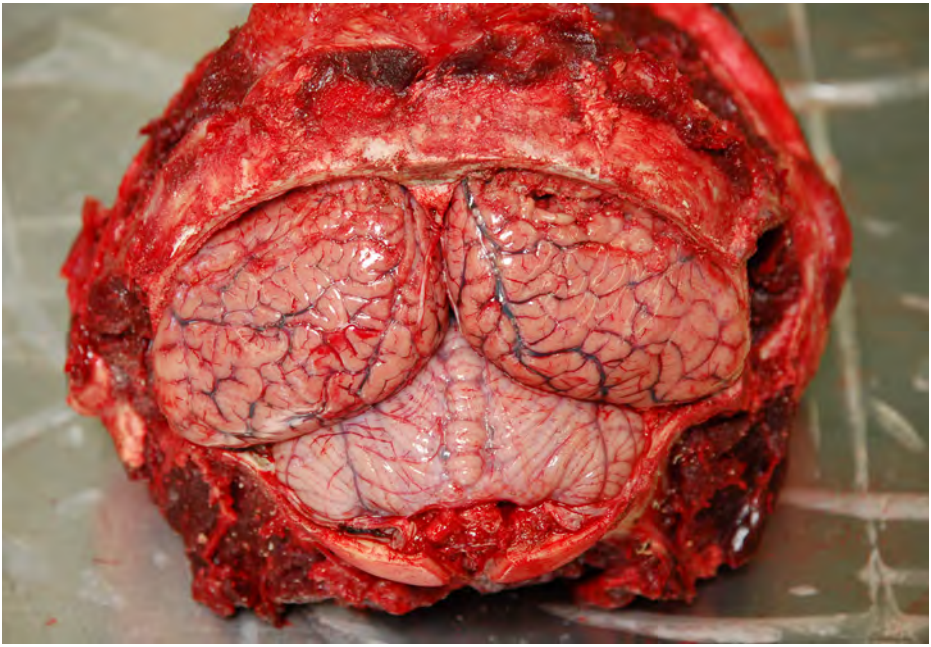

Fig. 3.77: Brain; opening the skull *Tursiops truncatus*

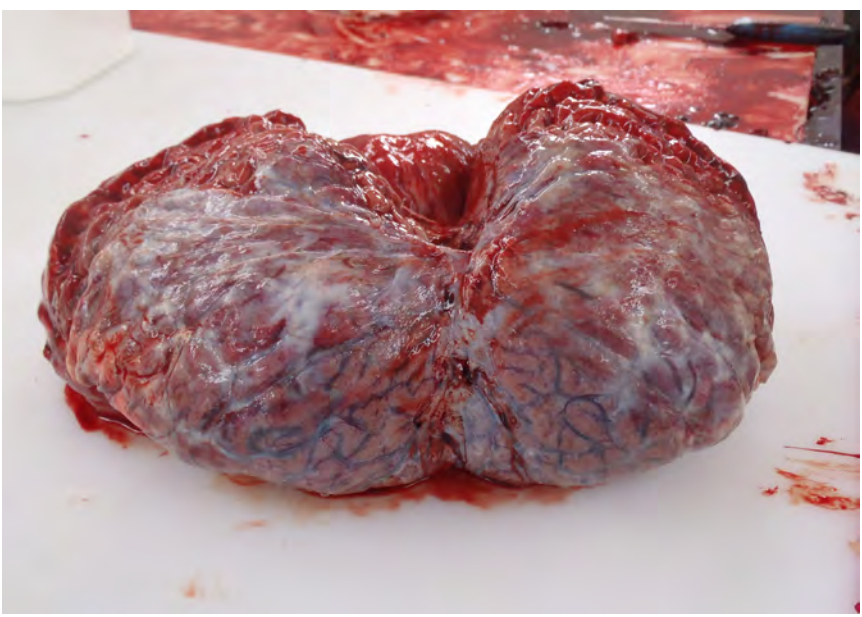

Fig. 3.78: Brain; multifocal meningeal fibrosis likely due to parasitic migration *Grampus griseus*

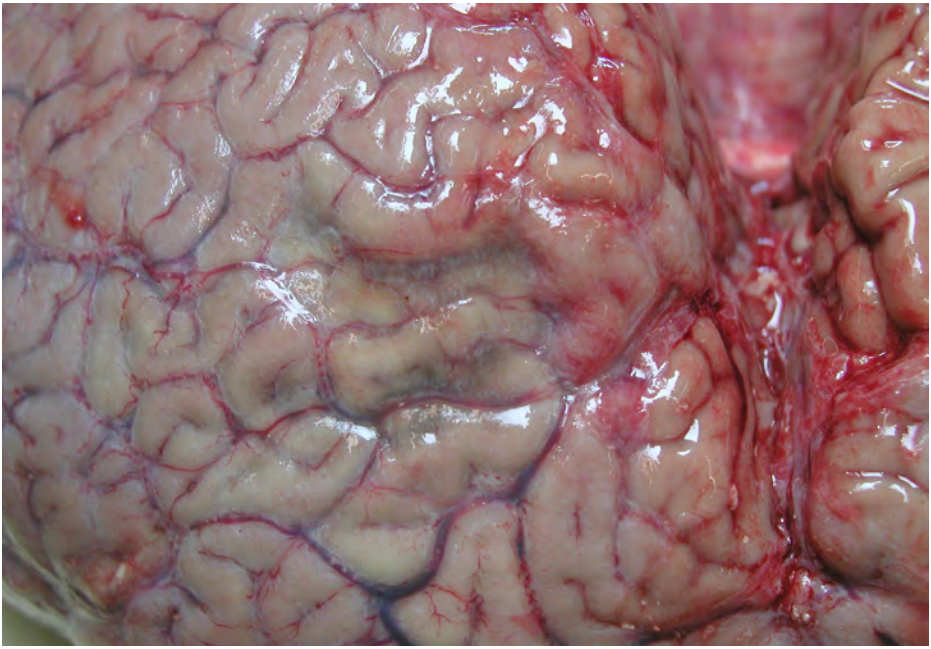

Fig. 3.79: Brain; malacic areas *Tursiops truncatus*

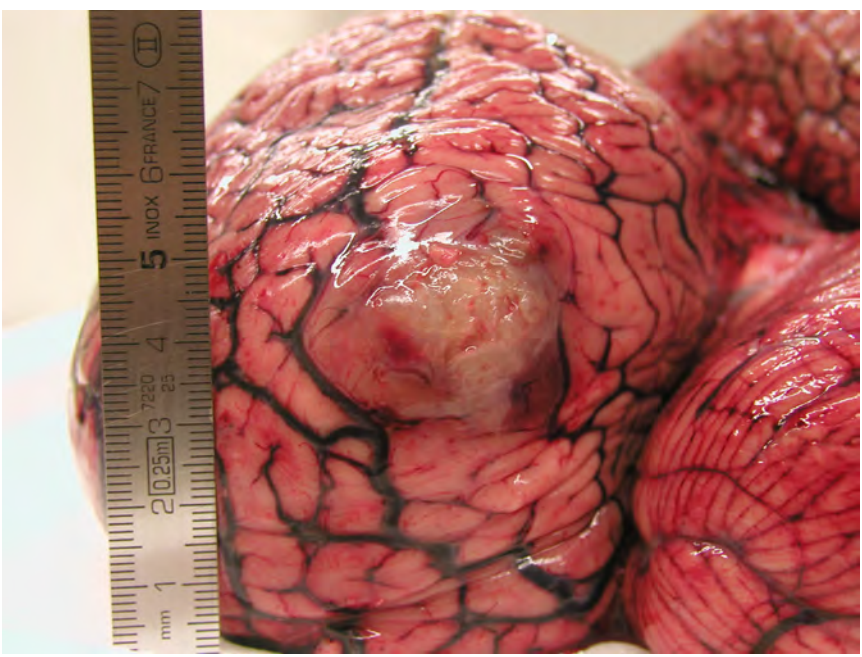

Fig. 3.80: Brain; cholesteatoma *Tursiops truncatus*

Fig. 3.81:Brain; microasbcesses due to *Staphylococcus aureus* in choriod plexuses *Tursiops truncatus*

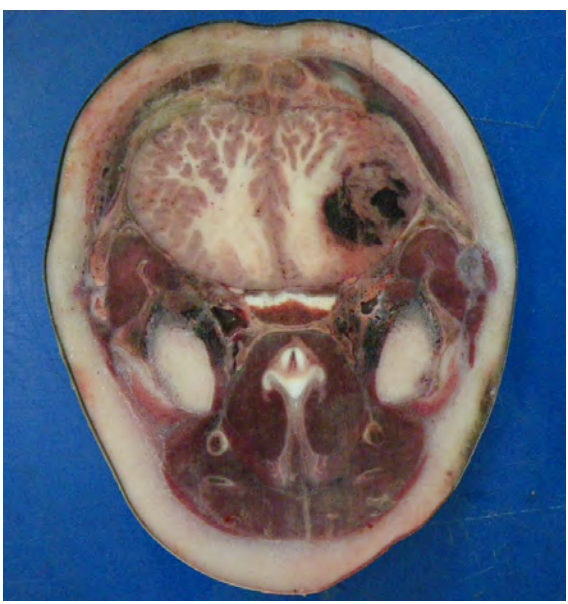

Fig. 3.82:Brain; cerebral hemorrhage due to trauma *Stenella coeruleoalba*

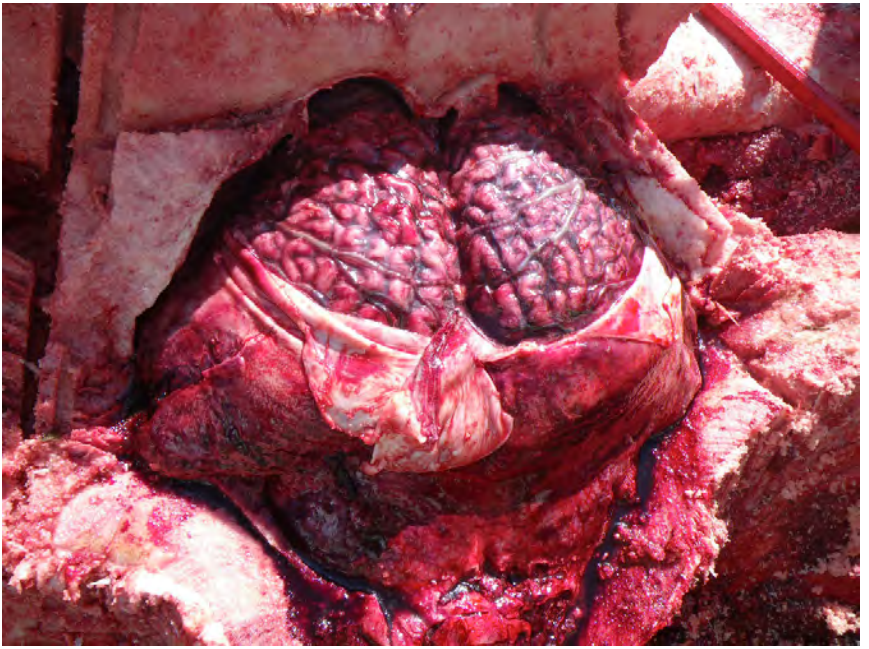

Fig. 3.83: Brain; severe diffuse meningeal hyperemia due to morbilliviral infection *Balaenoptera physalus*

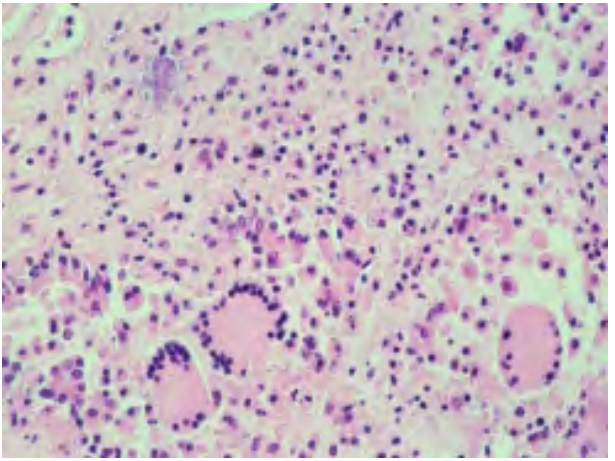

Fig. 4.1: Lung, histological slide (ematossilin and eosin, 40X); multinucleated syncytia *Warthin-Finkelde Stenella coeruleoalba*

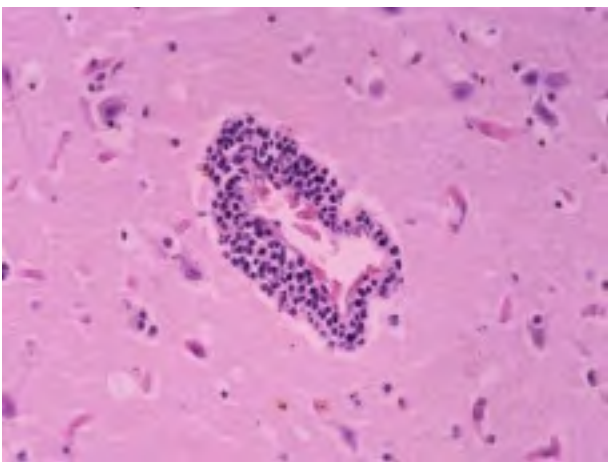

Fig. 4.2:Brain, histological slide (ematossilin and eosin, 40X); moderate cronic encephalitis with inflammatory peri-vascular cuffs *Stenella coeruleoalba*

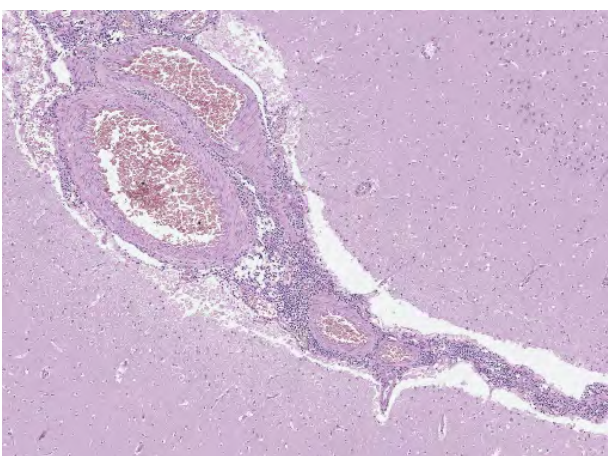

Fig. 4.3:Brain, histological slide (ematossilin and eosin, 10X); moderate diffuse non-suppurative meningitis due to *Brucella spp*. infection *Stenella coeruleoalba*

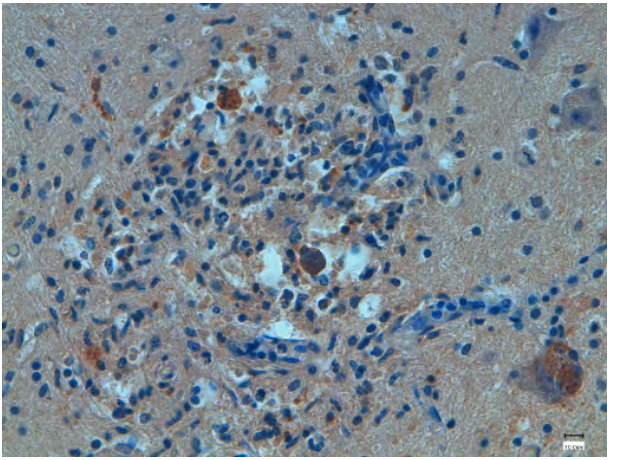

Fig. 4.4: Brain, histological slide (IHC, 40X); micro-granulomas with gliosis in the cerebral cortex, note the presence of the parasite *Tursiops truncatus*

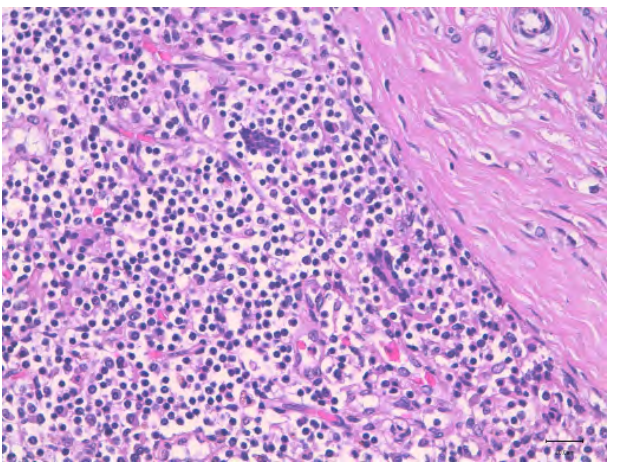

Fig. 4.5 Brain, histological slide (ematossilin and eosin, 40X); multinucelated giants cells *Tursiops truncatus*

Fig. 4.6:Acoustic fat; severe focal haemorrhage *Ziphius cavirostris*

Fig. 5.1: Field equipment in a hard-to-reach location.

Fig. 5.2:Fin whale hoisted on the bank using cranes.

Fig. 5.3: Fin whale hoisted on the bank using cranes. In case of large animals it is better attaching the carcass in two different points to avoid any damage

Fig. 5.4: Fin whale tugged on a sandy shore using two tractors. The carcass could be damaged during these operations.

Fig. 5.5:“Clean” dressing area close to the warehouse one.

Fig. 5.6: Centralized sampling area between the clean area and the necropsy one

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