netcet

STANDARD PROTOCOL FOR POST-MORTEM EXAMINATION ON CETACEANS

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PREFACE

Many protocols have been developed for marine mammals necropsy and sample collections, some for taxonomic groups and others aimed to reveal any human interaction. This protocol is a summary of these procedures and our experience providing a practical approach for networks conduction routine data and sample collection. Furthermore, This protocol is designed to improve knowledge about diseases of free-ranging cetaceans by providing guidelines for people to complete more comprehensive necropsies and disease testing.

Using standardize necropsy protocols is important to facilitate comparison of data among stocks or population. Screening for specific pathogens(i.e., *Brucella*, DMV, and *Toxoplasma gondii*) is of increasing importance for assessing population health and the presence of potential zoonoses. In some cases, negative results are as meaningful as positive ones. It is our hope that once people on the Adriatic Sea realize the need to learn more about diseases of cetaceans, this protocol also will increase the number of complete postmortem necropsies performed on stranded dolphins.

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1. EXTERNAL EXAMINATION

1.1 Condition code

Before initiating the necropsy, carcass condition must be determined. Preferably, necropsies are performed on fresher carcass (within 48 hours of death); however, environmental conditions can greatly impact condition code.

If human interaction is suspected or forensic data are of value, necropsies should be performed irrespective of tissue quality. Carcasses are classified in one of five code categories depending on the level of decomposition.

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

Code 2: Fresh carcass (< 24 hours post mortem). Normal appearance, usually with little scavenger damage, fresh smell, minimal drying and wrinkling of skin, eyes and mucous membranes, eyes clear, carcass not bloated, tongue and penis not protruded.

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

Code 4: Advanced decomposition. Carcass may be intact, but collapsed, skin sloughing, often severe scavenger damage, strong odor, blubber or muscle easily torn or falling off bones, liquefied internal organs.

Code 5: Mummified or skeletal remains. Carcass completely desiccated, often with dried skin draped over bones.

Condition code 1

Condition code 2

Condition code 3

Condition code 5

1.2 Nutritional condition

The body condition of a cetacean can be assessed by looking along the dorsal axis of the animal. The dorsal muscle mass (epaxial muscle) to either side of the dorsal fin of a robust animal will be rounded or convex. A thin animal will have a slight loss in epaxial muscle girth and could have a minor sunken aspect to the dorsal-lateral body. An emaciated animal will have a greater loss of epaxial muscle girth and will be concave down the dorsal-lateral body. Emaciated animal may also have more prominent indentation at the nape.

1.3 Sex determination

To determinate the sex of a small cetacean, examine the ventral midline of the animal. Both male and female cetaceans possess a genital silt between the umbilicus and anus. For female cetaceans, there should generally be less than 10 cm distance between the centers of the anal opening and the genital slit. Whereas with a male, the distance between the anus and genital slit is much greater.
A single short mammary slit can be seen on either side of the genital slit in most female cetaceans, though some males may also possess this feature.
A more definitive method to sex a cetacean is by blunt-probing the genital slit. If the probe angles forward it has entered the vagina and is, thus, a female. If the probe angles backward it has entered the penile opening of a male (often the distal end of the penis can be felt as well). When probing, be sure that your finger has penetrated past the first knuckle in order to ensure accurate sex determination.
It is important to note also, that different species are easier to probe than others; common dolphins are often quite difficult to accurately probe due to very small genital apertures. Final confirmation of gender will always be a result of internal examination.

Genital slit and mammary slit - Female (*Stenella coeruleoalba*)

Penis - Male (*Tursiops truncatus*)

1.4 Age estimation

Estimation of age for specific animals is important from an epidemiological perspective, as well as important in understanding the basic biological characteristics of a particular species. Currently age is estimated primarily from counts of growth layer deposited in several persistent tissues, primarily teeth and, less often, bone. Saving teeth or other tissue for aging from known-age animals is also important, because these tissue are used to validate the interpretation of growth layers for specific taxa.
Relative measures of age, such as tooth wear, pelage or skin color, or fusion of cranial sutures, which allow individuals to be placed in age groups, are helpful. Age class or maturation status may be estimated using body size (length), fusion of epiphyses, pelage color, or reproductive parameters.
Among the physical parameters, length definitely helps to define the estimated age. The average length allows firstly to differentiate whether it is a subject newborn or adult. Few days' infants can be identified by the presence of lingual papillae and a navel patent. The intermediate length between an adult and a newborn allows to classifying the animal as young. Finally, an old animal is characterized by a size comparable to that of an adult one, associate with aspects of muscle atrophy along the trunk and teeth missing or excessively worn.

Species	Tot. length birth (cm)	Tot. length pup (cm)	Tot. length 1 year (cm)	Tot. length 2 years (cm)	Tot. length weaning (cm)	Tot. length adult (m)
Stenella coeruleoalba	93-100	100	166	180	170	2.2-2.6
Tursiops truncatus	117	100-130	170-200	170-225	225	2,2-3 cost. 2,5-6 pel.
Grampus griseous	110-150	120-160				3-4
Delphinus delphis	80-90	80-100			110-120	2,3-2,5
Ziphius cavirostris	270	200-300				6,7-7

Estimated age based on animal's length

1.5 Integument

An external examination should include the investigation and description of the eyes, mouth, blowhole, umbilicus, genital opening and anus.
When examining the eyes, look for discoloration, injuries, or discharge.
Document any lesions, parasites, and the mucus membrane color in the mouth. Make note of worn, broken or missing teeth.
Describe color and amount of discharge from blowhole as well as the presence of parasites or obstructions. Obtain culture swabs.
Examine the umbilicus in neonates for signs of infection and degree of healing.
Look for lesions, discharge or growths around the genital opening and anus. Obtain samples of abnormalities for histology, microbiology and molecular investigations.
If the animal has mammary glands, attempt to express milk and note color, consistency and estimate amount in cc's or mls. Milk can be expressed by pressing on the body about 10cm dorsal and cranial to the mammary slit and massaging downward toward the slit.

1.6 Teeth

Teeth from the center of the lower left mandible are collected for life history analysis. Using a scalpel blade, transversely cut in between and around 5 -7 teeth. Teeth can be extracted by inserting tooth extractor or a flat head screwdriver in the incision made between the teeth and wiggling the tool down to the base of the mandible until the entire, undamaged, tooth becomes loose. Avoid snapping or crushing the tooth, as such damage can render the sample useless for analysis. In some species and in older animals, a sturdy knife may be advisable over a scalpel to avoid breaking the blade

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Teeth (*Tursiops truncatus*)

Teeth (*Stenella coeruleoalba*)

Teeth (*Grampus griseus*)

Teeth (*Ziphius cavirostris*)

1.7 Skin and Blubber

Examine and document any scars, abscesses, ulcerations, erosions, wounds and parasites on the skin. Make note of the size (length x width x depth/height), shape, color, texture, location and distribution of all abnormalities.

Remove about two cm^2 of skin from the tip of the dorsal fin or flukes for genetic (frozen and DMSO) and histology samples. A skin sample with no blubber attached is preferred. Trim the skin as cleanly as possible from the other tissue. (Be sure all morphometric measurements have been completed before collecting this sample.)

Skin with parasites (*Tursiops truncatus*)

Skin (*Tursiops truncatus*)

The first step to examine the body cavity of the animal is removal of the blubber. Position the animal left side up. Using a scalpel blade or knife, start just left of the dorsal midline posterior to the blowhole and make a longitudinal incision down the length of the animal ending at the dorsal tail stock. Do not penetrate into the skeletal muscle, cut only through the skin and blubber layers. Next, make a dorso-ventral incision perpendicular to the previous body length incision just cranial to the anterior insertion of the left pectoral flipper. Continue making perpendicular incisions down the length of the animal that are ~ 20 cm apart, creating a series of panels along the lateral body. At the top of each panel begin to separate the blubber from the muscle by cutting through the fascia or connective tissue. If you remain between the blubber/ muscle interface (fascia) and reflect the panel of skin down and away from the body, in a dorsal to ventral direction, the blubber should easily separate from the muscle.
Note the thickness, color and texture of the blubber. Look for parasites and abnormalities within the blubber layer.
Obtain blubber samples for histology and contaminants. When collecting these samples, be sure to collect blubber without any skin or muscle attached and be sure to take the sample from the same location on each carcass, generally from the dorsal mid-thoracic region.
Once the blubber has been examined, make a cut along each reflected panel at the ventral midline and discard the blubber.

Incisions to separate the blubber from the muscle (*Tursiops truncatus*)

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1.8 Skeletal Muscle

Examine the quality of the fascia and muscle on the body before removing it. Note the color, texture, thickness and abnormalities. Look for hemorrhage, post mortem pooling of blood in vessels (hypostasis or post mortem lividity) and bruising (hematoma). Bruising usually has a gelatinous texture and is deep maroon to purple.

Remove the large dorsolateral muscle mass or epaxial muscle that spans from the occipital ridge down to the tail stock. Use the dorsal and lateral spinal processes as landmark boundaries for this muscle. Trim away as much muscle as possible from the backbone and ribs.

Obtain muscle samples for histology and contaminants.

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2. INTERNAL EXAMINATION

2.1 Removal of the Scapula and Prescapular Lymph Node

Locate the prescapular lymph node prior to the complete removal of the scapula. The oval to triangular shaped, beige to peach tissue is located just underneath the cranial corner of the scapula, proximal to the location of 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 very slight differentiation between the cortex (outer layer) and medulla (center area). If the tissue begins to vary from the homogenous peach to tan it is indicative of a reaction.
Note the size, shape, color and texture of the prescapular lymph node. Be sure to distinguish changes of the cortex from changes of the medulla. Sample for histology, microbiology and molecular investigations.
Remove the left scapula and appendage by cutting through the connective tissue and muscle just underneath the bone. If you pull the scapula ventro-laterally, reflecting it down as done with the blubber layer, the scapula will detach easily. You should hear a crackling sound as you pull and cut indicating that you are in the correct spot between muscle groups.

2.2 Removal of the Rib Cage

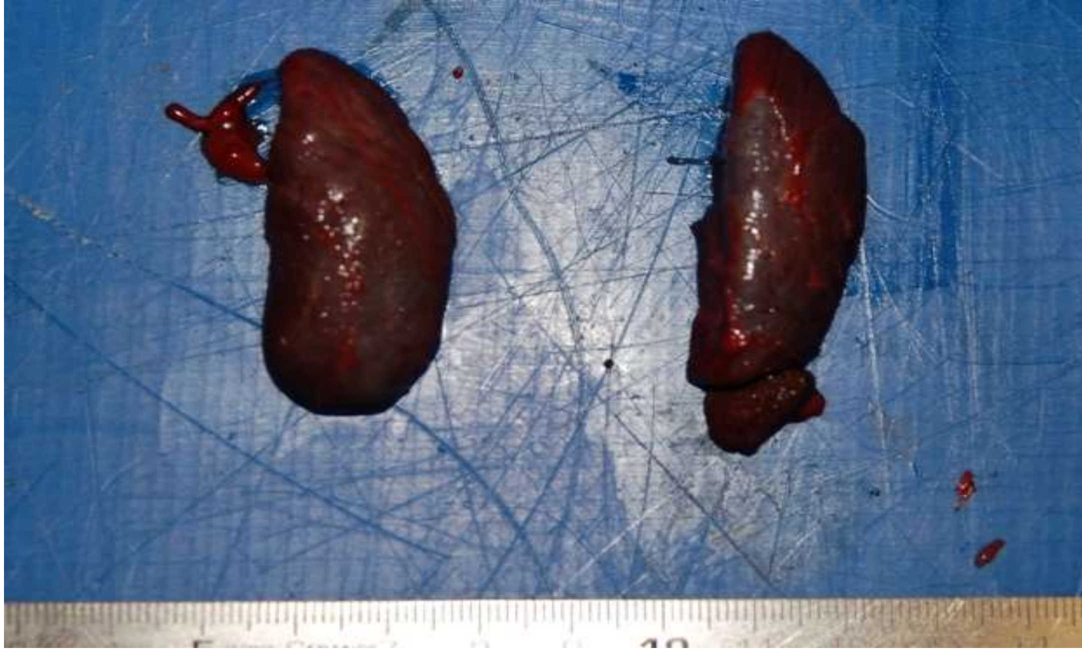
Before collecting any samples or cutting the ribs, the diaphragm should be punctured with a scalpel or scissors and deflation should be noted. If the diaphragm is already deflated, it is possible that a pneumothorax or severe pneumonia may be present.
To open the thoracic cavity, start at the caudal end of the left rib cage and feel for the articulation between each individual rib and vertebrae. The ribs and vertebrae should easily separate, without breaking, if you cut through the articulation with a scalpel blade or small knife. Also to note, age and disease may affect the way the joints disarticulate. Move cranially from rib to rib maintaining a constant angle with your scalpel as you cut and moving the rib to find the articulation.
Note that the most cranial ribs are double-headed along the vertebrae. The doubleheaded ribs can be removed by first severing the first articulation, then by sliding the scalpel along the inside of the second head to reach its articulation with the spine. The articulation can be cut by sweeping the scalpel parallel to the long axis of the animal. The rib articulations should feel smooth, not granular. Feel for fractures and bone spurs on the rib cage.
Removed in this manner, the skeleton may be of more value for future bone pathology studies, educational outreach or as museum specimens.
Once the rib cage is removed, examine the body cavity with all organs in place. Note any discoloration, lesions, adhesions, odor or fluids.

At this point, one needs to adhere to a systematic examination of the internal tissues. The organs may be removed as a pluck, or may be examined in situ. The method of sampling can be guided by sampling needs, condition code, and personal preference. It is recommended that internal fluids, such as those in the gastro-intestinal system do not contaminate other tissues.

Thoracic cavity (*Stenella coeruleoalba*)

2.3 Thyroid

The thyroid sits ventrally on the cranial trachea and spans the width of the trachea. The thyroid is one of the more difficult tissues to locate and identify. The color and texture are often similar to smooth muscle.
The parathyroid is a small, light colored tissue attached to the thyroid along the cranial margin of the thyroid and can aid in correct tissue identification if it can be found. Examine the tissue externally and internally.
Note the size, shape, color and texture. Sample for histology, microbiology and molecular investigations.



Thyroid (*Stenella coeruleoalba*)

2.4 Thymus

The thymus is a large, lymphoid organ, that is primarily found in neonates and some juveniles. It is situated at the base of the thoracic inlet, cranial to the anterior margin of the heart. The thymus is absorbed with time after weaning, thus is not usually visible in adult marine mammals. Examine the tissue externally and internally.
Note the size, shape, color and texture. Sample for histology, microbiology and molecular investigations.

THORACIC CAVITY

2.5 Tracheobronchial Lymph Node

The tracheobronchial 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. It is recommended that this tissue be identified and removed prior to removal of the lung or trachea, as it can be easily lost without anatomical landmarks. Examine the lymph node externally and internally. Describe any differences between the cortex and medulla.
Note any other changes in size, shape, color and texture. Sample for histology, microbiology and molecular investigations.

2.6 Esophagus

Trace the esophagus from the exposed caudal end to the mouth, opening the esophagus in the same manner as done with the trachea. Observe the serosal and mucosal surfaces of the esophagus.
Note, color, texture and contents. Sample for histology.

2.7 Trachea

The trachea is a long, firm, off-white, flexible, ridged, tubular organ that extends from the larynx to the tracheal bifurcation. Using scissors, cut through the entire length of the trachea from the bifurcation up to the apex of the thorax.

Examine the mucosa and identify and describe contents (froth, fluid, blood, color, etc.). Sample for histology.

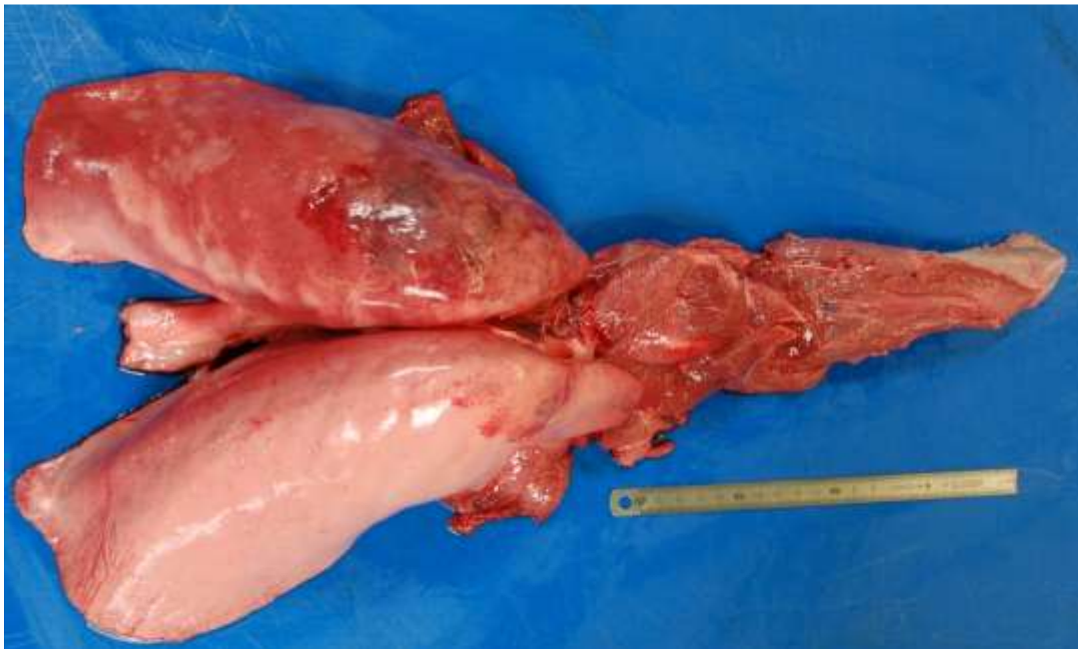
2.8 Lungs

The lungs occupy the majority of the thoracic cavity and are the large, normally bright pink, tissue with a consistent sponge-like texture. Detach the lung from the trachea at the bifurcation. Examine the pleural surface: note color pattern and texture. Normal, air-filled lung tissue should bounce back immediately after being depressed with a finger (like a sponge) and float when placed in water or formalin.

To examine the internal structures, using scissors, trace the trachea from the bifurcation along the bronchi and into the bronchioles of each lung. Note whether fluid, froth and/or parasites are present and describe amount, color, *etc*.

Next, make serial cuts into the tissue by "bread-slicing" (making multiple, parallel slices into the tissue) perpendicular to the long axis of the body to examine the parenchyma. This is best done with a long knife using a single sweeping cut in order to avoid tearing or serrating the lung tissue.

Examine the parenchyma and note color pattern and texture. Sample for histology, microbiology and molecular investigations.



Lung (*Tursiops truncatus*)

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2.9 Heart Muscle and Valves

Before handling the heart, observe and describe the pericardium. There should be a small amount of clear fluid within the pericardium to allow for lubrication. Note if there is excessive fluid and describe the characteristics. Also, note the presence of gas bubbles within the pericardium and vessels and note thickness of the tissue.

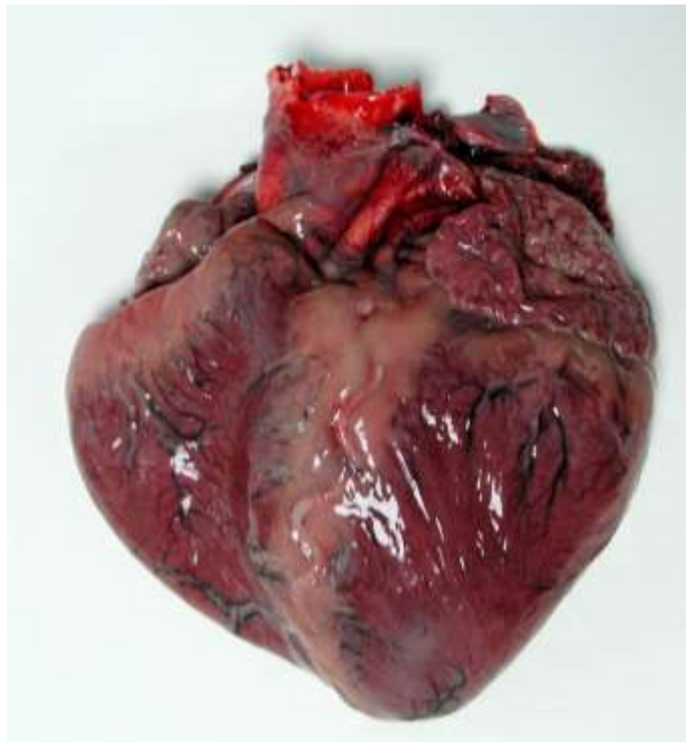
Trim away the pericardium and observe the epicardium (external surface of heart) in situ. Note size, color, and texture of each structure (right and left atria and ventricles, aorta, and pulmonary vessel).

Remove the heart by cutting transversely across the aorta and pulmonary artery leaving approximately 6.0 cm of each vessel still attached to the heart muscle. There are varying techniques for examining the internal structures of the heart. One way is to use scissors 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. Continue cutting along the right ventricle side of the septum until this chamber joins the pulmonary artery and cut up through the vessel. Next, snip the left ventricle side of the apex, cut through the muscle along the septum, and up through the aorta. This process leaves both sides of the heart intact.

A simpler way to examine the endocardium (inner surface of the heart) is by slicing the organ completely in half starting at the apex going laterally toward the vessels, so that it opens up like a book. Examine each chamber for the presence of worms or other foreign matter. Note the size/thickness of each atrium and ventricle, as well as color and texture. The left ventricle should be substantially thicker than the right.

Thoroughly examine the interior of the valves for changes in texture or thickness. Normal mitral and tricuspid valves should be thin and slightly opaque. Once the endocardium is examined, bread–slice the ventricles to examine changes in the myocardium.

Sample the right and left ventricles and atria, septum, apex, atria and aorta for histology.



Heart (*Tursiops truncatus*)

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2.10 Diaphragm

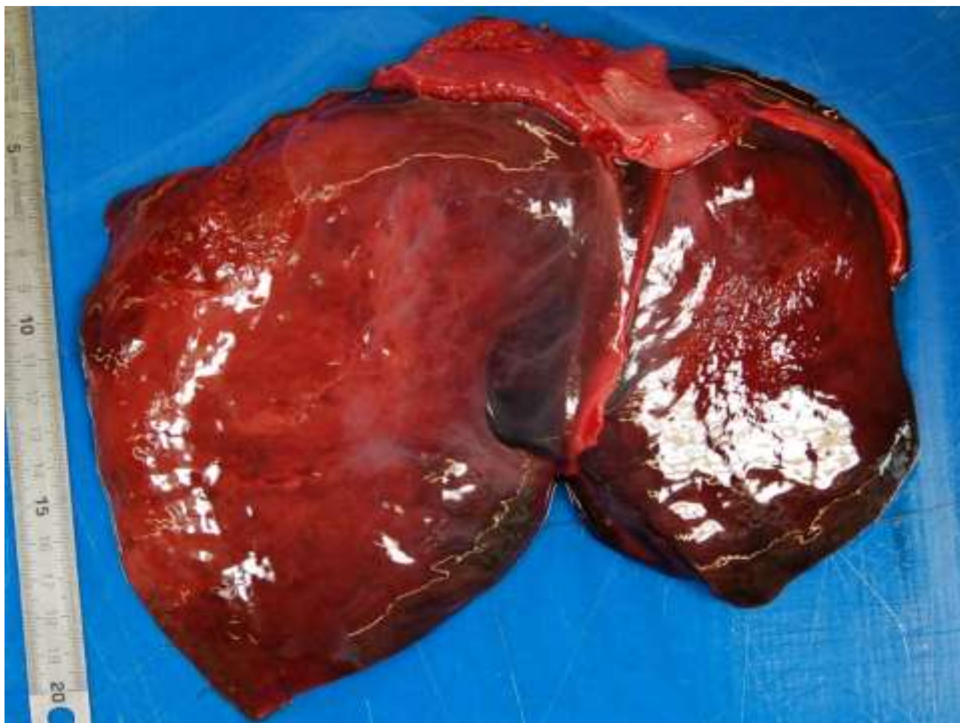
The diaphragm is the thin, smooth textured, dark maroon, expandable muscle that is attached to the caudal rib cage and separates the thoracic and abdominal cavities. Note the texture and color as well as any tears or adhesions. White striations over the surface of the diaphragm are normal. Trim away the diaphragm enough so that there is complete access to the abdominal organs. Sample for histology.

ABDOMINAL CAVITY

To expose the abdominal organs, incise the abdominal wall from the last rib mid-ventral to the level of the anus. Extend the most cranial cut laterally along the thoracic arch and reflect the abdominal musculature to expose the internal tissues. The orientation of the organs should be visually assessed and any free fluids aseptically collect in a sterile syringe prior to proceeding with the internal examination.

2.11 Liver

The multi-lobular, diffusely maroon liver is large, lies over the stomach and dominates most of the abdominal cavity. Examine the parietal (toward the body wall) and visceral (toward the organs) surfaces of the liver and note color pattern, texture and size of the lobes. Examine the parenchyma of the liver by bread-slicing through the tissue. Again, note the color and texture within.
Examine bile ducts for presence of parasites. Sample for contaminants, histology, microbiology and molecular investigations. All cetaceans lack a gall bladder.



Liver (*Tursiops truncatus*)

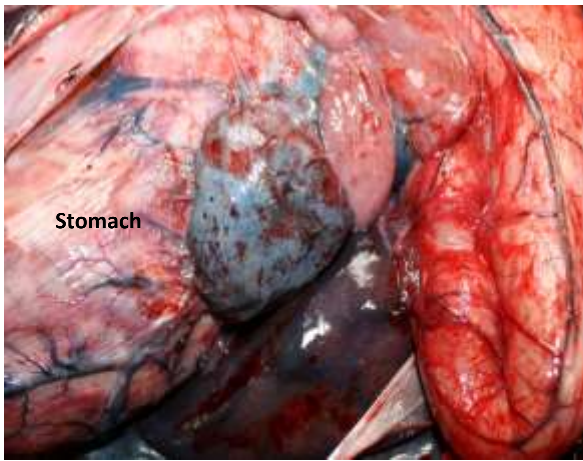
2.12 Spleen

The shape and size of the spleen vary among cetacean species. The spleens of most dolphin are palm-sized, spherical and mottled dark purple to white with a smooth external texture. For other species, the spleen may share these characteristics or be smaller and oblong.

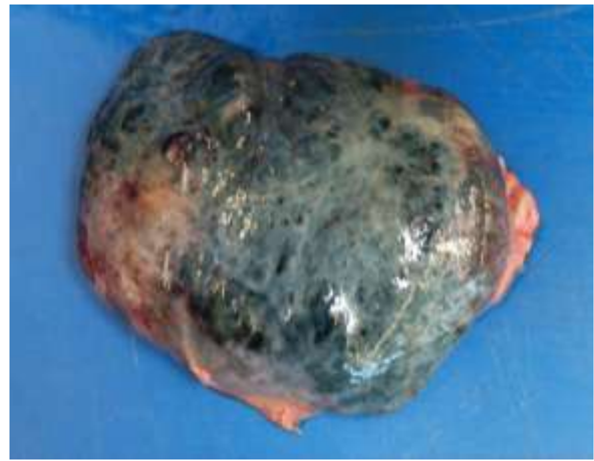
Regardless of physical characteristics, the organ is always located underneath the main stomach toward the left side of the body. Remove the spleen by detaching it from the omentum (thin, web-like, connective tissue).

Note size, shape, color and texture of both the surface and the parenchyma of the spleen. In some cases, smaller (0.2—1.0cm), accessory spleens may be attached to the visceral surface of the spleen. These smaller spleens share the same characteristics as the larger spleen.

Sample for histology, microbiology and molecular investigations.



Spleen (*Stenella coeruleoalba*)



Spleen (*Tursiops truncatus*)

2.13 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. Remove the pancreas from the cavity by detaching it from the connective tissue and duodenum.

Note the size, shape, color, and texture of the surface. Cut into the parenchyma and note changes in color or texture. Examine ducts for parasites.

Sample for histology, microbiology and molecular investigations.

2.14 Mesentery and the mesenteric Lymph Node

The mesentery is a broad sheet of connective tissue which attaches the intestines (and other viscera) to the mesenteric root. This connective tissue should be translucent and show some resistance when attempting to bluntly dissect. Examine the mesentery for parasitic or fungal attachments or other abnormalities. Note thickness and opacity.

The mesenteric lymph node is a finger-like, gray to tan colored, larger lymph node that is centrally attached to the mesentery. Remove the lymph node by detaching it from the mesentery.

Note the size, shape, and color of the mesenteric lymph node. Examine the external surface and internal structures for changes in color and texture. Unlike previous lymph nodes discussed, the mesenteric lymph node tends to have a more defined cortex and medulla. Be sure to describe each structure separately.

Sample for histology, microbiology and molecular investigations.

2.15 Stomach

The stomach of most odontocetes is comprised of three compartments: the fore stomach, the main stomach, and the pyloric stomach. There is also the omentum, net-like connective tissue that is attached to the visceral side of the stomach.

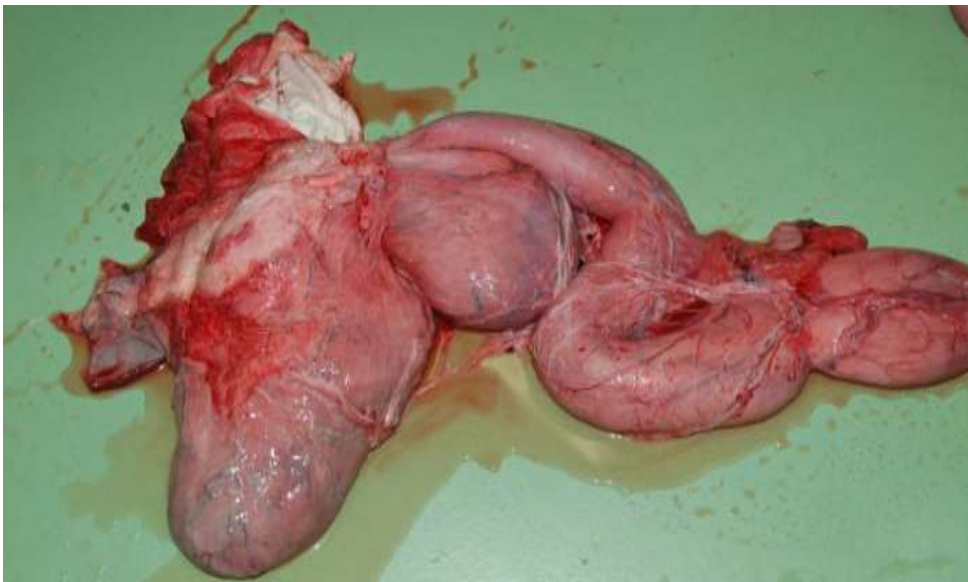
To avoid contaminating the remaining tissues in the body cavity or losing contents, it is necessary to tie off both ends of the stomach prior to extracting. With some twine, tie a tight, secure knot at the location of the attachment of the esophagus to the fore stomach. A second piece of twine can be tied just below the base of the pyloric stomach where the small intestines begin. Remove the stomach from the carcass by cutting beyond both knots. Examine the serosal (external) surface of the stomach for discoloration and lesions. If an internal pathology is present, the peri-gastric lymph nodes attached to the stomach should be noticeably enlarged. Sample for histology, microbiology and molecular investigations and make note on the sample inventory list if this is the case.

Otherwise, remove all excess attached tissue from the exterior of the stomach and weigh the stomach full.

Using a scalpel, make an incision through the wall along the greater curvature of each stomach large enough to allow examination of the contents and the entire mucosal surface. Describing the contents of each compartment separately, note the composition of stomach contents (fluid; whole or partially digested fish; fish bones; parasites; foreign objects). Be sure to describe amounts, color and texture. Prior to further manipulation, collect a sample of contents for biotoxins. The remaining contents can be emptied and rinsed into a sieve to ensure solid material is not lost and is thoroughly examined. Save all foreign objects for human interaction documentation.

Once empty, examine the mucosa of the stomach. Note the color and texture of the mucosa of each compartment separately. The mucosa of the fore stomach is composed of squamous tissue and is usually white. The wall of the main stomach is stratified and usually thicker than that of the fore stomach. The mucosa is usually dark red. The pyloric stomach tends to be thin walled, glandular, and the mucosa is pink or stained with bile. Look for ulcers, areas of discoloration and other abnormalities. Weigh the stomach empty.

Sample each compartment for histology.



Stomach (*Tursiops truncatus*)

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2.16 Small intestine

Examination of the intestines is preferably left until the end of the necropsy so as to not contaminate the other organs. Examine the serosal surface of the small intestine first. Look for areas of hemorrhage or discoloration as well as parasites. The inside of the small intestine can be examined by spot checking: at 5 – 10 random, separate areas, using scissors to cut about 10 cm down the length of the lumen. Note color, consistency and amount of contents as well as thickness of the lumen and the texture and color of the mucosa.

Sample several sections for histology.

2.17 Large intestine

To locate the beginning of the large intestine, look for the ileo-cecum-colic junction, which usually is a ridged junction between the smaller diameter small intestine and the larger diameter large intestine. The large intestine can be examined in the same manner as the small intestine. Note any discoloration or the presence of parasites. Describe the color, consistency and amounts of contents. Note the thickness of the lumen as well as texture and color of the mucosa.

Sample for histology.

2.18 Colon

Examine the serosal surface of the colon for areas of discoloration. Cut through the lumen of the colon from the anus to the large intestine. Describe the color, consistency and amount of contents. Note the thickness of the lumen as well as texture and color of the mucosa. Sample for histology.

Collect feces for biotoxins analysis.

2.19 Adrenal Gland

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 possessing irregular furrows over the surface. Locating and extracting the adrenals prior to removing the kidneys is highly recommended, as they can be difficult to locate without the kidneys as an anatomical reference.

To remove the adrenals, grasp and pull the tissue away from the body wall and cut the surrounding connective tissue. Before sectioning, measure (LxWxH) and weigh each adrenal. When cut in half, a normal adrenal will present a distinct darkened center (medulla) with a lighter perimeter (cortex).

Note size, shape, color and texture of the external and internal tissue. Also, note relative size of the aperture, or opening in the medulla, which would indicate usage of the vessel. Normal apertures should be no larger than the tip of a pin. Sample each adrenal for histology investigations.

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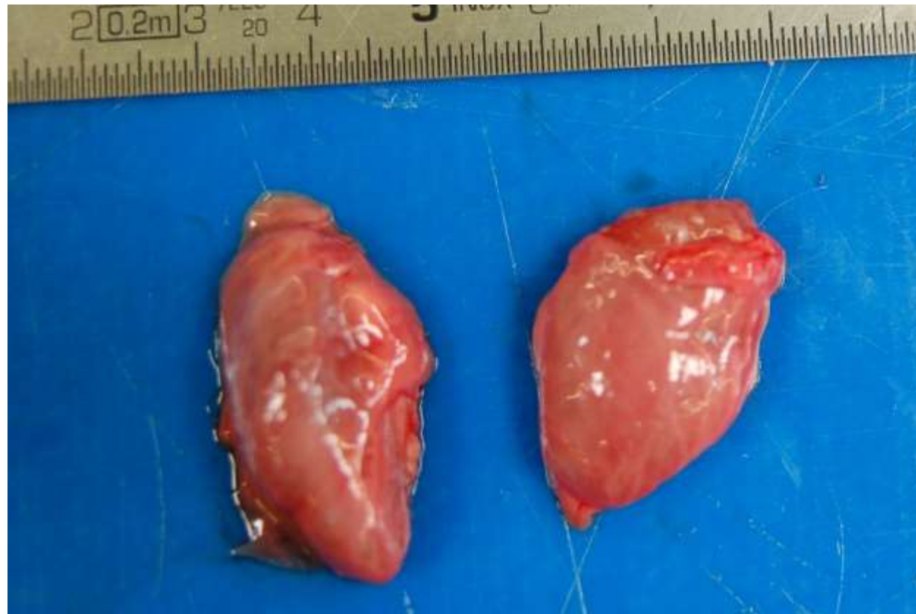


Adrenal gland (*Tursiops truncatus*)

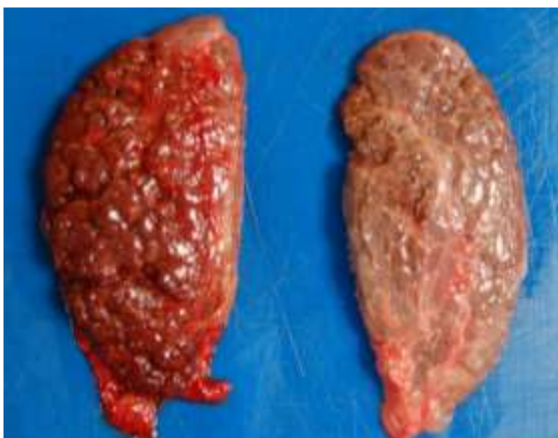
2.20 Kidney

The left and right kidneys are maroon, ovoid, tissues comprised of numerous, clustered *reniculi* (*miniature kidneys)* and are attached to the caudal dorsal abdominal wall. Examine the capsule (connective tissue surrounding the kidney) for the presence of fluid, hemorrhage or bubbles and note color, thickness and opacity. Create a longitudinal incision through the capsule and reflect the margins to asses for adhesions or sub-capsular hemorrhage. Detach the kidney from the abdominal wall and remove the capsule to examine the external surface.

Note the size, shape, external color and texture of each kidney. Examine the internal structure of each kidney by bread- slicing. Note color and presence of stones.

Observe the degree of differentiation between the cortex and medulla as well as the medulla/cortex ratio within each *reniculus*. Each *reniculus* should be well demarcated but clustered together within the kidney itself.

Sample for contaminants, histology, microbiology and molecular investigations.



Kidney (*Tursiops truncatus*)

Kidney (*Stenella coeruleoalba*)

2.21 Urinary Bladder

The bladder is a smaller, light pink, organ that is found along the ventral body wall. The organ may appear as a thick walled, muscular organ, but if distended with urine, the walls may be thinner and semi-translucent.

Before removing the bladder from the body, extract contents using a sterile syringe and medium gauge needle. If none are available, be sure to clamp the bladder using a hemostat before removing the organ in order to retain urine. Note color, consistency, and amount of urine.

Remove the bladder and examine internally by cutting along the length of the organ to expose the mucosal surface. Note color and texture of the mucosa.

Sample the cranial tip of the bladder for histology.

2.22 Reproductive tract

2.22.1 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 will vary in size and thickness depending on the maturity of the animal and its reproductive history. Note size, shape, color and texture of the external and internal surfaces of the organ.

If a fetus is present and is too small for a sufficient individual necropsy, incise the abdomen, collect microbiology and molecular samples, then preserve fetus whole in formalin. If the lung tissue floats in formalin (or water), this signifies that bronchiole expansion of the fetal lungs has occurred.

An off-white spindle-shaped ovary is attached to the end of each uterine horn. Detach the organ from the uterus and examine the external surface. Note size, shape, color and texture.

A mature ovary will possess random darkened notches or scars (corpus albicans) which signify previous ovulations. The ovary of a pregnant female will possess a corpus luteum or a large yellow mass attached to the ovary. Before internal examination, measure (LxWxH) and weigh each ovary. Also count and note the number of scars and presence/absence of a corpus luteum. Examine the tissue internally and note color and texture.

Sample both the uterus and ovaries for life history, histology, microbiology and molecular investigations.

2.22.2 Male: Testis and Penis

The elongated, spindle shaped, off-white paired testes are located within the caudal abdominal cavity along the ventral wall, posterior to the kidneys, each one just off the ventral midline. Remove the testes (with the epididymus attached) from the body. Obtain measurements (LxWxH) and weight of each one. Examine the size, shape, color and texture externally and internally. Section epididymus for the presence/absence or sperm. Obtain samples of each testis for life history, histology, microbiology, molecular and ancillary investigations.

Examine the penis externally and look for discharge or the presence of papillomas or other lesions.

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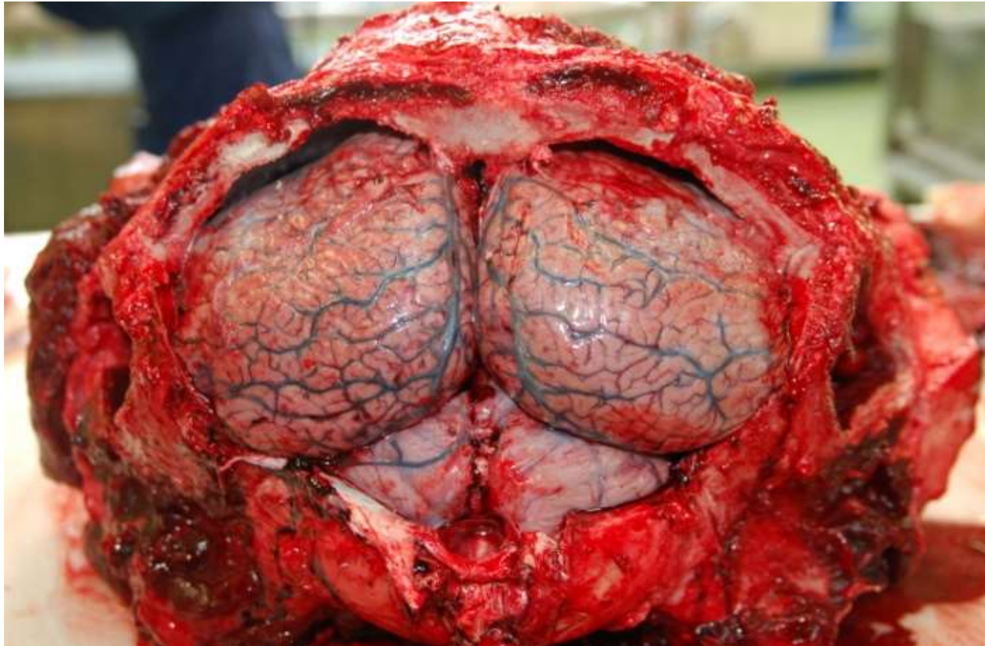
Uro-genital Tract (*Tursiops truncatus*)

SKULL

2.23 Removal of the Brain

The brain is the most fragile and easily disrupted tissue in the entire body, thus extreme care must be taken when removing the brain from the skull. Before removing the head, cerebrospinal fluid (CSF) can be collected for cytology and culture. To do so, remove the overlying soft tissue at the back of the head and neck to gain access to the atlanto-occipital joint. Insert a sterile needle and syringe and collect the clear, viscous CSF.

First the head must be detached from the body to safely remove the brain. Do so by cutting behind the blowhole down to the joint between the skull and cervical vertebrae, and then completing the cut ventrally. Once separated, remove all excess skin, blubber, muscle and connective tissue from around the dorsal and caudal skull. Then, using a Stryker saw or a hacksaw, make cuts from left to right through the middle of each occipital condyle, up each side of the lateral skull, and then across the dorsum, just posterior to the marked transverse ridge at the apex of the skull. Be sure to fully penetrate the bone, but avoid contact with the brain. This can be very difficult, so proceed with caution. It will take some practice to successfully remove the cranium without penetrating the brain. Carefully place a chisel between the cut bone and turn the tool to crack the remaining bone until the back of the skull comes away in one piece. Be careful to pull it off evenly, without using one edge as a lever, otherwise the bony shelf (the *tentorium cerebellae*) that is positioned between parts of the brain will penetrate the tissue and damage the brain. Using fingers, gently tease the meninges (thin membranes enveloping the brain) away from the skull, and work under the brain to sever each cranial nerve. Inversion of the head often allows the brain to gently descend in to the palm of your hand.



Brain (*Grampus griseus*)

2.24 Examination of the Brain

Again, the brain is the most delicate tissue in the body and will fall apart if handled excessively.

Observe the external surface of the brain and note symmetry of each distinct structure (right and left cerebral hemispheres, cerebellum, and brain stem) while noting the color, texture and presence of worms or lesions. Vascular congestion can be a result of positioning or post mortem lividity.

Cut through the brain in one long motion, cranial to caudal, using a large, thin knife so that the two hemispheres evenly separate. Again, note symmetry, color, texture and the presence of worms or lesions. Each section of the brain has a distinct pattern. The cerebrum is comprised of two distinct lobes and is the most cranial section of the brain. The cerebellum is the most caudal portion and sits dorsal to the brain stem. The brain stem originates from the ventral midline of the brain and extends into the spinal cord.

Sample the cerebrum, cerebellum and spinal cord for microbiology and molecular investigations. Fix the remaining brain tissue for histology. It is important to include a sample of normal and abnormal meninges in the histology sample set.

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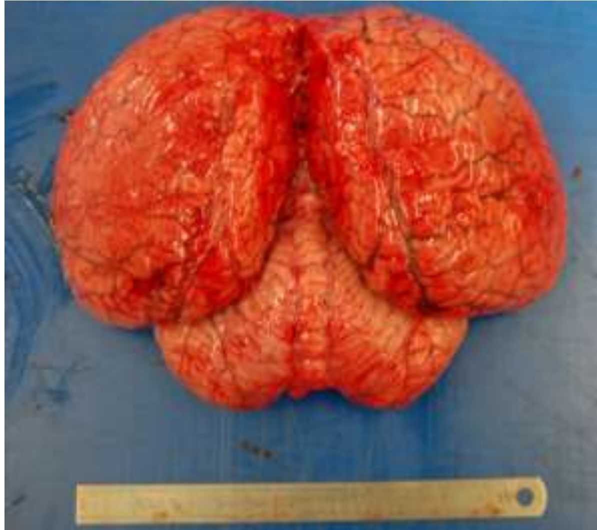
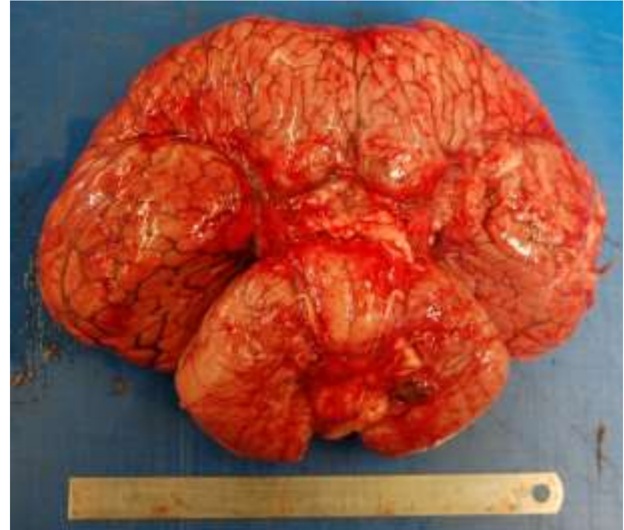


Brain (*Stenella coeruleoalba*)



Brain (*Stenella coeruleoalba*)

2.25 Pituitary Gland

Once the brain has been removed, immediately under the crossover of the optic nerve, the usually small pituitary gland can be extracted after incision through the overlying dura.
The organ is within a bony recess and has to be lifted out using a scalpel blade and small forceps.
Sample for histology and other priority testing.

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3. SOUND EXPOSURE

The possibility that exposure to sonar stimuli could lead to the stranding of cetaceans is an issue of increasing interest because of recent strandings temporally and geographically coincident with naval sonar operations. Nonauditory link between strandings and sonar exposure is proposed to occur when tissues are supersaturated with dissolved nitrogen gas, and bubble growth–facilitated diffusion is stimulated within tissues. Bubble growth could result in emboli-induced tissue separation and increased localized pressure in tissues, the presumed cause of decompression sickness (DCS) in human divers. DCS is the result of the supersaturation of body tissues with nitrogen gas and the subsequent release of nitrogen gas bubbles.
No studies have specifically addressed whether nitrogen bubble formation in tissue occurs in diving marine mammals. Recently researchers have presented strong evidence of chronic, gas bubble lesions in the liver and kidney. These lesions of different stranded cetacean species suggest that gas bubbles formed in vivo can persist and generate fibrosis in diving cetaceans.
Hemorrhage in the brain, ears and acoustic fat was reported as the main lesions.

3.1 Fat emboli

The presence of gas emboli is an important finding in human DCS, but pulmonary fat emboli have also been reported with DCS-related, severe cardiorespiratory disturbances. Systemic fat embolism is a secondary effect of the abrupt pressure changes observed with dysbaric osteonecrosis (DON), a condition initiated by the evolution of gas bubbles in nitrogen-supersaturated fatty marrow after inadequate decompression. The clinical manifestations of fat emboli depend on the volume of fat reaching the lungs and other affected tissues.
The pathogenesis of fat embolism is not fully understood, and it is likely multifactorial. Two mechanisms have been proposed for the development of fat emboli. First, direct entry of fat emboli into the bloodstream after trauma may cause direct, toxic injury in the lung and produce respiratory insufficiency when free fatty acids are released from fat tissues. A second mechanism involves the generation of fat emboli from plasma lipoprotein disruption and coalescence of lipid at the intravascular gas bubble interface.
In particular, hemorrhages should be detected and reported in acoustic fat in lower joint, around temporo-mandibular joint, around middle and inner ear, in the sub-cutaneous tissue, in inner cavities, around meningeal tissue or around eyes.
Tissues should be collected and stored in buffered 10% formalin and later fixed and preserved for routine histopathologic examination or immediately frozen . Tissues from stranded animals include: brain, cervical spinal cord, lung, liver, kidney, heart, middle and inner ear.
Histologic stains used on frozen or on formalin-fixed tissues to detect fat emboli are oil red O stain (ORO) and Sudan black B stain. Furthermore pulmonary tissue and periotic veins could be processing according to Osmium tetroxide post-fixation technique in order to detect fat emboli within vessels.

3.2 Gas-bubble

Gas-bubble lesions have been described in cetaceans stranded in spatio-temporal concordance with military maneuvers. Authors described an acute and systemic gas and fat embolic syndrome similar to decompression sickness (DCS) in human divers. A behaviorally induced decompression sickness-like disease was proposed as a plausible causal mechanism, although these findings remain scientifically controversial. Investigation into the constituents of the gas bubbles in suspected gas embolic cases are highly desirable.
Gas chromatography has been demonstrated as a valid method to discriminate putrefaction gases from air embolism and has been used as a forensic tool in humans for this purpose.
Although gas extraction can sometimes be performed at the stranding site, gas analysis must always take place in a laboratory, a situation that requires proper storage and transportation of gas samples. Gases

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likely to be of scientific interest might be found in sites within body cavities (such as intestinal gas, air in sinuses, pneumothorax, subcapsular gas or gas pockets), inside vessels (emboli) and/or mixed with blood inside the heart. Sampling of gas from these body sites will require the use of different techniques.
A standard necropsy protocol is used, with some modifications necessary to preserve bubbles for gas collection and storage. Skin and blubber are removed taking into account possible gas embolism within the subcutaneous veins. If bubbles are seen, vessels are explored to confirm that they have not been cut during dissection. As soon as a bubble is detected, a photograph is taken and the bubble is sampled for gas analysis. In order to have access to the body cavities, dissection is done avoiding cutting of medium-large vessels (usually larger than 3 mm, although it varies among species). The abdominal cavity is opened first and mesenteric and renal veins as well as the lombo-caudal venous plexus are screened for bubbles.
The thoracic cavity is then opened to permit access to the heart and coronary veins are explored. After bubble exploration and gas sampling from the different localizations, systemic vessels can be subsequently cut and the routine necropsy protocol can be completed.
When sampling gas from sites within body cavities, the 5-mL additive-free vacutainer is directly applied to cavities with its appropriate plastic holder or adapter and a double-pointed needle with a rubber barrier on the tube puncture side. To avoid atmospheric air, the needle is preliminarily inserted into the cavity for purging; the vacutainer is then pushed against the double-pointed needle and, finally, the vacutainer is removed before the needle is released from the cavity. This technique allows adequate gas sampling from head sinuses, the digestive tract and even from heart chambers if post mortem autolysis ranged from grade 3 to grade 5. The pericardial sac is always filled with distilled water to avoid contamination with atmospheric air.
If the carcass preservation status is fresh or very fresh (putrefaction grades 2 or 1, respectively), the aspirometer is used to separate the gas from the blood found in the heart.
In order to properly sample bubbles in the rest of the cardiovascular system (blood vessels), disposable insulin syringes (BD Plastipak U-100 insulin) are used to sample bubbles and their contents are promptly injected into a vacutainer. One new syringe and one new vacutainer are used for each bubble.

a. The new design of the aspirometer – b. close-up view of intravascular bubbles in the coronary heart veins of a beaked whale – c. abdominal cavity with close-up view of the spleen showing chronic gas embolism. ***SCIENTIFIC REPORTS | 1 : 193 | DOI: 10.1038/srep00193***

3.3 Ear

Ear sampling could be used in both in case of sonar exposure or other sounds exposure.
Initially, to collect the ear, disjoint the jaw to expose the tympanic bulla and clean it from the soft tissues. Helping levering with a scalpel or knife, cut the ligament between the tympanic complex, the occipital and temporal bones up to completely remove the whole bone. Once removed, locate the eardrum and slowly inject a solution of 10% Formalin (better for fall).
To evaluate the micro and macroscopic examination of the inner ear, it's necessary to decalcify the bones using a fat commercial decalcifier (RDO®), based on hydrochloric acid, according to the protocol proposed by *Morell et al*. (2009). Times in RDO® must be adapted to different species and size of the bone.
Decalcification finished, proceed to carry serial section of the ear itself.
In larger species should remove the tympanic complex to facilitate the decalcification of the periotic process.

4. SAMPLING PROTOCOL

4.1 Life History

Code 1, 2 and 3, ideal; 4 and 5 limited.

- Age determination
  - Teeth, should be kept frozen.
  - Eyes, should be kept frozen.
  - Ear plugs, preserve in 10% NBF.
- Reproductive Status
  - Gonads and Uterine samples fixed in 10% NBF.
- Feeding Habits
  - Stomach contents can be collected into a sealable plastic bag or jar, freeze.
  - Skin, for stable isotopes should be kept frozen. What the animal has been eating recently.
  - Baleen, provides a multiple year record of the animal's diet.

4.2 Genetics

Codes 1, 2-4 are suitable. Only one of the following needs to be collected, unless there are specific requests.

- Skin, Heart, Liver - Better to freeze tissue samples, in case the tissue is used for something other than genetics. Genetic tissue samples can be fixed in DMSO saturated with NaCl.
- Blood - Can only be collected from Code 1 and 2 animals. Minimum amount is ~10 ml; 50-100 ml is optimal for DNA studies.

4.3 Microbiology

Code 1 and 2 are ideal, 3 limited, 4 and 5 useless. Take separate samples for bacteriology and virology. Lesions should be sampled from several distinct locations, include normal tissue with the infected tissue sample.

- Bacteriology – Avoid freezing samples for bacteriology if avoidable. Refrigerate samples at 4°C. Freezing at –70°C is preferable to decomposition.
  - External samples can be taken with a swab from the eye, blowhole, throat, anus, and genital opening. Culture swab in a bacterial transport medium.
  - Internal samples can be taken from the heart, lungs, liver, spleen, lymph node, bone with marrow, and tissues showing pathological changes. 6 x 6cm sample placed in a sterile container.
  - Fluid samples can be taken from the pleural fluid, peritoneal fluid, urine, blood, fluid from abscesses. Store in appropriate aerobic or anaerobic vial.
- Virology – Refrigerate samples at 4° C.
  - External samples use a sterile swab dipped in viral transport medium. Take samples from the blowhole, rectum, genital opening. Place swabs in the vial that contains the viral transport medium.
  - Internal samples can be taken from the CNS tissues, lungs, liver, spleen, kidney, placental/fetal tissues, tissues with pathological changes, intestinal contents. 6 x 6cm sample placed in a sterile container.
  - Fluid samples from pleural fluid, peritoneal fluid, pericardial fluid, urine, fluid from skin vesicles, blood from heart, cerebrospinal fluid.

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4.4 Parasites

Samples taken from Code 1-4 animals are suitable for examination.

- Barnacles – first fix in 10% NBF, for no more than 24 hrs, then transfer to 70 % EtOH.
- Copepods & Amphipods – place directly into 70% EtOH.
- Nematodes (roundworms) – Fix in GAA for 5-10 minutes first if possible. Otherwise use 70% EtOH or 10 % NBF. If formalin is used, fix only for a few hrs. Then transfer to 70% glycerin alcohol.
- Trematodes (flukes/flatworms) – Dead or alive, fix in AFA for up to 3 days, transfer to 70% EtOH. Do Not use glycerin alcohol.
- Cestodes (tapeworms) – Fix for 5-10 min. in AFA solution and water, 4:1 ratio. Transfer to 70% EtOH. Include cestode head when removing from the host, if necessary cut host tissue.
- Acanthocephalans – Fix in AFA for up to 24 hrs. Then transfer to 70% glycerin alcohol.

4.5 Histopathology

Code 1, 2 and 3 are ideal. Rare / Endangered species should be thoroughly sampled. Lesions, fractures, lacerations, and gunshot wounds of any code should be sampled in this manner.

- Tissues should be preserved in 10% NBF.
- Tissue samples should be no larger than 3 x 3 cm and approximately 1 cm in thickness.
- Ideally, histo samples should be cassetted and placed into a labeled jar for the appropriate Institution/Researcher. Individual requests should be noted and tracked.
- Samples of gross lesions should include abnormal and normal tissue.

4.6 Contaminants / Biotoxins

(organochlorines, heavy metals)

Code 2 is ideal, 1 and 3 is limited; 4 and 5 questionable to useless.

- Biotoxin Analysis – Code 2 animals only. Collect stomach contents, liver and/or kidney tissue. Freeze.
- Contaminant Analysis – All of the samples should be frozen in plastic zip lock bags.
    - Blubber (include skin), Muscle, Liver, Kidney, Brain (if possible include cerebrum and cerebellum).

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6. NECROPSY REPORT

Event Info	Animal Info
Strand Date: ______________________________	Sex: M F CBD
Recovery Date: ___________________________	Length: _____________cm / in / ft
Euthanized / Died	Weight: _________lbs / Kg
Date & TOD: ______________________________	Pup / Calf / YOY / Sub-adult / Adult
Necro Date & Time: _______________________	Condition at Stranding: 1 2 3 4 5 6
Storage Prior to Necropsy: ________________	Condition at Necropsy: 1 2 3 4 5 6
Stranding Location: _______________________	Human Interaction: Yes / No / CBD / NE
____	Mass Stranding: Yes / No
Lat/Long: _____________N/______________W	# Animals: ______________

CARCASS DISPOSITION:

HISTORY:

COMMENTS:

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

EXTERNAL EXAM

Body Condition: Robust [5] - Normal [4] - Moderate [3] - Thin [2] - Emaciated [1]

Skin / Hair Coat (color, condition):

Wounds / Scars:

Lesions:

Parasites:

Nostrils / Blowhole:

Mouth (tongue, teeth condition, ulcers) / Mucous membranes (color):

Eyes (discharge, color, ruptures):

Ears:

Genital slit / anus:

Umbilicus: Pink Open Healed:

EXTERNAL EXAM

MUSCOLO/SKELETAL SYSTEM

Blubber:

Muscle:

Diaphragm:

Skeletal:

CIRCULATORY SYSTEM

Pericardium:

Heart:

Vessels:

PULMONARY SYSTEM

Trachea:

Bronchi:

Lungs (color, condition, edema, congestion, consolidation, granulomas, emphysema, lesions):
(R)

(L)

GASTROINTESTINAL SYSTEM

Esophagus:

Stomach (contents, ulcers, mucosa, parasites):
Weight Full: _______ Weight Empty: ________

Small Intestine:

Large Intestine:

Colon:

Omentum, Mesentery, Peritoneum:

Liver (color, congestion, lesions, size):

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

Pancreas:

URINARY/REPRODUCTIVE SYSTEMS

Kidneys (reniculi differentiation, color, condition):
(R)

(L)

Bladder:

Testes / Ovaries: Immature / Mature

(R) **L x W x H cm:**

(L) **L x W x H cm:**

Mammary glands:

Uterus / Cervix / Vagina:

Pregnant? : Y N NA (male)

LYMPHATIC SYSTEM

Thymus:

Spleen:

Scapular Lymph Node:

Tracheobronchial Lymph Node:

Mesenteric Lymph Node:

Other Lymph (list location):

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ENDOCRINE SYSTEM

Thyroid:

Adrenals:
(R) **L x W x H cm:**

(L) **L x W x H cm:**

Other:

CNS

Spinal Cord:

Brain:

Pterygoid Sinuses:

OTHER

Thoracic Cavity:

Abdominal Cavity:

Internal Parasites (location, type, number):

Differential Diagnosis from Gross Exam:

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7. PROTOCOL FOR EXAMINING CETACEANS FOR SIGNS OF HUMAN INTERACTION

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

ND: Not Determined – **NE**: Not Evaluable

2. EXTERNAL EXAM			
a. Body condition			
Emaciated	Not emaciated	ND	NE
b. Sings of fishing net or lines. (indicate if YES, NO, ND, NV for each area and in the positive case describe the lesion)			
Head	Dorsal fin		
Pectoral fin left	Pectoral fin right		

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<table>
<tr><th colspan="4">2. EXTERNAL EXAM</th></tr>
<tr><td colspan="2">Caudal peduncle</td><td colspan="2">Other</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="2">c. Presence of fishing nets on the animal</td><td>YES</td><td>NO</td></tr>
<tr><td colspan="2">Fishing nets have been preserved?</td><td>YES</td><td>NO</td></tr>
<tr><td colspan="4">d. Penetrating wounds</td></tr>
<tr><td>YES</td><td>NO</td><td>ND</td><td>NE</td></tr>
<tr><td colspan="4">Describe gunshot wounds, puncture wounds, from harpoon, etc.</td></tr>
<tr><td colspan="4">e. Mutilations</td></tr>
<tr><td>YES</td><td>NO</td><td>ND</td><td>NE</td></tr>
<tr><td colspan="4">Describe ctus, tears, cracks in the body wall, missing appendages, etc.</td></tr>
<tr><td colspan="4">f. Hemorrhages and hematomas</td></tr>
<tr><td>YES</td><td>NO</td><td>ND</td><td>NE</td></tr>
<tr><td colspan="4">Describe extension and area.</td></tr>
<tr><td colspan="4">h. Post-mortem damage from scavengers and opportunists</td></tr>
<tr><td>YES</td><td>NO</td><td>ND</td><td>NE</td></tr>
<tr><td colspan="4">Describe extension and area.</td></tr>
</table>

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<table>
<tr><th colspan="8">3. INTERNAL EXAM</th></tr>
<tr><td colspan="8">a. Sub-epidermal hemorrhages</td></tr>
<tr><td colspan="2">YES</td><td colspan="2">NO</td><td colspan="2">ND</td><td colspan="2">NE</td></tr>
<tr><td colspan="8">Describe extension and area.</td></tr>
<tr><td colspan="8">b. Fractures</td></tr>
<tr><td colspan="2">YES</td><td colspan="2">NO</td><td colspan="2">ND</td><td colspan="2">NE</td></tr>
<tr><td colspan="8">Describe.</td></tr>
<tr><td colspan="8">c. Content of airway and lung</td></tr>
<tr><td colspan="2">AIR</td><td colspan="2">FLUID</td><td colspan="2">FOAM</td><td>ND</td><td>NE</td></tr>
<tr><td colspan="8">Describe lungs’ appearance (heavy, consolidated areas, color variations, etc.) and airway’s content.</td></tr>
<tr><td colspan="8">d. Stomach content</td></tr>
<tr><td colspan="8">Describe stomach content, amount, presence of parasites and foreign bodies.</td></tr>
<tr><td colspan="4">Stored in frozen</td><td colspan="2">YES</td><td colspan="2">NO</td></tr>
<tr><td colspan="4">e. Histopathology</td><td colspan="2">YES</td><td colspan="2">NO</td></tr>
</table>

3. INTERNAL EXAM			
f. Presence of macroscopically visible lesions			
YES	NO	ND	NE
Describe.			
g. DIAGNOSTIC HYPOTHESIS:			

8. GAS SAMPLING

ID________________ **SPECIES**____

DATE OF DEATH ________________________ **DATE OF NECROPSY**___________________________

CONSERVATION CODE **1** **2** **3** **4** **5**

FRESH OR FROZEN_______________________________________

STRANDED ALIVE	
STRANDED DEAD	
FOUND DEAD	

GAS SCORE

SUBCUTANEOUS VEINS[1]	
VENOUS PLEXUS LOIN-CAUDAL[1]	
MESENTERIC VEINS[1]	
CORONARY VEINS[1]	
SUBCAPSULAR ENPHYSEMA[2]	
TOTAL	

[1]:

SCORE	DEFINITION
0	No gas bubbles / Presence of congested veins
1	Occasional presence of gas bubble
2	Some bubbles / Small discontinuities along the vessel's course
3	Abundant and large discontinuities along the flow in the vessel, but not only filled with gas
4	Moderate presence of gas bubbles within a specific vein
5	Abundant presence of gas bubbles
6	Complete sections of the vessels are filled with gas

[2]:

PUNTEGGIO	DEFINIZIONE
0	No gas bubbles
1	Low presence of gas bubbles (UN only organ involved)
2	Moderate presence of gas bubbles (MORE THAN ONE organ involved)
3	Abundant presence of gas bubbles (MANY organs involved)

SUBCAPSULAR EMPHYSEMA LOCALIZATION: ____

____

SAMPLES

N°	LOCALIZATION	METHOD	VOLUME (mL)
1	Subcutaneous veins	Insulin siringe	
2	Venous plexus loin-caudal	Insulin siringe	
3	Coronary veins	Aspirometer	
4	Heart – Right ventricle	Aspirometer	
5	Heart – Left ventricle	Aspirometer	
6	Aorta	Aspirometer	
7	Polmonary trunk	Aspirometer	
8	Mesenteric veins	Insulin siringe	
9	Proximal intestine	Insulin siringe	
10	Middle intestine	Insulin siringe	
11	Distal intestine	Insulin siringe	
12			
13			
14			
15			
16			
17			
18			
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20			

The project is co-funded by the European Union, Instrument for Pre-Accession Assistance

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9. STANDARD SAMPLES

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

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