Appendix I. Dissection Protocol and Tissue Sampling for Small Cetaceans in Southern Africa

1. General Comments

Before starting the post mortem, the collection of some general information on the carcass is necessary to obtain as much information as possible, and to ensure that usable samples are obtained. This section lists the type of information and measurements that should be collected, as well as sampling techniques, and should be referred to throughout the procedure.

1.1. Relevant History

Examination of the tissues under the microscope (histopathological interpretation) can be facilitated by knowledge of the circumstances surrounding the animals' death. This may be related to any recent unusual weather events or circumstances (such as storms, red tides, chemical spills) in the area where the animal was found and, in the case of a live stranding, any clinical signs exhibited by the animal prior to its death (such as uncoordinated behaviour, heavy breathing, muscle tremors), any treatments given (such as specific drugs, amounts and time they were administered), time of death, and cause of death, if known (such as euthanised or incidentally caught). The geographic location, including the geographic coordinates, if available, of the animal is also important and should be added to the history. A section is provided in the necropsy report (Appendix II) for the history.

1.2. Morphometric and other biological measurements

Morphometric and other data collection is important for a range of biological investigations, such as age, growth rate and determination of reproductive status, investigation of disease processes, and taxonomy. To ensure that data are comparable to other studies, it is extremely important that morphometric measurements are consistently taken in the same way. The total body length (tip of upper jaw to deepest part of notch, measurement 1 in Figure 1) is important as it can be used to estimate age from existing growth curves (Pugliares *et al.* 2007; Norris 1961). General guidelines, from Norris (1961) and Pugliares *et al.* (2007), for measurements include:

- All measurements, except for girths, should be straight measurements parallel to the long axis of the body, and not following the contours of the body.
- For dolphins, straight lengths are measured from the tip of the upper jaw (maxilla).
- All measurements should be in centimetres.

A full list of measurements for morphometric studies (to be used in conjunction with Figure 1) can be found in Appendix IV.

In addition, organs should be weighed entire before any samples are taken for histopathological investigations; samples for detection of infectious agents should then be taken from the inside of the organ to avoid possible contamination. Baseline data on organ weights are scarce, but such data may over time assist in indicating whether gross changes have occurred due to disease (Turner *et al.* 2006; Plön *et al.* 2012). Furthermore, other biological sample collection should be accommodated wherever possible after consultation with the various researchers or research groups.

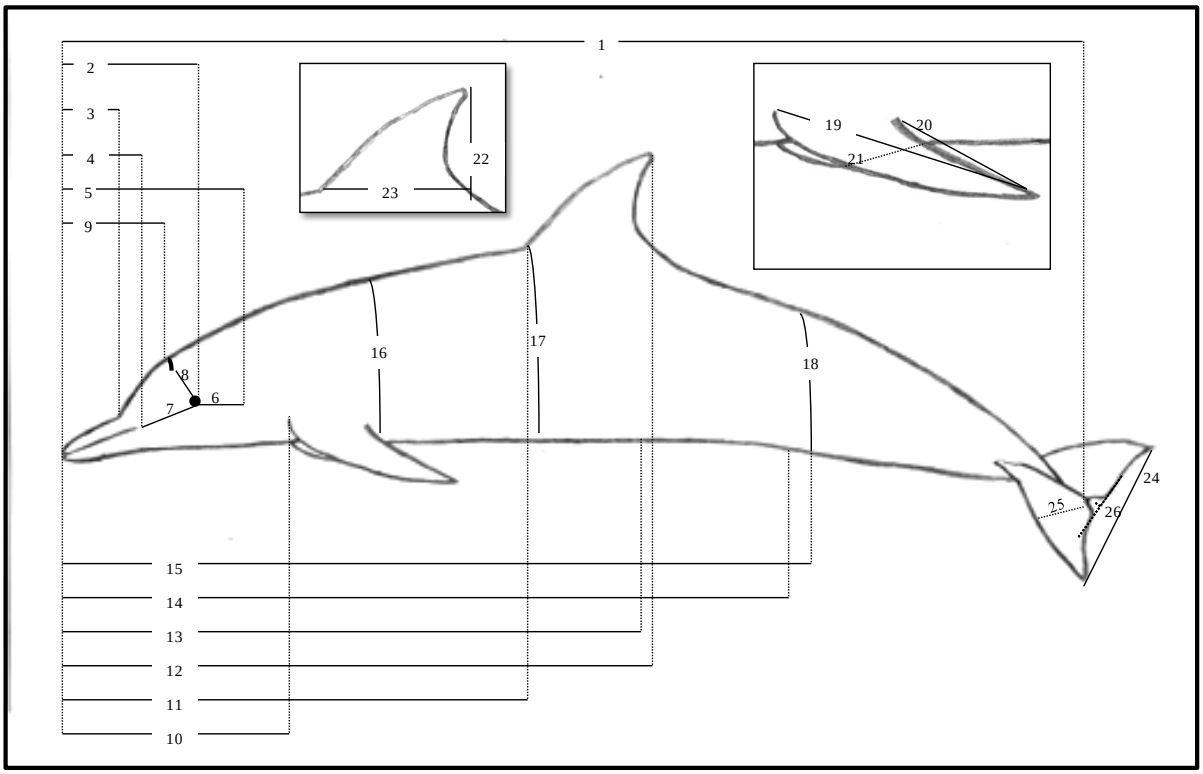

Figure 1: Standard measurements for morphometric studies (after Norris, 1961). For approximate age estimation only measurement 1 is required - tip of upper jaw to deepest part of fluke notch. Key: see Appendix IV.

1.3. Photography

Photographs of animals, organs, and abnormalities are of great value to document findings and should accompany written descriptions of abnormalities. Some general guidelines for photography include:

- Photographs should be taken without the use of a flash, but with sufficient natural light to see all anatomical detail.
- External photographs of the whole carcass should include both left and right views (Figure 2).
- All abnormalities should be photographed prior to sampling.
- Photographs of abnormalities should contain a label with the identification of the animal that is being dissected, and a scale if one is available. Alternatively a common object, e.g. matchbox, can be used to infer scale (Figure 2).

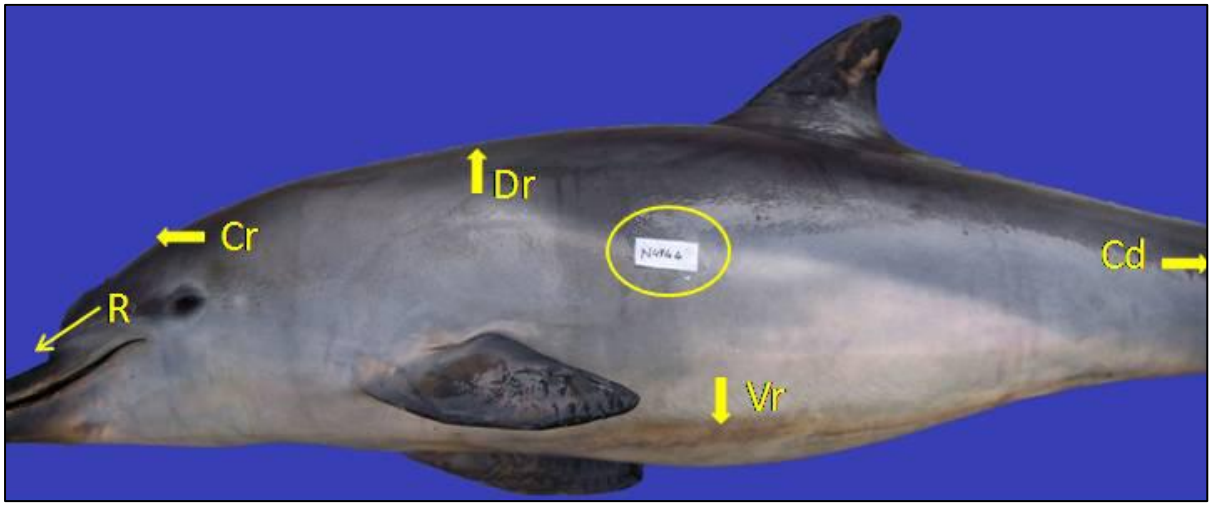

Figure 2: External photograph of dolphin lying on right side, with a label and scale identifying the animal (circled), and directional nomenclature indicated. Cranial (Cr): towards the head of the animal. Caudal (Cd): towards the tail of the animal. Dorsal (Dr): towards the top or back of the animal. Ventral (Vr): towards the belly of the animal. Rostral (R): Towards the tip of the beak.

1.4. Descriptions of Abnormalities

The most important aspect of macroscopic descriptions is to describe what is seen, without adding any interpretation or assumptions. Descriptions aid substantially in the later interpretation of

histopathological results. Any description should include at least the following parameters, where applicable.

- Location: exactly where the abnormality was seen and the organ or body cavity affected.
- Number: single, many, approximate numbers or, in the case of liquids, the approximate volume in millilitres or litres.
- Size or extent: length x width x depth or area covered.
- Colour, consistency and opacity of liquids: thick or thin, liquid or mucoid, and cloudy or clear.
- Colour, consistency and texture of the affected tissue: firm (like tendon), hard (like bone), soft, or fluctuant; friable, viscous, gritty or rough. It is important to note that abnormalities should be sampled prior to being handled and palpated.
- Characteristics of the shape of an anomaly, such as knobbly, finger-like, or wart-like.

There is no such thing as too much information; abnormalities should be described in as much detail as possible, using everyday English, and avoiding the use of jargon or interpretive terms. If there is doubt whether something is truly abnormal, it is better to err on the side of caution and describe the possible abnormality. Examples of descriptions are given in Figure 3.

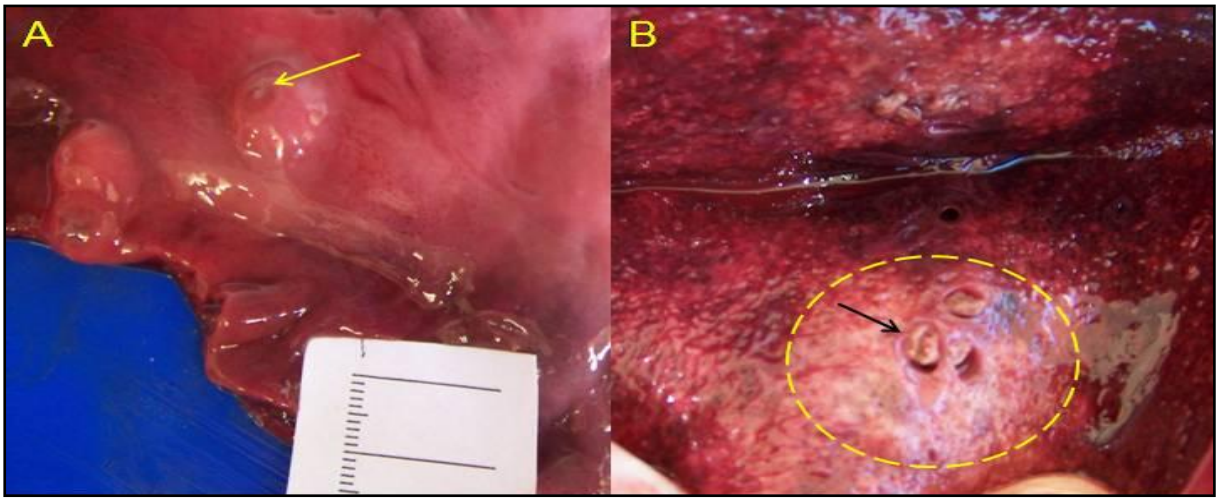

Figure 3: Examples of macroscopic descriptions. A: Pyloric stomach contains a small number of spherical, firm, pink nodules extending into the lumen and up to 7 mm in diameter, with a small pore (arrow) ca. 1 mm in diameter opening into the lumen. B. Lung contains a single, irregular, firm, white area (circled) around three bronchi that is filled with, and almost occluded by, white, pasty material (black arrow). The rest of the lung is mottled red to dark red.

1.5. Histopathology sampling

- Macroscopically normal tissue should be sampled from all organs listed in the dissection guide and checklist (Table 2).
- Standard samples should be taken from approximately the same area of each organ to aid in interpretations and so that samples from different cases may be more readily compared with each other.
- All samples should be less than 2 cm in diameter. If a large abnormality is present, several representative samples from different portions of the abnormality should be taken.
- Any abnormality sampled should include macroscopically normal appearing tissue as well as abnormal tissue.
- All samples should be stored in 10% buffered formalin, with a 10:1 formalin to tissue ratio, particularly the brain, where a 10:1 formalin to tissue ratio is essential for proper fixation (Figure 4).
- All sample containers should be labelled with internal and external labels, clearly stating the identification number of the animal, and, if possible, the sex, age, date of collection, and contents. Internal labels should be written in pencil, and placed inside the container. The exception being the microbiology samples (Section 1.6), which should not be labelled internally as this will compromise the sterility of the sample.

Figure 4: Sample containers with 10% buffered formalin showing a formalin: tissue ratio of 10:1.

1.6. Pathogen Sampling (bacteria, viruses, fungi and protozoa)

- Standard samples are listed in the checklist (Appendix II, Table 2), and these should always be taken regardless of any visible pathology.
- Any abnormality, over and above the standard samples (listed in Appendix II, Table 2), with a possible infectious cause (etiology) should also be sampled for microbiology. If uncertainty exists whether an abnormality has a possible infectious etiology, a sample for microbiology should be taken.
- Samples for microbiology should be taken with sterile instruments, and aseptic (sterile) technique should be observed.
- Equipment can be sterilised between samples by immersion in formalin and subsequent air drying. This is done by dipping the instrument tip that has been used into 10% buffered formalin for a few seconds, and allowing it to air dry in a well ventilated area. The surface of a handled organ can be sterilised by wiping with formalin and then drying it with clean paper towel.
- Fluid samples, such as discharges, should be placed in a serum (red top) blood collection tube or a sterile syringe.
- If no refrigeration facilities are available, the sample should be kept cool in a cooler box with cooler blocks or on ice.
- Tissue samples should be placed in an empty Ziploc® bag and chilled (see next point whether in fridge or freezer) as soon as possible.
- If bacteriological or fungal cultures will be carried out within 24 hours, the samples should be kept refrigerated until submission to the relevant laboratory. If processing will be delayed, the samples should be frozen (-25°C) as soon as possible after collection.
- All samples should be clearly labelled on the outside of the bag; for microbiological analysis no labels should be placed inside the bag as this will compromise the sterility of the sample.

1.7. Parasitology Sampling

- Description of parasites, location, distribution, approximate number of parasites, and associated abnormalities should be described and recorded.
- Any parasites noted at any location should be collected in 70% ethanol. An attempt should be made to collect as many parasites as possible.
- Samples from different locations on or in the body should be kept separately, with the location clearly indicated on the label.

2. Necropsy Technique

2.1. Preparation of the carcass

To gain the most insight from the examination of carcasses, the following guidelines were developed, taking South African weather conditions into account:

- Assessment of the state of decay of the carcass will assist in the decision whether the carcass can be frozen and later sampled for histopathological and health investigations (possible for bycaught or freshly dead animals, although necropsies are always better on unfrozen material) or not (futile if the animal has been dead in excess of two days).
- If a carcass cannot be immediately examined, it should be frozen as quickly as possible after death to delay autolysis and putrefaction.
- If a cold room is available the carcass may be kept there for a day or two before dissection.
- Carcasses should be defrosted slowly, preferably indoors (in a cool place or the cold room of a freezer) and out of direct sunlight.
- On average, an adult dolphin (over 230 cm long or weighing more than 140 kg) takes three days to defrost, while a smaller animal takes two days, at an environmental temperature of 25°C.
- Similar consideration should be paid to freezing carcasses i.e. larger dolphins/ whales will take longer to freeze entirely than smaller animals.

2.2. Standard safety precautions

Standard safety precautions should be followed at all times as freezing of any carcasses may not prevent the potential of disease transmission from animals to humans. Safety precautions consist of basic hygiene and safety, especially with regards to using sharp objects, and should include:

- Wearing of protective clothing, including gumboots, plastic aprons and gloves. Many individuals feel more comfortable using a face mask, but this is only essential in certain circumstances (see next point).
- Face masks and goggles should be worn when the reproductive tract, foetus or brain is handled due to the potential risk of *Brucellosis*.
- Knives and other instruments should be kept sharp, so that excessive force is not required during dissection, as this may result in accidents.
- Disinfection of all equipment with a medical grade disinfectant after each necropsy examination.
- Safety precautions when handling fixatives, such as formalin and alcohol, to prevent spillage, splashing, inhalation and fires. Many fixatives are carcinogenic and should be treated with extreme caution for this reason.
- Eating, drinking, smoking, and using a cell phone during a necropsy should be avoided. Persons performing the necropsy should thoroughly wash their hands and any other body part that may have come into contact with the carcass, tissue or body fluids, subsequent to the necropsy.
- Persons performing the necropsy should change into a clean set of clothes before leaving the premises/location where the necropsies were performed. Specifically pregnant women should not come into contact with carcasses or biological material from cetaceans.

2.3. External measurements

A number of different measurements are routinely taken for morphometric studies by institutions like the Port Elizabeth Museum (see Norris, 1961; Figure 1).

2.4. External Assessment and Sampling

- Weigh the animal if possible, and record the body mass on the necropsy report.
- Determine and record the sex of the animal. In small cetacean females, the distance between the centre of the genital slit and the centre of the anus is less than 10 cm, and small mammary slits are seen on both sides, lateral to the genital slit. Small cetacean males have a distance greater than 10 cm between the centre of the genital slit and the anus, may have the penis protruding from the genital slit. Mammary slits are mostly absent, although some males may have mammary slits. Gender should be confirmed on internal examination during the necropsy (Pugliares *et al*. 2007).
- Describe and photograph any external scars, wounds (including those caused by nets, boat strikes, predators or scavengers), or abnormalities (Section 1.4).
- Describe and swab any fluids draining from orifices or external abnormalities for culture (Section 1.4).
- Collect any external parasites that may be present for later identification (Section 1.7).
- Position the animal lying on the right hand side.
- Take full blubber thickness measurements from the sites illustrated in Figure 5.
- Collect a sample of the skin and blubber adjacent to the lateral blubber measurement (site d in Figure 5). The same location should be sampled in each necropsy as the histological appearance of the skin varies at different sites of the body.
- In females, examine the mammary glands and place a sample in formalin.

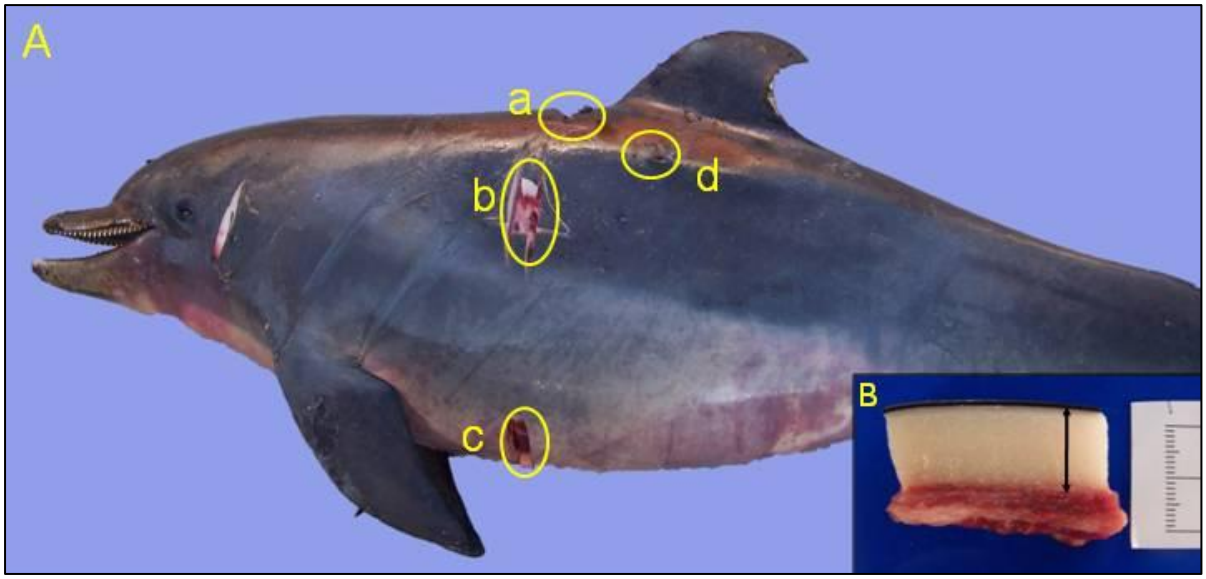

Figure 5: A: Location of blubber thickness measurements to be taken in a line: (a) in front of the dorsal fin; (b) in front of the dorsal fin half-way down the body; (c) in front of the dorsal fin at the bottom. Skin sample for histopathology is taken at site (d). B: Blubber thickness is measured from below the skin (black) to the muscle (red).

- Keeping the left eye intact to conduct morphometric measurements, remove the right eye with as much of the optic nerve attached as possible and, using a needle and syringe, remove 0.25 ml of the fluid in the front of the eye and replace it with an equal volume of 10% buffered formalin to facilitate fixing. The fluid that is removed should be placed in a labelled sterile container and frozen. Then place the eye in 10% buffered formalin.
- Place at least five teeth from any part of the mandible in a separate, labelled vial in 70% ethanol for age determination and other, potentially destructive analyses (such as ancient DNA analysis, isotope analysis etc.)

2.5. Opening of the Carcass

- With the animal lying on its right side, make a roughly oval cut through the skin and blubber to the underlying muscle, extending from the neck to the anus, following the border of the spinal muscles and the ventral midline (Figure 6). The lateral skin and blubber can then be divided into suitable portions and removed. Removing the left flipper and the scapula facilitates adequate exposure to the underlying thorax.

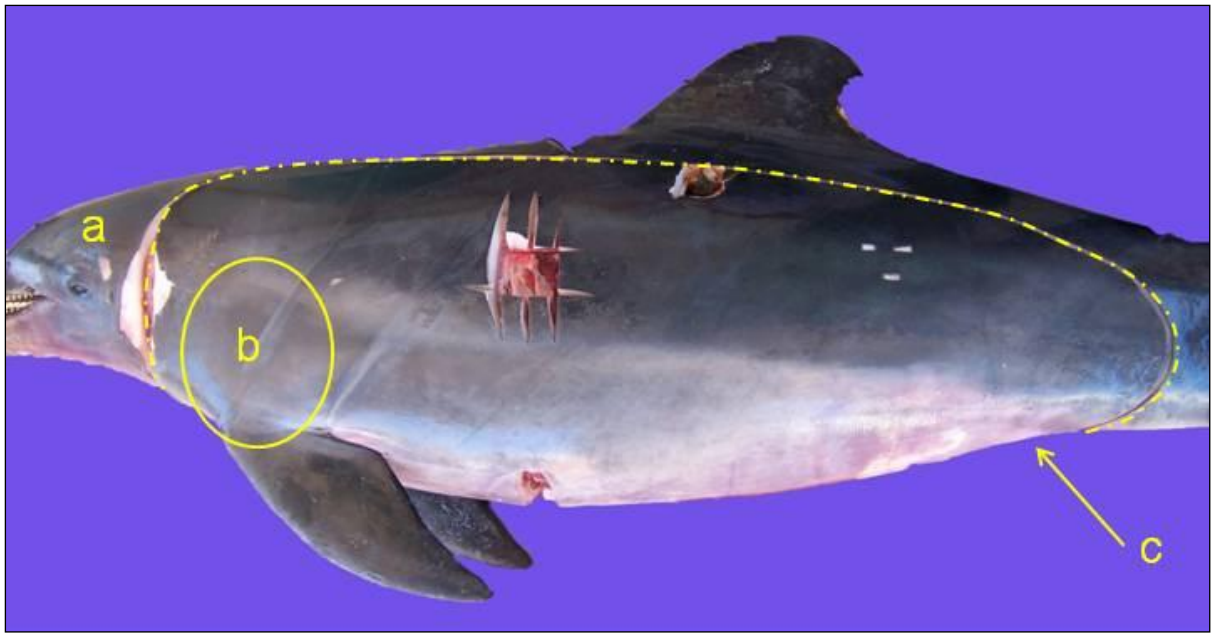

Figure 6: Dolphin lying on the right side. The yellow dashed line indicates the cut to follow when opening the carcass, extending from behind the head (a) (just in front of the shoulder (b)) to the anus (c), following the border of the spinal muscles and the ventral midline.

- Examine the blubber for parasites by making multiple cuts in the blubber on the skin sections that have been removed (Figure 7). Count or estimate the number of parasites found and collect as many as possible in 70% ethanol for molecular analysis and 10% buffered formalin for morphological analysis. Place any abnormalities in formalin.

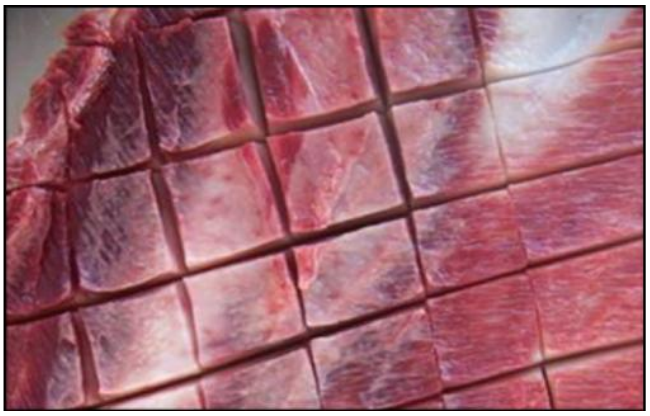

Figure 7: Multiple cuts are made into the blubber to evaluate for parasites.

- Examine muscles for bruising, or abscesses. This may be facilitated by making multiple parallel incisions into the muscle.
- Place a sample of the spinal muscle, just behind and below the dorsal fin, in formalin.
- Examine and place a sample of the left cervical lymph node in formalin (Figure 8).
- Incise and examine the shoulder joint of the left flipper that was removed (see above) for any abnormalities.

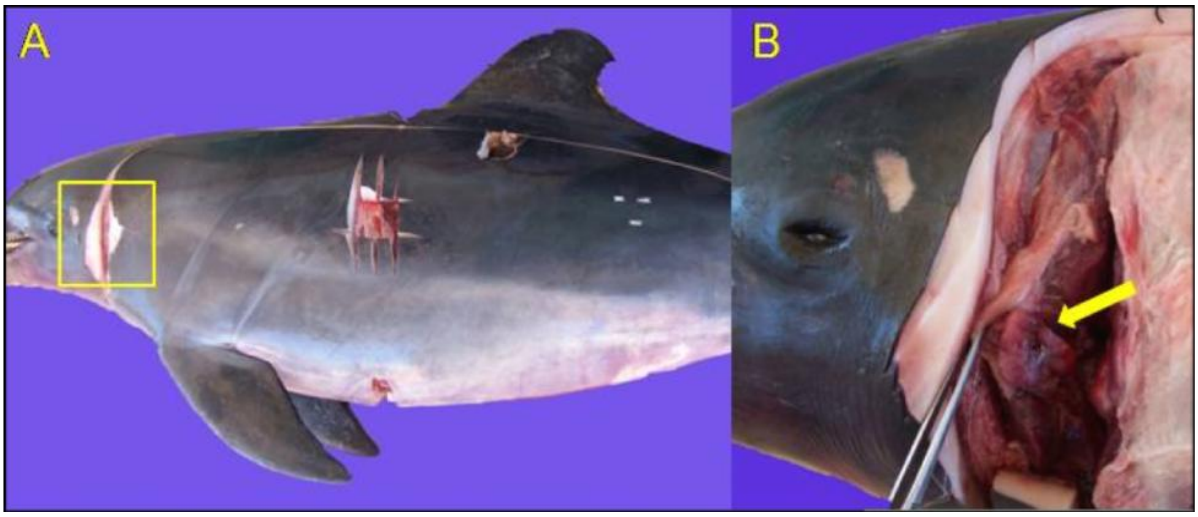

Figure 8: Location of the cervical lymph nodes. A: The cervical lymph nodes are located 6 – 10 cm in front of the shoulder blade (Cowan & Smith 1996), approximately at the level of the joint between the head and the first cervical vertebra. B: Arrow indicates the position of the cervical lymph nodes.

- Expose the abdominal organs by making a Y-shaped cut in the body wall (Figure 9): make a small cut just behind the last rib. Extend this upwards to the spinal muscles (point a; see also Figure 10), and downwards following the curve of the last rib (point c). Cut just below the spinal muscles (see Figure 6), extending from the previous cut to just behind the anus (point d). Care must be taken not to cut any organs. The abdominal muscles can now be pulled down to expose the abdominal organs. Do not cut along the ventral midline, as there is the risk of cutting the uterus.
- Puncture the diaphragm to evaluate the negative pressure in the thorax, by making a stab incision into the diaphragm and listening for a sudden intake of air. If there is no intake of air, there was no negative pressure in the thorax, and this should be recorded as it might indicate puncture of the lungs or damage to ribs.

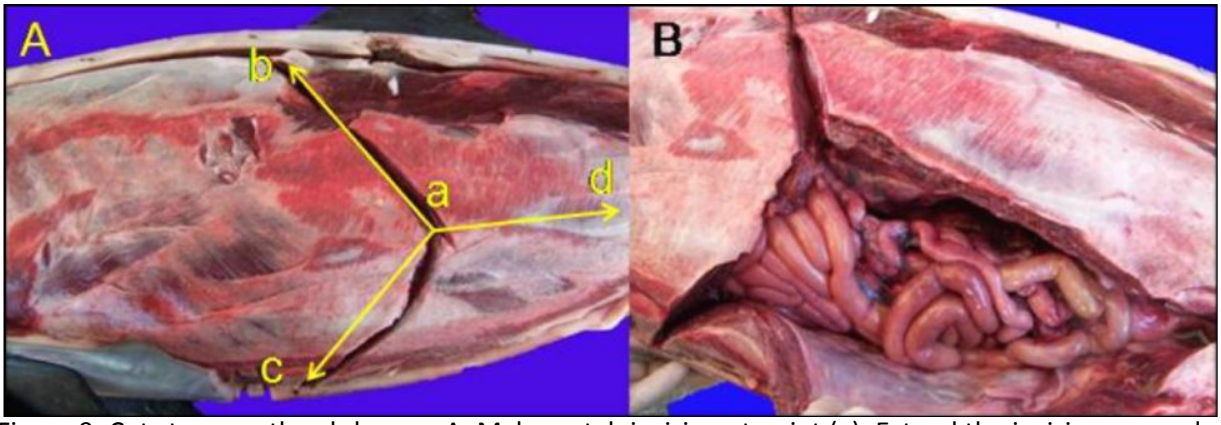

Figure 9: Cuts to open the abdomen. A: Make a stab incision at point (a). Extend the incision upward towards the dorsal fin to point (b), and down towards the umbilicus to point (c). The final incision is made from point (a) towards the tail to point (d) to end with a Y shaped incision (B).

- Using a pair of large garden shears, cut through the ribs at the levels of the breast bone and below the spine, and remove the thoracic (chest) wall.
- Examine any fluid noted in the thoracic or abdominal cavity, and, if present, collect a sample in a serum tube or syringe for microbiology.

2.6. Internal Examination

- Assess and record the stage of decomposition of the carcass, following published guidelines (Geraci & Lounsbury 1993).
    - Code 1– Live animal: no necropsy performed.
    - Code 2 – Carcass in good condition (fresh): normal appearance, fresh smell, minimal drying and wrinkling of skin, blubber firm and white, muscles firm and dark red, no evidence of hemolysis, little or no gas in intestines.
    - Code 3 – Fair (decomposed, but organs basically intact): carcass intact, but bloated, skin sloughing, mild odour, membranes dry, eyes sunken, blubber blood-tinged and oily, muscles soft and poorly defined, , organs soft and mottled, gut has some gas distension.

- Code 4 – Poor (advanced decomposition): carcass may be intact, but collapsing, skin peeling off, blubber soft and full of gas, muscles easily torn, blood thin, organs often identifiable, but easily torn, gas filled intestines.

- Code 5 – Mummified or skeletal remains: skin draped over skeleton, indiscernible or dried out tissues.

- Examine and sample any immediately visible abnormalities before handling any organs. Gently push away the intestine and locate the adrenal glands, ureters, urinary bladder, spleen, liver, reproductive tract and the pancreas. For superficial topography of the abdomen see Figure 10. Move aside the lung to find the heart, bronchi, and trachea (Figure 11). Also examine for any firm and pink or fine, soft and red abnormal attachments between the lung and the body wall.

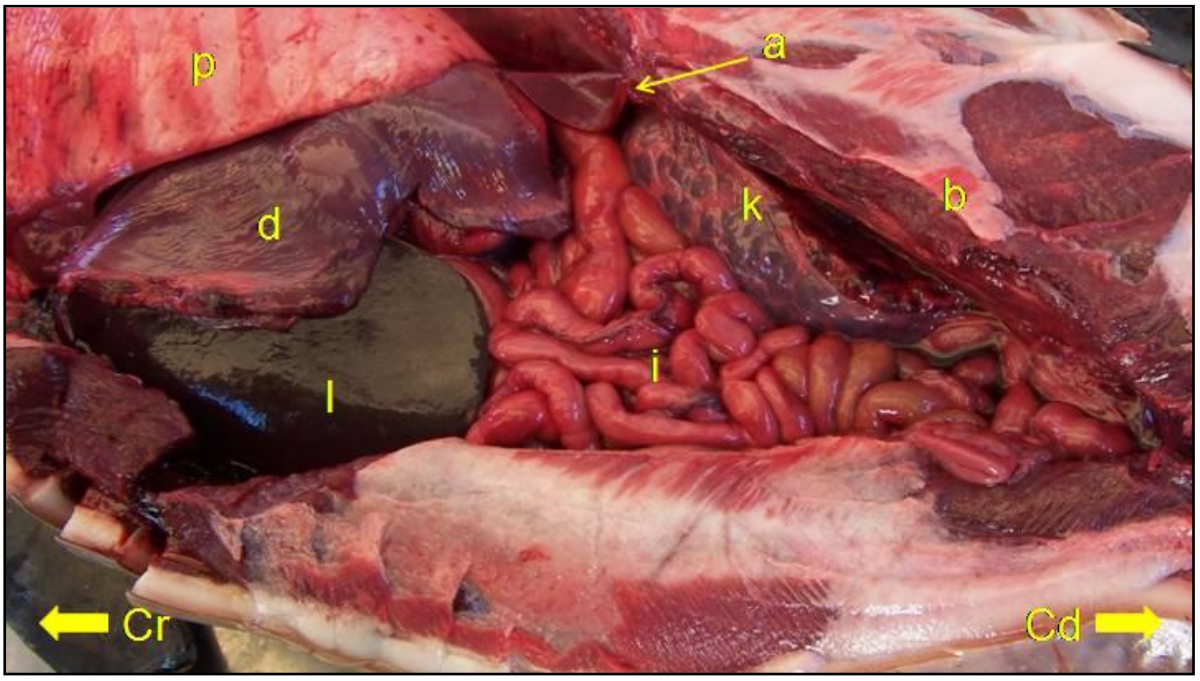

Figure 10: Abdominal topography. From cranial (Cr) to caudal (Cd) in the abdomen is the diaphragm (d), liver (l), intestinal loops (i) and kidney (k). (a) indicates the approximate position of the adrenal gland. The spinal muscles that serve as guidance for the cut into the abdomen are indicated by (b). The border of the left lung (p) can be seen on the left.

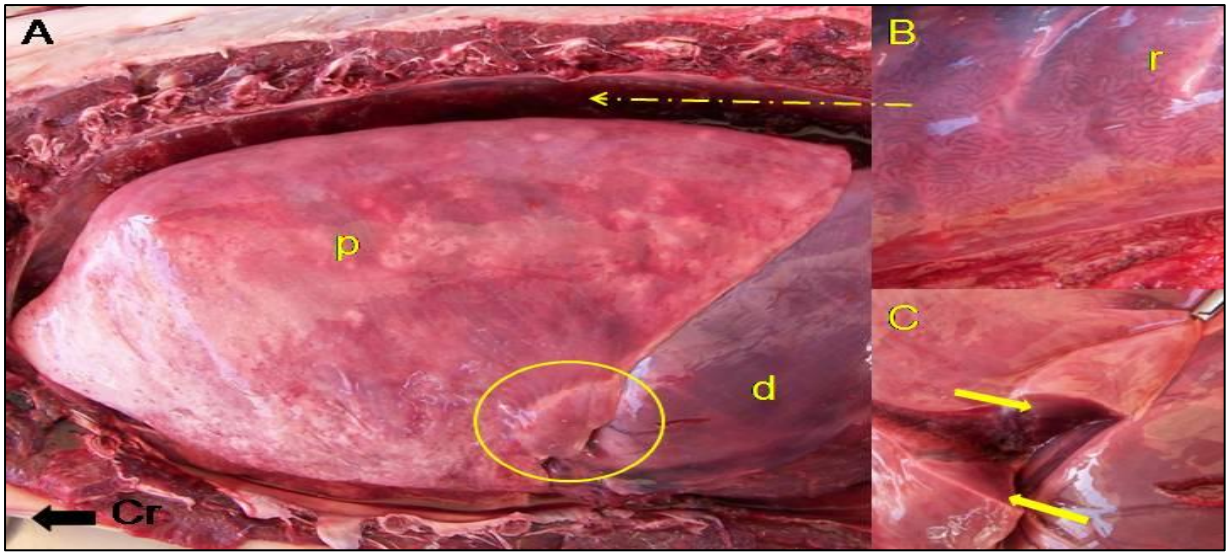

Figure 11: Thoracic topography. When the ribs are removed, the lungs (p) cover most of the thorax in front of the diaphragm (d). The marginal lymph node of the lung is circled (left), and the arrows indicate the cut surface (bottom right). The *rete mirabile* (r) is above the head of the ribs (arrow and top right).

2.7. Abdominal Examination and Sampling

- Examine the diaphragm and place a sample in formalin.
- Tie off the colon twice about 2 cm apart, as it approaches the anus and cut through the colon between the ligatures.
- Do the same with the small intestine as it exits the stomach and cut through the intestine between the ligatures.
- Remove the intestines, starting at the colon, by cutting the attachment to the body wall. Handle the intestine very gently to prevent damage to the tissue. Remove the fat attached to the intestines with associated lymph nodes (Figure 13). Evaluate and sample the lymph nodes for histopathology and microbiology. Place the lymph node on tissue paper and label the tissue paper with pencil, identifying the lymph node (i.e. mesenteric lymph node), before placing it and the tissue paper into the formalin.

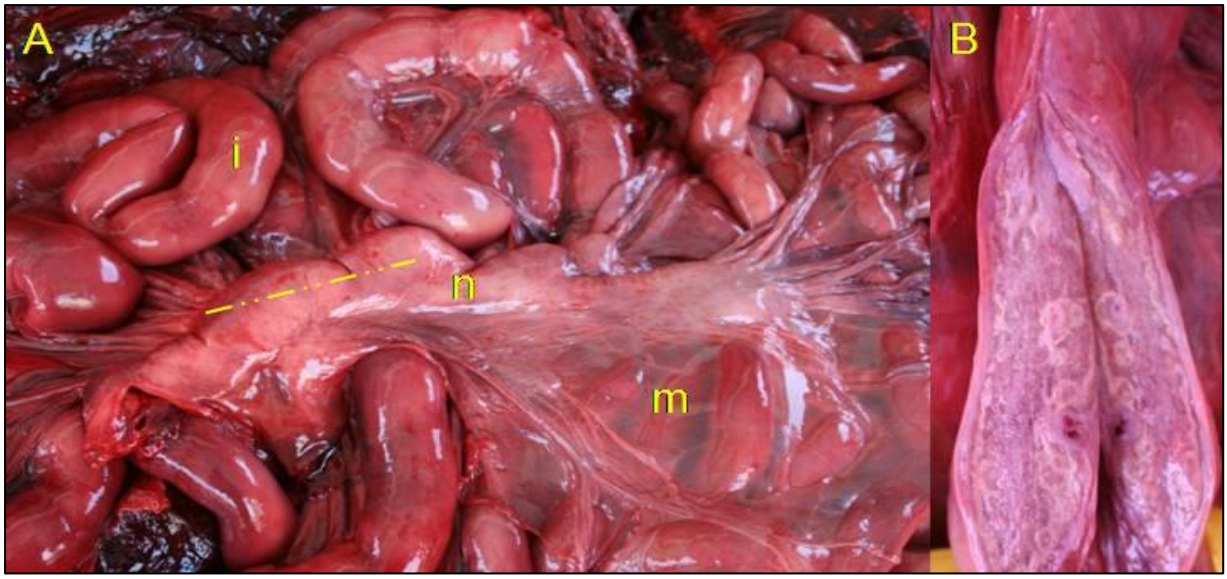

Figure 12: A. Mesenteric lymph nodes (n), with cut surface (B), along dashed line, situated in the mesentery (m) surrounded by intestine (i).

- Examine the intestine as follows:
    - Arrange the intestine in serial loops on a flat surface (Figure 12).
    - Sample one section from the cranial, middle and caudal parts of the intestine for histopathology and microbiology before opening the entire length. Sample these sections from the intestine where it is closest to the stomach, from the middle part, and close to the anus.
    - Open the rest of the intestines, and sample any parasites or abnormalities in the intestinal wall.
    - Intestinal contents should be collected in an empty sample bottle. One third of this should be frozen, and the rest diluted and preserved with 70% ethanol (at a 3:1 ratio) for parasite identification.

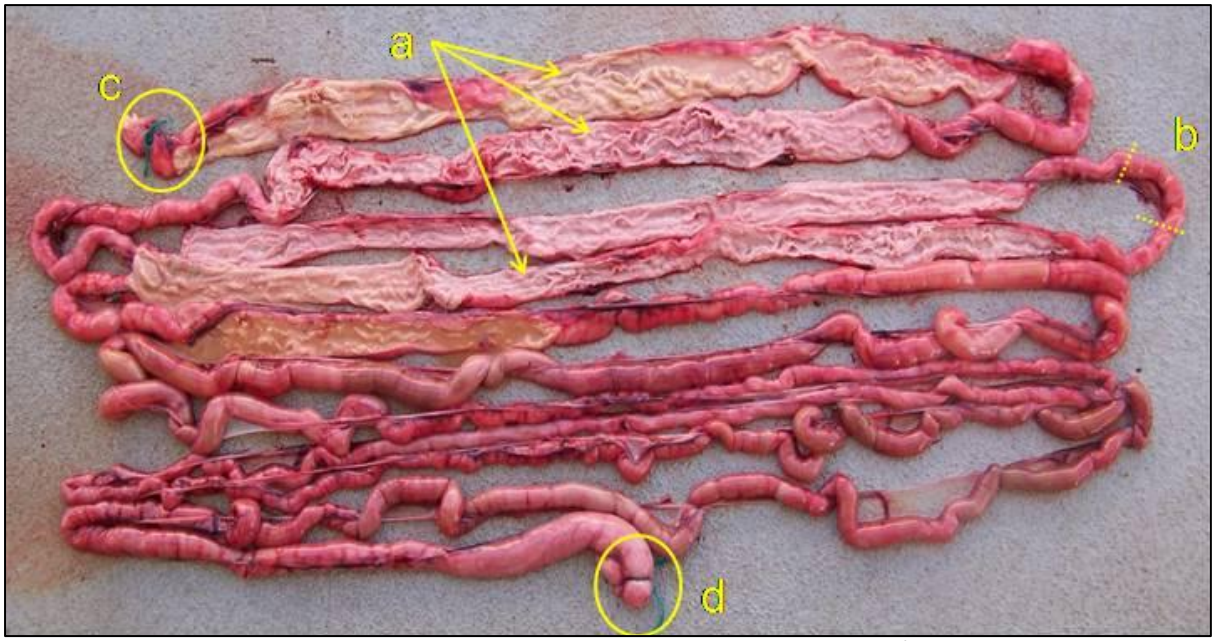

Figure 13: The entire intestinal tract from the duodenum (d) to the colon/rectum (c) is arranged in parallel loops on a flat surface and then sampled by taking a closed section of intestine, as illustrated by (b), where two cuts are made along the dashed lines. The intestine is then opened (a) and examined. Tying off the colon (c) and duodenum (d) prevents faecal contamination of the carcass.

- Remove the oesophagus and stomach by cutting through their attachments, leaving the spleen and other organs in the abdominal cavity. Open the stomach and evaluate and sample any parasites. If the stomach contents are not processed immediately (which requires trained personnel), the contents should be kept frozen to prevent further digestion. All the stomach contents, and any contents that may be in the oesophagus, should be kept in a labelled container. Gently rinse the stomach wall and take a sample from each of the glandular, muscular, and pyloric sections in formalin. The relevant anatomy of the stomach is shown in Figure 14 (Harrison *et al.* 1970).

- Examine and sample the liver, both left and right lobes, with bile ducts, for histopathology and microbiology.

- Examine and place a sample of the pancreas in formalin. The pancreas can be found attached to the intestine as it exits the stomach. It is a light pink to tan organ, quite thin and elongated.

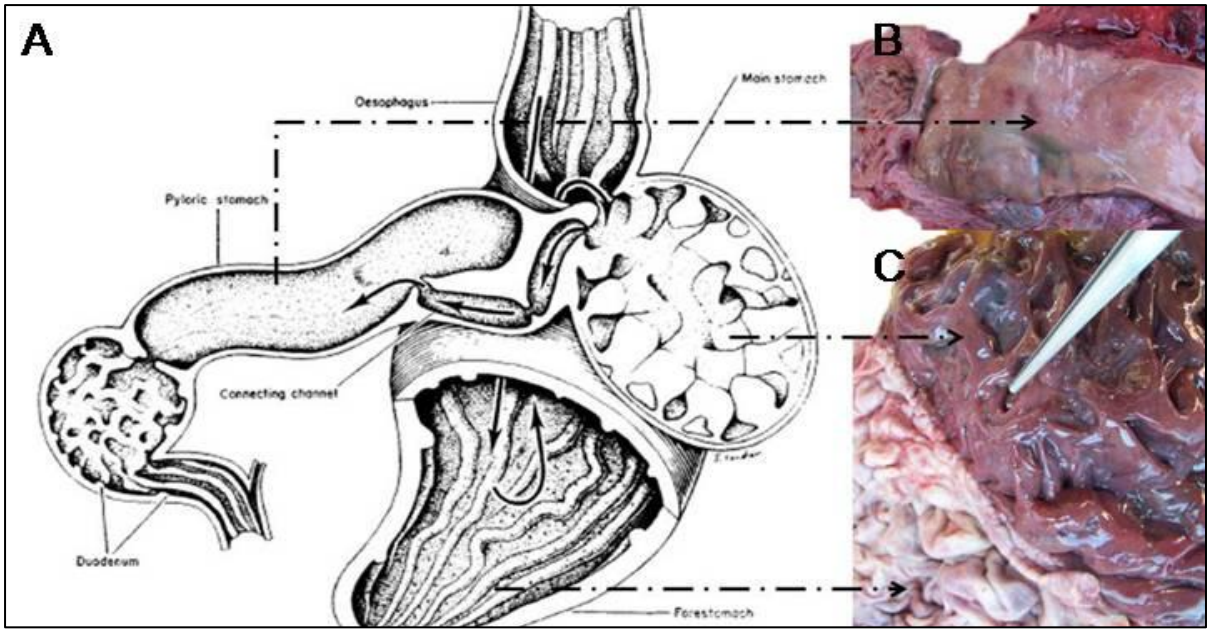

Figure 14: Anatomy of the dolphin stomach. A: (from Harrison, 1970) illustrates the different compartments of the dolphin stomach; the forestomach (muscular part), main stomach (glandular part), and pyloric or third compartments. B and C: The different appearances of the various compartments.

- Examine and sample the spleen for histopathology and microbiology. The spleen is typically very dark red to black in colour, approximately 5 cm in diameter. It is usually located just behind the stomach and liver. Dolphins usually only have one spleen, although accessory spleens are common (Cowan and Smith 1999). The number, size and weight of the accessory spleen (s) should be recorded on the necropsy reports, and sections of the accessory spleen(s) should also be placed in formalin.

- Examine, weigh and take a full-thickness sample of both left and right adrenals, in formalin (Figure 15). The adrenals are located just in front of the kidneys, below the spine, and are relatively small (approximately 3 cm x 2 cm x 2 cm).

- Examine and take a full thickness sample of both left and right kidney for histopathology and microbiology (Figure 15).

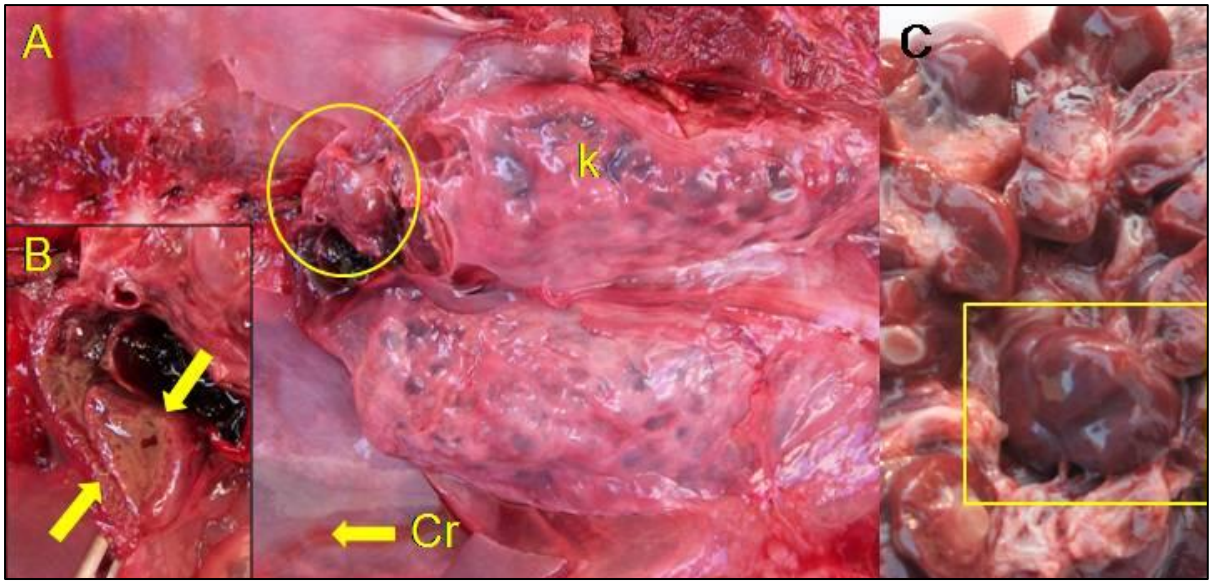

Figure 15: Location of the kidney and adrenal gland in a small cetacean. A: The adrenal glands (circled in A, arrows in B) are located just in front of the kidneys (k). C: sectioned kidney and multiple lobules (square). Pictures by David Zimmerman.

- Examine and place a sample of the bladder wall in formalin. Store urine, if present, for urine and microbiological analysis either in a sterile urine container suitable for freezing or a sterile syringe. It may be refrigerated or stored on ice before it is sent to the laboratory. If the carcass is fresh and has not previously been frozen, urine can be sampled for urine analysis and should not be frozen, while urine for microbiological analysis may be frozen. Urine from a previously frozen carcass may only be used for microbiology and may be re-frozen.
- Remove, examine and sample the entire reproductive tract (Figure 16) as follows:
    - In juvenile animals, the entire reproductive tract is placed in formalin for histopathology.
    - The weights of both testes should be recorded independently before sampling. A transverse section of both testes should be placed in formalin.
    - In adult females the whole reproductive tract is kept in a separate sample bottle and preserved in 10% buffered formalin for reproductive studies. During sub-sampling, a longitudinal section from both ovaries and transverse midsections from both uterine horns, and the body of the uterus should be placed in formalin. (Figure 16).

- If a female is pregnant, particular attention should be paid that investigators wear protective clothing, as there is currently uncertainty about the presence of *Brucella* spp. in dolphins in South Africa. The foetus should be dissected in the same way as an adult animal, and the reproductive tract of the female/cow sampled *in toto* in the same manner as a non-gravid uterus.

- Several sections of the placenta should also be evaluated and sampled for histopathology and microbiology.

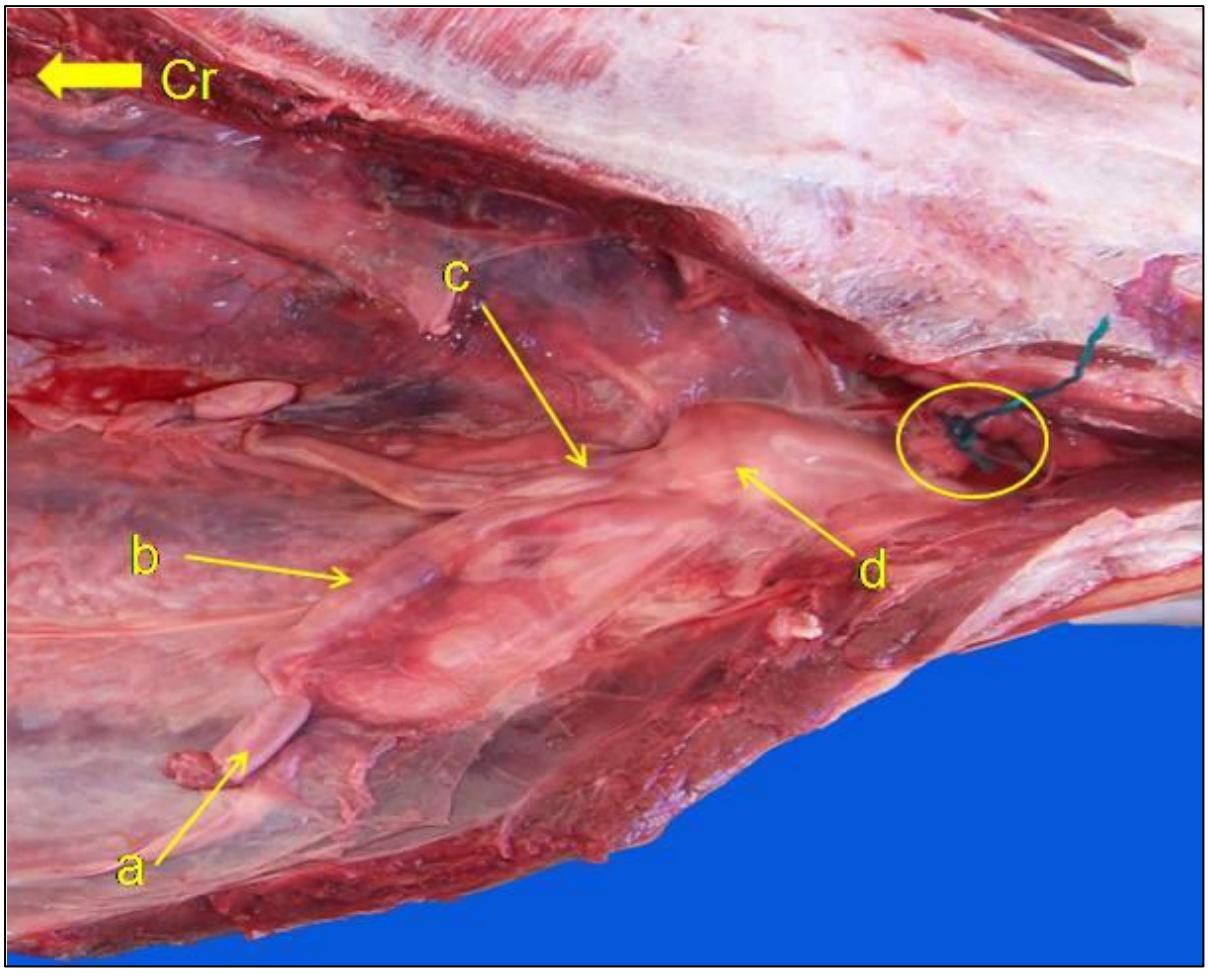

Figure 16: Female reproductive tract topography. The female reproductive tract is located on the ventral body wall with the ovary (a), uterine horn (b), uterine body (c) and the cervix (d). Circled is the tied off colon.

2.8. Thoracic Evaluation and Sampling

- To remove the pluck (heart, lungs and trachea), cut the blubber and skin between the lower jaw and the tongue, freeing the tongue. Extend the cuts towards the thorax, cutting through the fine bones just behind the tongue, and the pharynx that leads to the blowhole, ensuring that the larynx ('goose beak') is removed together with the pluck. Free the heart, lungs, thymus and trachea all together from the thoracic cavity.

- Place the head of one rib in formalin. Examine the rib joints and spine for signs of abnormality (e.g. arthritis etc.).

- Examine and sample the *rete mirabile*, a meshwork of blood vessels attached to the ribs (Figure 11).

- Examine the tongue and place a section of the middle third of the tongue in formalin (Figure 17).

- Open the larynx and the pharynx. Pay particular attention to the pharynx as the tonsils are embedded in the wall (Figure 17). A section of the tonsils or tonsillar area should be placed in formalin.

- Locate the thyroid, which is attached to the trachea, between the larynx ('goose beak') and the lung (Figure 18). Examine and place in formalin.

- If the animal is young, locate the thymus, which lies in front of the lungs and the heart (Figure 18), examine and place a sample in formalin. In adult animals the thymus may resemble fatty tissue, so tissue from just in front of the heart may be sampled as this frequently contains thymic remnants. Examine and sample the following before opening the trachea and feeling the lungs for small abnormalities (Figure 19):

    - the trachea behind the 'goose beak' for histopathology; cranio-ventral lung lobe from both the left and right lungs for histopathology and microbiology
    - upper parts of the lung lobes from both the left and right lungs in formalin for histopathology
    - Open the trachea along its entire length as well as the smaller airways. Make multiple cuts in the lung and feel it carefully to examine for abnormalities and parasites. As lung nodules may be small, the cuts should be approximately 1 cm apart.

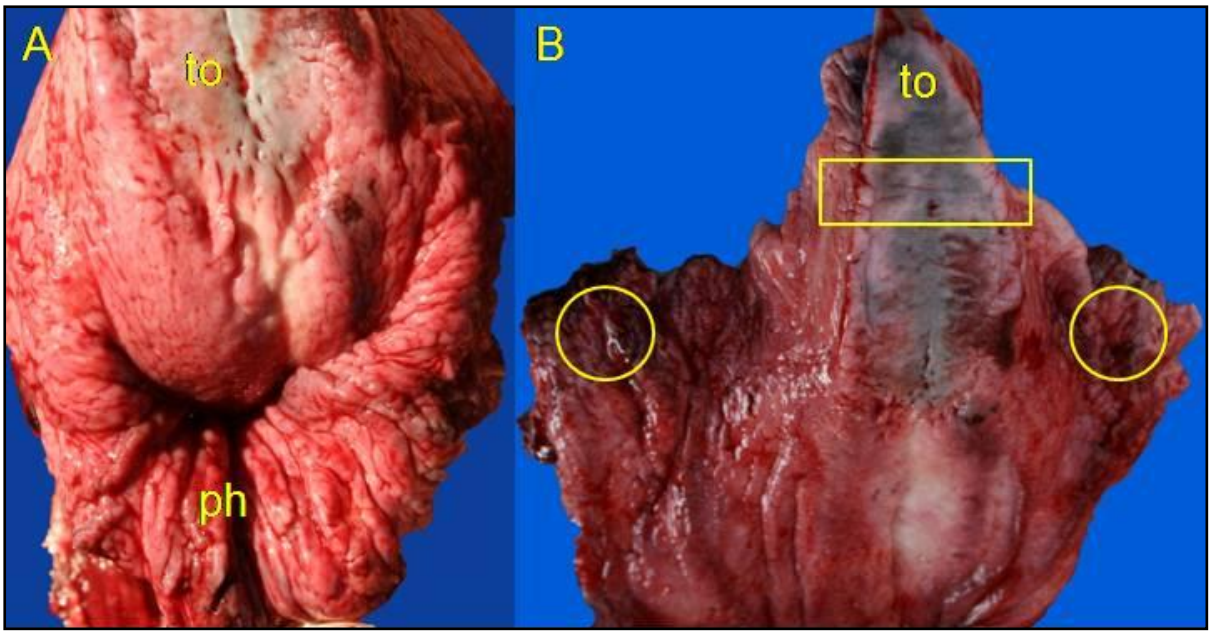

Figure 17: Anatomy of the pharynx and location of the tonsils. A: Pharynx (ph) before it is opened (B), containing the tonsillar area (circled). The tongue (to) sample for histopathology is taken from the middle third (square).

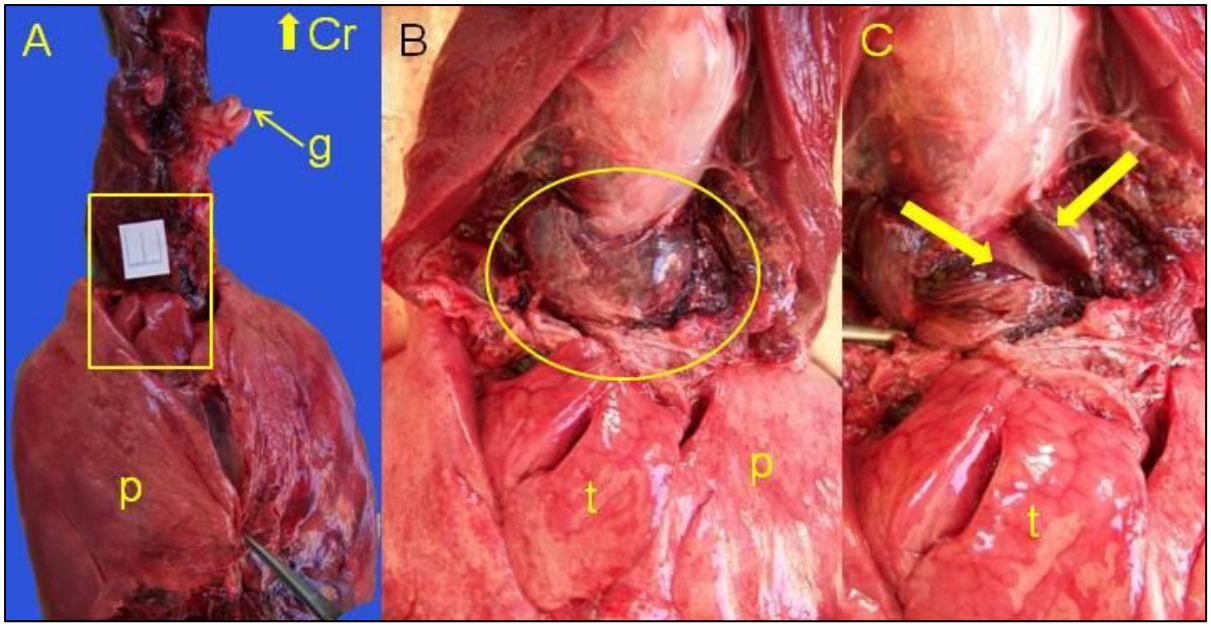

Figure 18: Location of the thymus (t) and thyroid (circled in B, and arrows in C), both of which can be found between the lungs (p) and the larynx ('goose beak')(g in A) on the ventral side of the pluck (A).

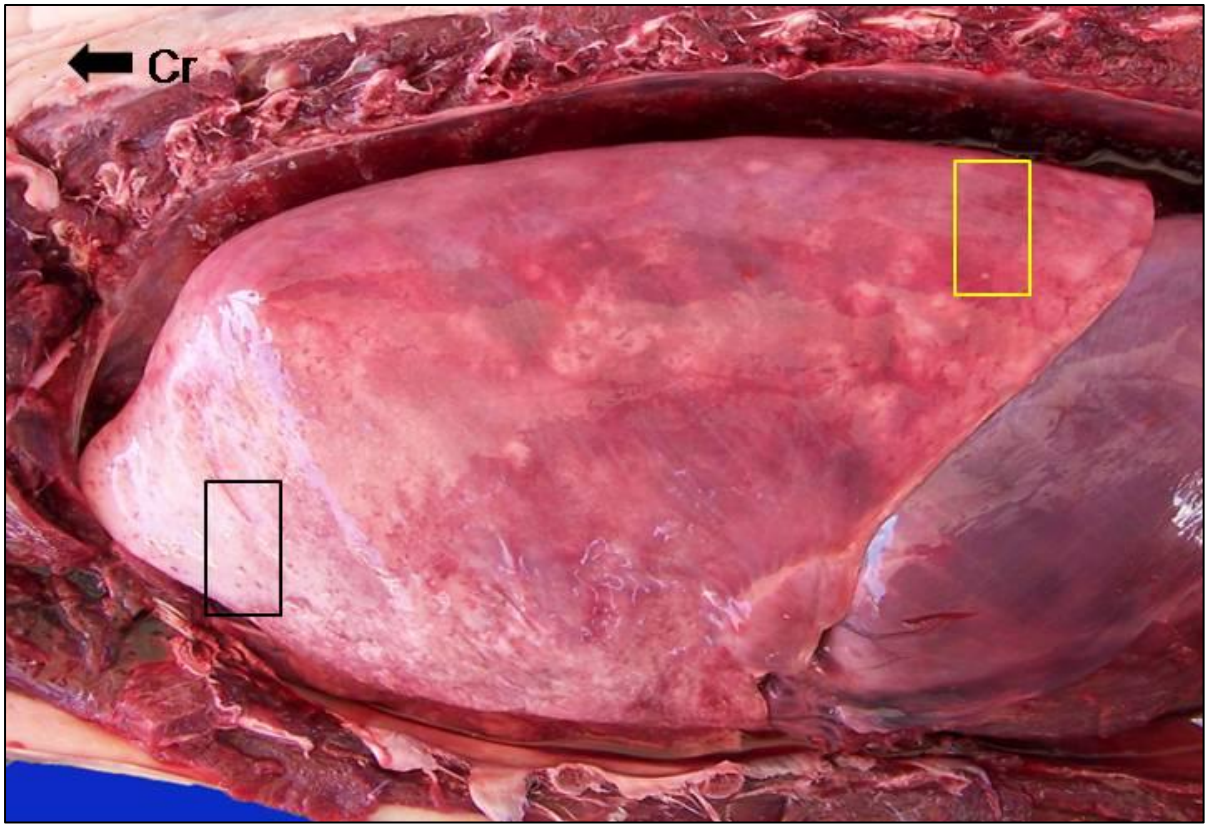

Figure 19: Lung with rectangles indicating the site of sampling; black: cranio-ventral lung field. Yellow: caudal lung field.

- Examine and place a sample of the marginal lymph node of the lung that lies on the outside edge of the lung (Figure 11) in formalin.

- Open the heart by cutting along the *vena cava*, entering the right atrium, and cut along the right ventricle circumference, following the blood vessels in the interventricular groove, ending in the pulmonary trunk (for an overview of the anatomy of the heart see Figure 20). Then start an incision in the left atrium, extending straight down into the left ventricle. Open the aorta as it leaves the heart, by cutting through the heart valve. Examine the heart muscle, and inner and outer linings as well as the valves. Place samples of the following in formalin:

    - right ventricle wall and pulmonary semilunar valve
    - left papillary muscle
    - interventricular septum
    - aorta

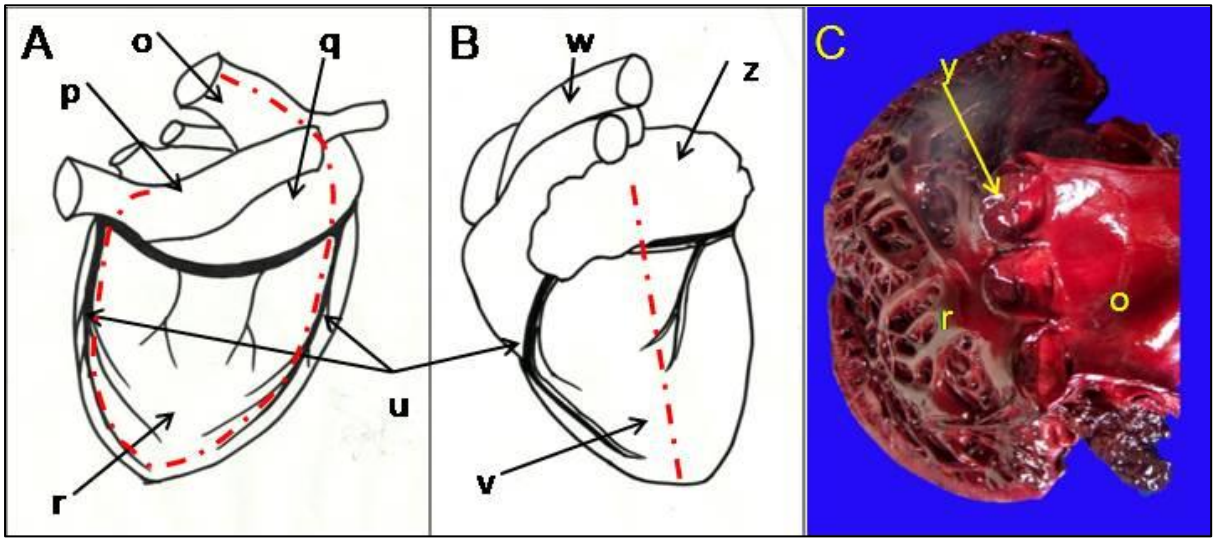

Figure 20: A and B: Anatomy of the heart showing the pulmonary trunk (o), vena cava (p), right atrium (q), right ventricle (r), blood vessels in interventricular groove (u), left ventricle (v), aorta (w) and left atrium (z). The red dashed line indicates the incisions made to open the heart (A: incisions in the right side of the heart. B: incision in the left side of the heart). C: The appearance of the endocardium (right ventricle, r), the heart valves (y) and the major blood vessels (pulmonary trunk, o). Illustrations by Ingrid de Wet.

2.9. Skull Examination and Sampling

- Remove the head by cutting through the joint between the spine and skull (Figure 21). Examine the joint for any abnormalities.
- Incise and examine the melon (Figure 21).
- Cut into the airsacs located under the blowhole, and further down to include the 'monkey lips' (internal nares).
- Remove as much muscle and fat from the skull as possible.

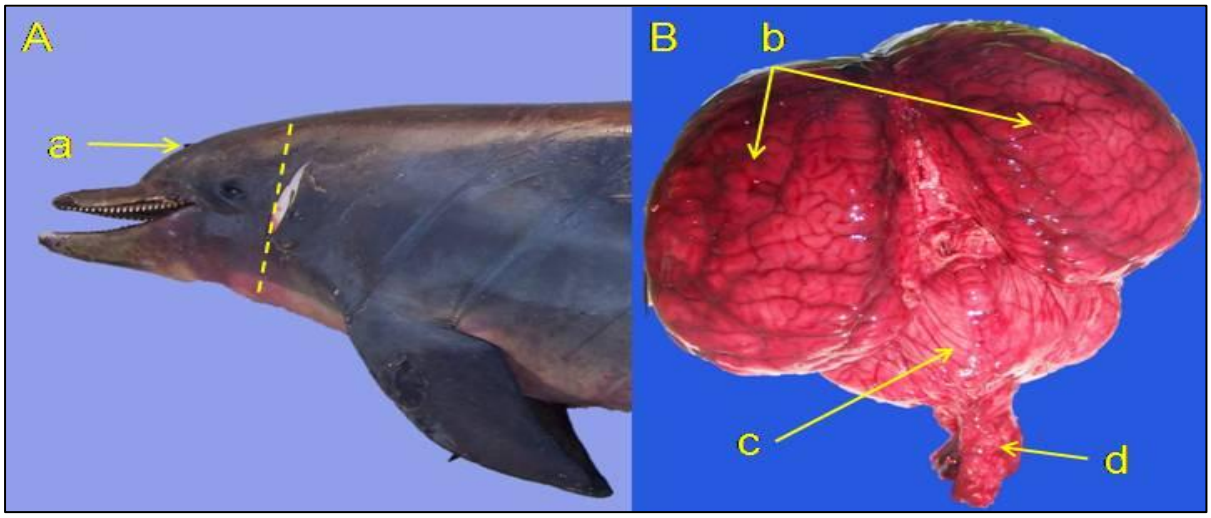

Figure 21: A: Dashed line shows approximate incision to separate the head from the body, and the melon (a). B: Anatomy of the brain showing the cerebral hemispheres (b), cerebellum (c) and spinal cord (d).

- Removal of the ear requires some practice and may best be achieved with some prior training. However, examination of the ears is increasingly important and thus sampling should be attempted at all costs. The ear is located behind and below the eye, just above the jaw joint. Remove the lower jaw. Place the head upside down and locate the ear, just next to the jaw joint. Cut around the bone of the ear, taking care not to damage the bone. Gently remove the attachments of the ear to the skull, examine (particularly for the presence of parasites) and place in formalin; Sample any parasites in the area.

- Examine and place samples of the auditory fat along the outer wall of the lower jaw bones in formalin. Pay close attention to any haemorrhage that may be present.

- Open the skull by making a circular cut on the back of the skull using a saw or axe (Figure 22). Once the bone has been removed, cut the fibrous membrane around the brain, cut through the cranial nerves that attach the brain to the skull and remove the whole brain. Examine and sample the cerebrum for microbiology and place the rest of the brain in formalin. If the container opening is not large enough to fit the brain without it touching the opening, the brain should be cut in half longitudinally. If only small sample bottles are available, take a sample from the front of the cerebrum, the mid-section of the cerebrum, the cerebellum and the spinal cord.

- Examine and place the entire pituitary in formalin. The pituitary can be found in a small midline recess on the floor of the skull after the brain has been removed (Figure 23).

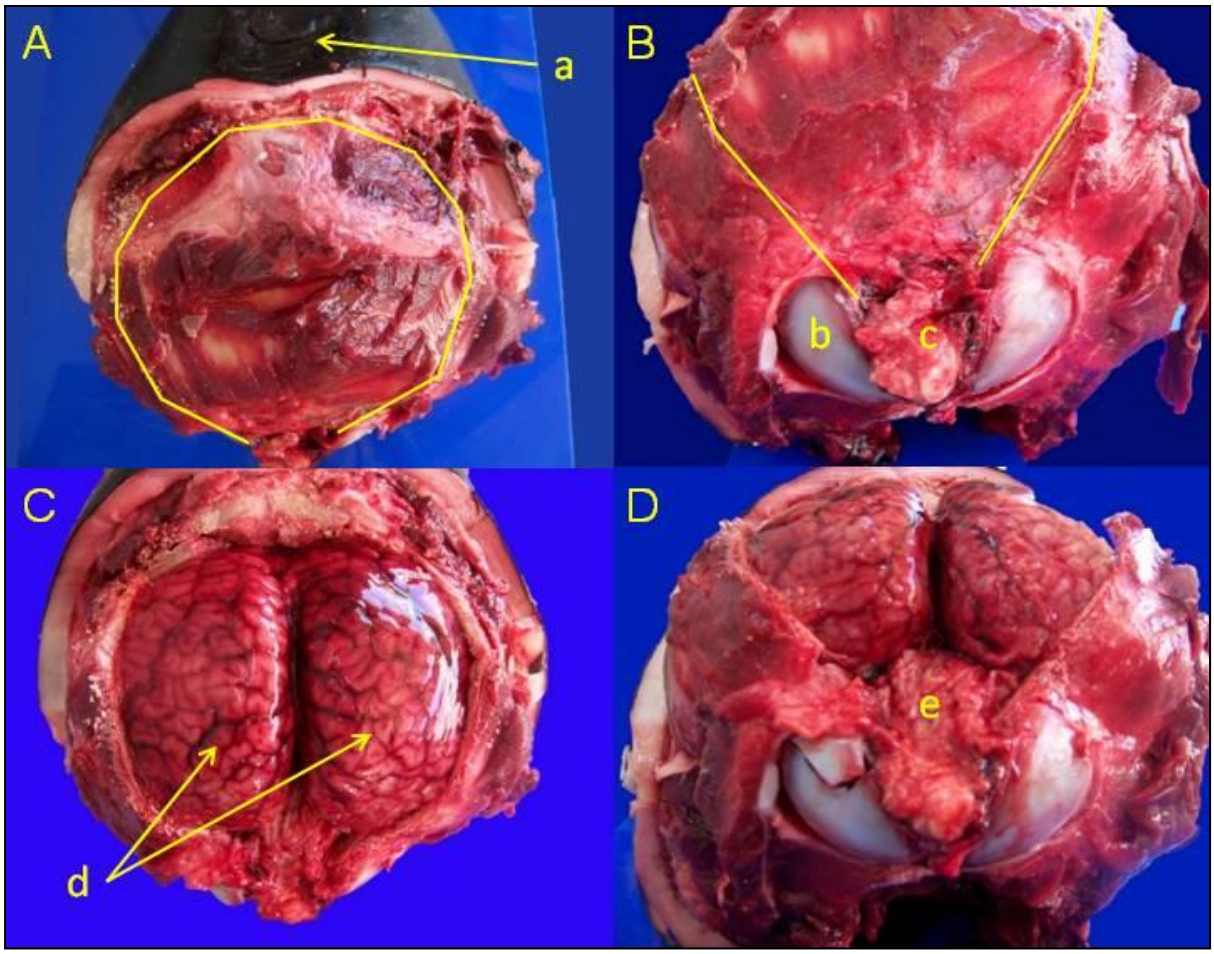

Figure 22: Opening the skull. A: Make a roughly circular incision on the dorso-caudal aspect of the skull behind the blowhole (a). B: Extend the cut to the *foramen magnum* (b), through which the

spinal cord extends, by cutting above the joint surfaces (c). C and D: The completed cuts with the brain exposed showing the cerebrum (d) and the cerebellum (e).

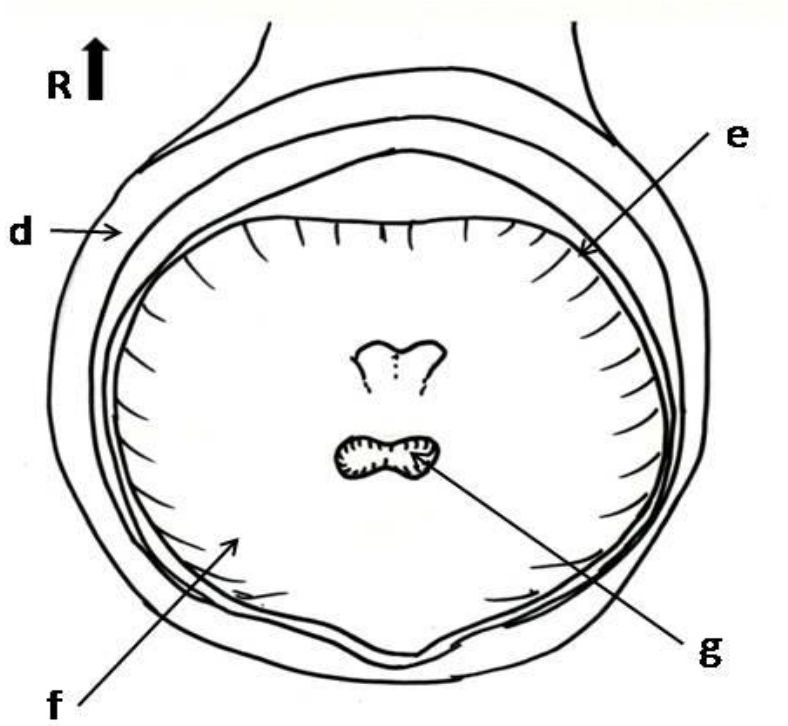

Figure 23: Back of the skull (towards the blowhole, R), showing the location of the pituitary gland, located in a recess (g) in the floor of the skull (f), visible after the brain has been removed by making the cuts in the skull to expose the brain (e), and the layers of muscle and skin (d). Illustration by Ingrid de Wet.

Appendix II. Reporting

This report has been designed specifically to follow the protocol designed for South African cetaceans. Reports should be as detailed as possible, with all information recorded. If information is not available, or an organ is not evaluated, a reason should be given to aid in further development and modification of this protocol and report.

Table 1: Detailed post-mortem record form to be used for individual necropsies.

Post Mortem Record		**Animal**	
		Identification:	
Person conducting the PM:			
Date:			
Location:			
Species:			
Sex:			
Age:			
Body weight (kg):			
History:	**(circumstances leading to the animals' illness/clinical signs/death)**		
Measurements (cm): see 2.1, Appendix IV	**Upper jaw to notch of tail fluke:**		
Stage of decomposition	**(1=Fresh, 5=advanced decomposition) see 2.4**		
External:	**see 2.2**		
Blubber thickness (cm): Fig 5	**Lateral to dorsal fin**		
	In front of dorsal fin (dorsal midline)		
	Cranial to dorsal fin (lateral midline)		

	Cranial to dorsal fin (ventral midline)	
Mammary Glands:	**see 2.2**	
Right Eye:	**see 2.2**	
Teeth	**see 2.3**	
Blubber:	**Fig 7, see 2.3**	
Muscle/subcutis:	**Fig 7, see 2.3**	
• **Cervical Lymph Nodes: Fig 8**		
Abdominal cavity: see 2.4, Fig 10		
Diaphragm see 2.5		
Intestines:	**see Fig 12**	
Mesentery and lymph nodes: see Fig 13		
Oesophagus:	**(contents/ulcers/parasites)**	

Muscular stomach (first compartment)	**see Fig 14**
Glandular stomach (second compartment)	**see 2.5**
Pyloric stomach (third compartment	**see 2.5**
Liver:	**see 2.5**
Pancreas:	**see 2.5**
Spleen:	**see 2.5**
Adrenal:	**see 2.5, Fig 15**
Kidneys:	**see 2.5, Fig 15**

Bladder:	**see 2.5**

Reproductive tract:	**see 2.5, Fig 16**

Thoracic cavity: see 2.6, Fig 11

Thoracic cavity and organs:

Bone marrow (head of rib)	**see 2.6**
***Rete mirabile*:**	**see 2.6, Fig 11**
Tongue:	**see 2.6**

Tonsils:	**see 2.6, Fig 17**

Thyroid gland:	**see 2.6, Fig 18**
Thymus:	**see 2.6, Fig 18**

Lungs:	**see 2.6, Fig 19**

Marginal lymph node of the lung:	**see 2.6, Fig 11**

Heart/pericardium:	**see 2g, Fig 20**

Skull: see 2.7, Fig 21	
Melon:	
Blowhole/airsacs:	**see 2.7**
Ear and auditory fat	**see 2.7**
Brain & hypophysis:	**see 2.7, Fig 21, 22, 23**
Suspected cause of death:	
Post mortem changes and incidental findings:	
Clinically significant findings:	
Cause of death:	

Name and contact number of person who conducted the PM:

Table 2: Checklist for necropsies.

CHECKLIST		**Identification**							
		Smpl	√	B			Smpl	√	B
Measurements					Blood	Serum and EDTA			
Stage of decomposition					Bone marrow				
Blubber thickness						Rib	H		
Blubber sample including skin		H			*Rete mirabile*		H		
					Tongue	Middle third	H		
External abnormalities		H			Thyroid Gland		H		
		M			Thymus	If present	H		
Any discharge?		M			Oesophagus				
Ectoparasites		P				Contents	PEM		
Mammary glands		H					P		
		M				Cranial third	H		
Right eye		H			Marginal lymph node of the lung		H		
Blubber		P			Tonsil		H		
Skeletal muscle		H			Trachea	Cranial third	H		
	Caudo-lateral to dorsal fin				Lung	Cranio-ventral lung lobe L	H		
Cervical lymph nodes		H					M		
Teeth	Age determination	PEM				Cranio-ventral lung lobe R	H		
In situ examination							M		
Fluid	Abdominal/thoracic cavity	M				Dorsal lung lobe L	H		
Intestines		H				Dorsal lung lobe R	H		
	Cranial, middle and	M			Heart	R ventricle & pulmonic valve	H		
	Caudal	P				L papillary muscle	H		
Mesenteric lymph nodes		H				Interventricular septum	H		
		M				Aorta	H		
Stomach					Diaphragm		H		
	Parasites	P			Blowhole				
	Stomach contents	PEM			Melon				
	Glandular stomach	H			Ear		P		
	Muscular stomach	H			Brain		H		
	Pyloric stomach	H					M		
Liver	Left and right lobes with	H			Hypophysis		H		
	bile ducts	M			Virology				
Pancreas		H							
Spleen		H							
		M							
Kidney	Left	H			Abnormalities				
		M							
	Right	H							
		M							
Adrenal	Left	H							

	Right	H							
Bladder	Tip of bladder	H			Key:	Sample	Smpl		
Reproductive tract						Completed task	√		
	Uterus	H				Bottle (container) number	B		
	Ovaries	H				Histopathology	H		
	Testes	H				Microbiology	M		
	Penis	H				Parasitology	P		
	Foetus	PM				Post Mortem to be done	PM		
	Placenta	H				Analysis by PE Museum	PEM		
		M							

Appendix III. Suggested Equipment List

The equipment required to complete a necropsy on cetaceans largely depends on the individual situation, the facilities available, and the type of animal. Basic equipment should include:

- Standard necropsy instruments such as scalpel handles, scalpel blades, scissors, forceps, knives, knife sharpeners, pruning shears (for cutting through ribs), and manual saws.
- String or twine for tying off the the stomach and intestine (approximately one metre per dolphin)
- Sterile instruments for collection of samples for microbiology.
- Sample bottles of various sizes.
- 10% buffered formalin for collection of histopathological samples (1.5 litres per animal).
- 70% ethanol for parasite and teeth collection (100 ml per animal).
- Plastic zipper storage bags, e.g. Ziploc®, for microbiology samples (10 per animal).
- EDTA tube or syringe for sampling of any discharges (at least 5).
- Labels (both on waterpaper and sticky labels).
- Permanent markers and pencils for labelling.
- Data sheets for recording findings (see Appendix II, Table 1).
- Protective clothing, including waterproof aprons, gloves, gumboots, overalls, protective eyewear and face masks.
- Garbage bags, disinfectant, paper towels and other clean-up equipment.
- Cooler box and ice blocks if a fridge or freezer is unavailable.
- Digital camera for photographic documentation of abnormalities and animals.
- First aid kit.

Appendix IV. Standard Measurements

Table 1: Standardized measurements for full morphometric studies that should be taken before every necropsy (after Norris, 1961) to go with explanatory diagram (Appendix I, Figure 1). All measurements are taken in a straight line parallel to the long axis of the body, except if marked with *, which indicates a direct measurement.

No.	Measurement	Centimetres (cm)
1	Total length (tip of upper jaw to deepest part of fluke notch)	
2	Tip of upper jaw to centre of eye	
3	Tip of upper jaw to apex of melon	
4	Tip of upper jaw to angle of gape	
5	Tip of upper jaw to external auditory meatus	
6	Centre of eye to external auditory meatus*	
7	Centre of eye to angle of gape*	
8	Centre of eye to centre of blowhole*	
9	Tip of upper jaw to blowhole along midline	
10	Tip of upper jaw to anterior insertion of flipper	
11	Tip upper jaw to anterior insert of dorsal fin	
12	Tip of upper jaw to tip of dorsal fin	
13	Tip of upper jaw to midpoint of umbilicus	
14	Tip of upper jaw to midpoint of genital aperture	
15	Tip of upper jaw to centre of anus	
	Projection of lower jaw beyond upper jaw	
16	Girth at axilla*	
17	Girth at maximum*	
18	Girth at anus*	
19	Length of flipper, anterior insertion to tip*	
20	Length of flipper, axilla to tip*	
21	Maximum width of flipper	
22	Height of dorsal fin, fin tip to base*	
23	Dorsal fin: length of base	
24	Tail flukes: tip to tip width*	

25	Tail flukes: notch to anterior border*	
26	Tail flukes: depth of notch	
	Length of eye	
	Length of mammary slits (right; left)	
	Length of genital slit	
	Length of anal opening	
	Dimensions of blowhole (width: length)	