Odontocete Salvage, Necropsy, Ear Extraction, and Imaging Protocols

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Two beaked whales: ©NAN HAUSER

Dwarf sperm whale on table: University of North Carolina at Wilmington Marine Mammal Stranding Program

PREAMBLE

The tissue-collection and -analysis protocol contained herein is designed to guide the collection of pathological and biological data in single and mass strandings of cetaceans, including those qualified as an unusual mortality event (UME). Its purpose is to provide for the systematic collection of data so that a post-hoc analysis of potential factors influencing cetacean stranding events can be performed. The protocol is not intended to promote a specific forensic diagnosis of causative factors contributing to a stranding event and cannot be used to determine whether a stranding event was caused by anthropogenic sound. The protocol can support long-term research activities investigating a range of natural and anthropogenic causes of cetacean strandings. The creators of the protocol disclaim any responsibility for the opinions, forensic interpretation, or misuse of data collected under this protocol related to stranding events putatively associated with anthropogenic sound.

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Chapter 1

INTRODUCTION

This protocol document has been developed to assist those people involved in cetacean-stranding-response activities to provide critical data for diagnosis of the event itself and to provide a better overall understanding of the role of human-made (anthropogenic) noise in strandings and stranding-related mortality. The primary purpose of this document is to develop practical guidance for immediate stranding-response action and ensuing data collection, tissue sampling, and preparation for analysis.

It is critical that diagnosis of any stranding event be approached classically without bias. This approach involves acquisition of available history; blood sampling as possible; careful external and internal gross examination; radiography and imaging as possible; sampling for histology, microbiology, and toxicology; and subsequent sample analysis, the steps of which are iteratively driven by the implications of accumulating data. Thus, each event must be analyzed without preconception. Only this approach makes possible full consideration and distillation of a list of differential diagnoses, in order to ultimately arrive at the most parsimonious conclusion regarding likely cause(s) of stranding and/or death.

So that the biology of acoustic-induced stranding can be placed in the most-consistent and best-possible context, the protocols provided in this document need to be applied, as practical, to future single and mass stranded cases of all cetacean species.

No single protocol can be applied to all situations. The detailed protocols are intentionally comprehensive to encourage collection of a full suite of samples and data under ideal conditions. The protocols herein are provided in an attempt to formalize and standardize the investigation of small cetacean strandings (including beaked whales) to better determine the causes of death.

There are numerous marine mammal necropsy protocols (Geraci and Lounsbury 2005; McLellan et. al. 2005), and several marine mammal programs have their own standard protocols. The authors recommend using the following protocols in conjunction with. Our protocols are an initial attempt to bring together the experience and varied approaches of some of the world's most experienced scientists and to begin to apply a rigorous, standardized approach to determining the case of death of stranded cetacean. These protocols are designed for use in the best-case scenario, where time is not limited. When time or resources are limited, or experienced personnel don't need the detail provided in these protocols, form-based protocols are also included. It is expected that the advice of those experienced in the field will continue to shape these protocols further. Thus, this document is a living document, and the intent is to periodically update it to reflect advances in stranding response, necropsy, and imaging protocols and clinical diagnosis. It is formatted such that the user has an area in which to take notes, provide comments, and highlight critical information.

STRANDING-RESPONSE REGULATORY REQUIREMENTS

As protected wildlife, odontocete (toothed) cetaceans are covered under local, federal, and international laws. A number of laws and regulations pertain to the recovery or possession of cetacean carcasses or their parts and to the rescue and recovery of cetaceans in distress. One must be aware of and follow these laws and regulations.

Stranding network responders are governed by a legal framework established by various federal, state or provincial, and regional authorities. The United States Marine Mammal Protection Act (MMPA) specifically prohibits the collection of animals (live or dead) or parts from them or any form of harassment, detention, or restraints—however temporary—without a permit. Exceptions include government officials acting in the course of their duties and other authorized individuals.

Marine mammal stranding networks in the United States make up one facet of a broader, more comprehensive program called the Marine Mammal Health and Stranding Response Program (MMHSRP), established in the late 1980s in response to

growing concern about marine mammals washing ashore in U.S. waters. Following are the MMHSRP goals:

- To facilitate collection and dissemination of data
- To assess health trends in marine mammals
- To correlate health with available data on physical, chemical, environmental, and biological parameters
- To coordinate effective responses to unusual mortality events

The MMHSRP program was formalized by the 1992 Amendments to the Marine Mammal Protection Act, and the National Marine Fisheries Service (NMFS) was designated as the lead agency to coordinate related activities. A NMFS authorization is required to respond to a stranded marine mammal; thus, NMFS works with stranding networks to respond to marine mammal strandings. Volunteer stranding networks were established in all coastal states and are authorized through letters of authorization from the NMFS regional offices. Through a national coordinator and five regional coordinators, NMFS oversees, coordinates, and authorizes these activities and provides training to personnel who are the first-line responders to stranded marine mammals.

PUBLIC HEALTH AND SAFETY ISSUES

Although there are few known zoonoses (diseases of animals transmissible to humans) associated with cetaceans, it is likely that some remain unknown and, therefore, are likely to be difficult to diagnose and treat if contracted. Of the known zoonoses, *Erysipelothrix rhusiopathiae* is the best known and is a frequent cause of "seal finger" or erysipeloid (a dermatitis or cellulitis of the hand) if humans are bitten or have contaminated cuts. This disease is very common in the marine environment on all marine mammals and fish and has been diagnosed in cetaceans on many occasions (Dunn et al 2001). Risk of entry is via breaks in the skin (Duignan 2000). Mycoplasmas and Vibrio (Dunn et al 2001) are also known to be transmissible from cetaceans to humans.

When collecting samples, safety precautions should include basic hygiene and care around sharp objects; however, researchers and others overseeing a stranding response should

be aware of their state's occupational health and safety requirements.

HEALTH AND SAFETY RISKS

- Handling heavy carcasses—Marine mammal carcasses range in weight from 30 to 50,000 kg. Each carcass presents a different handling challenge. Where possible and necessary, use heavy equipment for moving carcasses. Small odontocete carcasses can be usefully moved with a chain or strong line tied though a hole cut in the dorsal fin. Always be aware of the risk of failing line; nylon line especially can recoil when it breaks.
- Handling biological material of unknown infectiousness risk—routine personal protection measures should be in place:
  - No food or drink near an animal
  - Protective clothing used at all times: e.g., rubber boots, waterproof bib pants, heavy-duty aprons, surgical scrubs, disposable gloves, eye shields, and face masks as appropriate.
  - Tools should be cleaned carefully with detergent and then in disinfectant. Sharps should be disposed in a dedicated sharps container.
  - Tissue waste should be disposed of appropriately.
- Use extreme care in handling toxic fixatives such as formalin. It is best transported to the beach packaged within a sealed leakproof outer container such as a screw-top bucket with a rubber gasket (see Lifelatch at http://www.m-m-industries.com)
- A first aid kit should be available.
  - Seek medical attention for bites, cuts, and other injuries; inform medical attendants of the source of the injury.
  - Disinfect all cuts with iodine or alcohol 70%.
- Use of sharp instruments: prior to any necropsy, safety protocols should be reviewed and any new

participants be shown how to handle any tools they might use

NECROPSY AND SAMPLE-COLLECTION GEAR LIST

The following is a suggested necropsy and sample-collection gear list taken from McLellan et. al. (2005).

NECROPSY GEAR

- digital camera (w/ disc space for at least 100 images) **OR** SLR camera with 28–70mm lens, macro lens, 2 rolls each of 50 Fujichrome, 64 Kodachrome, 200 Ektrachrome
- Hi or digital 8 video camera and video tape for 8 hours and storage media
- extra batteries for all previously listed electronic equipment
- handheld GPS (not mandatory)
- ID photo board to insert in all photo images; alternatively, see paper reference scales in subsequent necropsy procedure
- 2 metric tapes: 30m long (note: check end to see where numbers start)
- 2–3 data clipboards (Rubbermaid makes a plastic, rust-proof model)
- 30m of 2-cm braided line (examine for any abrasions or weak spots and remove)
- 30m of 1-cm line (examine for any abrasions or weak spots and remove)
- 1 very heavy (10cm wide) nylon towing strap—e.g., used by large vessel travel lifts
- 4–6 high quality knives with 12" blades and blade guard between blade and handle
- 4–6 high quality knives with 8" blades
- 4–6 high quality knives with 6" blades
- 2 diamond “flat” steels
- 2 draw-through knife sharpeners
- 2 pair ball shears or large boning shears
- 4 metal meat hooks: 2 feet long
- 4 metal meat hooks: 8 inches long
- 4 scalpel handles and a box of blades (take care loading and unloading blades onto or from handles)
- 4 large rat-tooth forceps
- 4 small forceps
- 2–4 plastic rulers: 15cm
- 2 plastic rulers: 30cm

- 2 plastic turkey basters for collecting urine and fecal samples
- 1 bow saw used for trimming tree branches: 1 meter long
- 1 long pry bar used for carpentry
- aerobic and anaerobic culturettes
- 100 Tyvek® tags for labeling
- fine point and large indelible ink Sharpies®
- permanent ink pens
- #2 pencils for recording data onto datasheets and histo cassettes
- several plastic 5-gallon buckets to store and wash gear
- 2 rolls of duct tape
- 2 boxes of 50-count, 2-gal. Ziploc® bags with write-on label
- 2 boxes of 100-count, 1-gal. Ziploc bags with write-on label
- 2 boxes of 100-count, 1-liter Ziploc bags with write-on label
- 1 large box of 50-count heavy garbage bags
- 2 large plastic cutting boards for cutting and photographing tissues
- set of sawhorses and 2' × 8' section of plywood to make workstation
- 1 box each of large, medium, small latex gloves
- 4 pairs each of fish-cutting gloves in large, medium, and small sizes
- boots, coveralls, and rain gear
- 2 headlamps
- 5 medium-to-large plastic coolers
  - 2 for dry-equipment storage for transport
  - 2 for tissue-containment on site and transport (get ½ full with slush ice)
  - 1 for food cooler for drinks, etc. (do not mix with above)
- 2 large plastic transport boxes
  - 1 for transport of rain gear and boots

  - 1 for plastic trash bags and Ziploc bags
  - 4–5 scrub brushes for cleaning gear
- Dawn dishwashing detergent
- safety glasses for those "going deep" into the whale

Tissue and Fluid Collection

- 1 cube of formalin with pour spigot: 20 liter, 10% buffered
- 2 gallons of 95% alcohol
- 6–8 plastic 1-liter jars with wide mouths
  - 2 boxes of 50-count large Ziploc bags
  - 2 boxes of 50-count small Ziplocs bags
  - 2 bread-box-sized waterproof plastic boxes for gross tissue collection
- histology cassettes
- 10 plastic syringes: 60 cc each
- 10 plastic syringes: 20 cc each
- 10 needles: 16-gauge

PROTOCOL FORMS

The necropsy and sample collection protocols are form-based to facilitate their use on the beach and in the laboratory. The protocols are provided to guide and assist personnel in conducting the necropsy and ear extraction. These forms contain information on sample collection and preservation, and, if completed legibly, can be scanned and become an integral part of the electronic necropsy report.

INITIAL RESPONSE AND LIVE-ANIMAL CLINICAL EXAM AND SAMPLING PROTOCOL

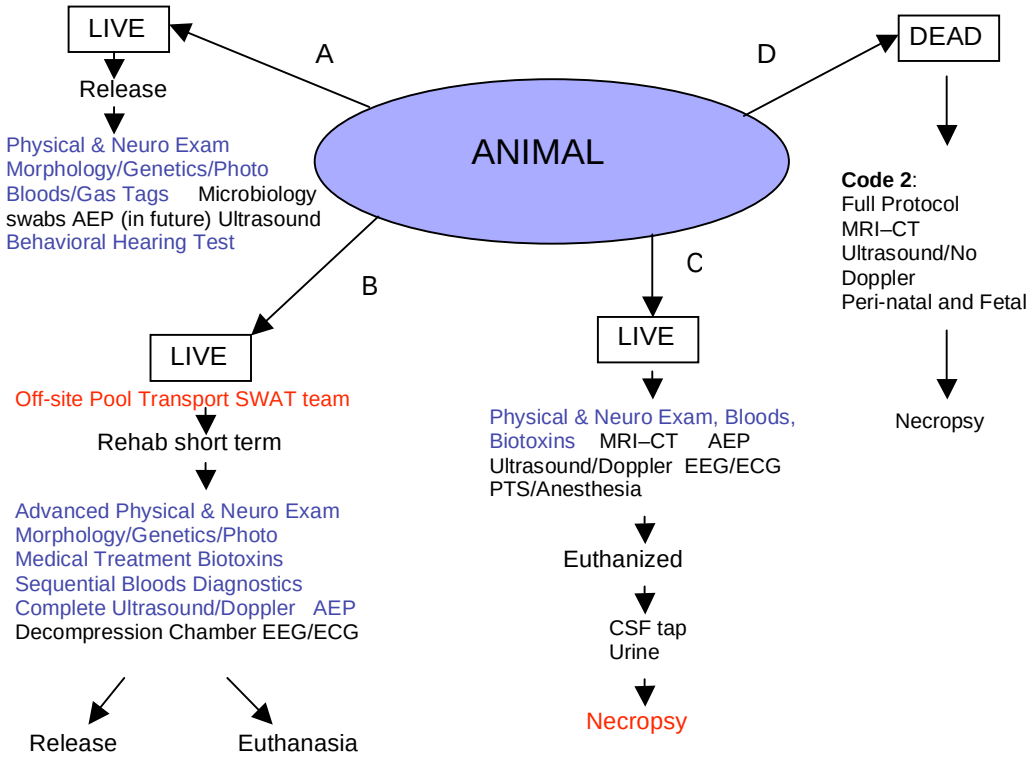

Figure 1.1 A decision tree for an organized triage approach. In this document we will provide a step-by-step process summary for each branch of the decision tree. The initial assessment should be rapid yet thorough. Under all branches of the decision tree, assessment should first include the collection of level A data.

Level A Data: Minimum Requirements (for each animal, complete NOAA Form 89-864 (see http://www.nmfs.noaa.gov/pr/pdfs/health/levela.pdf):

- Investigator: name and address (affiliation)
- Reporting source
- Species
  - Preliminary identification (by qualified personnel)
  - Voucher (supporting) material (photographs, specimens, including mandibles with teeth, tooth counts, and entire skulls)
- Field number
- Number of animals, including total and sub-groups (if applicable)
- Location
  - Preliminary description (local description)
  - Latitude and longitude (to 0.1 minute, if possible) with closest named cartographic feature as determined subsequently in the lab)
- Date (mm\dd\yy), time of first discovery and of data collection and specimen recovery
- Length (also girth and weight when possible)
- Condition (recorded for both discovery and recovery times)
- Code
  - **Code 1: alive**
  - **Code 2 (carcass in good condition):** fresh/ 'edible'; fresh smell; minimal drying or wrinkling of skin or eyes; eyes clear; no bloating, tongue and penis not protruded; blubber firm and white; muscles firm, dark red and well defined; brain firm; little gas in intestines.
  - **Code 3 (fair—decomposing but organs intact):** Some bloating may be present, possibly with tongue or penis protruded; mild odor, mucous membranes dry; eyes shrunken or missing; blubber blood tinged or oily, muscles soft and poorly defined; blood hemolysed; organs soft, friable, and mottled but still intact; gut dilated by gas; brain soft and fragile with reddish cast but surface features distinct.
  - **Code 4 (poor—advanced decomposition):** carcass collapsing; sloughing of skin; strong odor, blubber soft, possibly with pockets of gas or oil; muscle liquefying or easily torn; periosteum loose on bones; blood thin and black; organs identifiable but very friable, easily torn and difficult to detect; gut gas-filled; brain soft, dark red, containing gas pockets, pudding consistency
  - **Code 5 (mummified or skeletal remains):** skin may be draped over skeletal remains; any remaining tissues are desiccated
- Sex

LIVE-ANIMAL RESPONSE—BRANCH A

For live animals that are destined to be released, respondents should take four actions: (1) conduct a physical exam; (2) collect blood samples and perform a gas analysis on a subsample; (3) collect microbiology swabs; and (4) tag the animal. For the clinical examination, constantly record observations such as respiration rate and character; for other clinical signs, record observations as the opportunity arises (e.g., because you need to do some other procedure) or if there is a change in previously observed clinical signs. Changes in one clinical sign will likely result in changes to other clinic signs.

Physical Exam

After all Level A data have been collected, live animals should be given a physical exam. That includes the following:

- General Condition
  - Dorsal and cervical blubber and muscle mass. Photograph.
  - Record distribution and appearance of external lesions. Photograph and then record distribution on an outline drawing. Evaluate the condition of the skin; the condition will vary depending on the length of exposure. Note any drying, cracking, blistering, sloughing, and other traumatic injuries.
  - Awareness: Check for the presence or absence of reflexes, particularly the blowhole, which should be closed or should close if touched; the palpebral reflex to touch should result in lid closure. The anus should tighten when the surrounding area is touched.
  - Listen for vocalizations and note any audible sounds, apart from respirations, from blowholes. Whistles and high-frequency squeals, while possibly associated with stress, may be useful clinical indicators. Vocalizations diminish or cease in depressed animals.
  - Assess muscle tone by attempting to open the mouth or by resistance to traction of the

tongue or by assessing the ability of the cetacean to move.

  - Note any discharges.

- Cardiovascular and Pulmonary Systems
  - Record respiratory rate at regular intervals. The respiratory rate for cetaceans should be one to three per minute; however, there may be long periods of apnea especially if the animal is depressed or comatose. If the rate rises above 10/min when the animal is stressed (e.g., by handling), but then drops below this level once the stress is removed and the animal is made comfortable, it is a good sign. A consistently high rate carries a very bad prognosis.
  - Record heart rate.
    Listen to the heart or measure the heart rate by auscultating or palpating, medial to pectoral flipper at the level of the carpus (generally seen as a bend in the caudal border of the flipper). The heart rate may be very slow or weak and difficult to determine (e.g., may not be able to auscultate the heart at all in a medium-large cetacean). If the animal fails to exhibit tachycardia on inspiration, it is a grave prognostic sign.
- Body temperature.
  - This will only be useful if suitable rectal probes are available (minimum 30cm in length). Do not use glass. Normal values are 36.5° to 37.5° C (97.7° to 99.5° F). It may not be possible to take the temperature of a large cetacean in ventral recumbency.
- Neurological examination (see next page)

NEUROLOGICAL EXAMINATION

Date: **Time:** **Observer:** **Acc No:** **Total Score:**

Posture

1. Recumbent: sternal, dorsal, lateral: R/L
2. Body twisted: R/L (Stranding may result in neuromuscular damage causing the animal's body to
   to assume a "C" shape. Is the opening of the "C" to the left or the right?)
3. Opisthotonus (head and flukes pointing upward, limbs and body in rigid extension)

Mentation

1. Normal
2. Confusion and/or Disorientation
3. Obtunded
4. Coma

Abnormal Body Movements (each assigned 1 point)

1. Ocular: Nystagmus (slow drift of eye one way, rapid return):
   vertical/horizontal/postural/fixating/continuous (In which direction is the slow component?)
2. Strabismus: note position
3. Rapid blinking
4. Muscle fasciculations: note muscle or body part affected
5. Myoclonus (tremorlike activity)
6. Myotonus (longer duration muscle contractions)
7. Chorea (rapid, jerky, purposeful movements)
8. Athetoid (slow, writhing, purposeless movements)

Abnormal morphology of head (each assigned 1 point)

1. Drooping of lip on one side
2. Asymmetry of musculature (describe)

Responsiveness to a sound

1. Lifts and/or moves head
2. Moves eyes to observe
3. Nonresponsive
4. Seizures: note type of movements above

Locomotion

1. Able to swim normally
2. Able to swim slowly/reluctantly
3. Moves without use of fluke
4. Ataxic and/or Uncoordinated movements
5. Unable to use foreflipper and fluke on same side (hemiplegia)
6. Circling
7. Immobile

Blood Sampling

Blood samples provide an opportunity to evaluate the functional capacity of some organs. A broad spectrum of analyses can be performed, including plasma chemistry, hematology, antibody titers, and toxicology. Collect the following tubes of blood:

- 1 EDTA tube—*hematology*
- 1 serum separator tube—*serum chemistry*
  At least 1 mL of blood in a heparinized syringe or container—blood gasses. Separate the serum and freeze at –20° C for shipment, or refrigerate and ship using cold packs.
- 1 EGTA (ethyleneglycol-bis-N4-tetraacetic acid) tube or heparinized plasma—*catcholamines*
- 2 sodium citrate (clotting profile, glucose levels)—*clotting factors*
- 1 thrombin (FDP)—*clotting factors*
  - It is very important to make sure that the maximum draw has been obtained for the citrate and thrombin tubes.
  - Thrombin (FDP) tubes should be refrigerated but can only sit overnight before shipping.
- 2 serum separator tubes—*serum bank*

Record times of death and sampling. Place samples in a cooler or on ice, but do not freeze, and transport to the laboratory as soon as possible for processing.

When a delay of more than about 4 hours is anticipated, centrifuge the blood to separate the plasma or serum; these samples, free of red blood cells, can be frozen if delivery to the laboratory within 24 hours is impossible.

Allow blood in plain tubes or SST (serum tubes) to clot and for the clot to begin to shrink (room temp).

- Use EDTA blood to make three smears; chill remainder.
- Centrifuge all remaining blood samples (not EDTA) at 3000 rpm for 10 minutes. If no centrifuge is available, allow samples to stand, chilled, until the cells settle.

- Draw off clear liquid (plasma or serum) with a pipette, keep 2 mL of Li Heparin plasma and 0.5 mL of Fl Ox plasma aside; divide the remainder of all samples into 0.5–1 mL portions and freeze at –20° C or –80° C.
- Submit EDTA sample, the 2mL of Li Heparin Plasma, and the 0.5 mL of Fl Ox plasma to lab as soon as possible (must be processed at lab within 48 hours, preferably within 24 hours). Request full hematology and biochemistry profile.
- A blood smear is useful if samples for hematology cannot be analyzed within 24 hours.

Heart Blood in Plain Tubes (Disease, Toxicology, Hormones)

Freshly dead animals, including those euthanized by lethal injection, can sometimes be sampled in the same way as live ones. When procedures are carried out more than a few minutes after death, samples can be taken from the right ventricle of the heart with a syringe and needle (often the ventricles are contracted) In that case, collect blood from the caudal vena cava. 20–30 mL of whole blood is enough to run a comprehensive set of analyses.

- Allow blood in plain tubes (serum tubes) to clot and for the clot to begin to shrink
- Centrifuge at 3000 rpm for 10 min. If no centrifuge is available, allow the blood clot to shrink at room temperature for a couple of hours. Draw off clear portion (serum), divide into 0.5–1mL portions and freeze at –20° C or –70° C.

Collect Microbiology Samples

Using sterile swabs: Any orifices with discharges should be swabbed; also swab inside the blowhole as deep as possible. Place swabs in their accompanying transport media.

Chilled abnormal tissues (2–5cm cube in sterile container) (disease studies)

- Culturing and Submission of Tissues: Same Day
- Culturing by Swab:
  - Sear surface of tissue by dipping a knife or spatula in alcohol, lighting it (a small self-igniting propane torch also works well) and,

when it is hot, applying it to the surface of the tissue.

- Slice into the middle of the tissue through the seared region with a sterile scalpel.
- Insert a sterile bacterial swab into the middle of the cut, and then place the swab in culture medium.

Or, if no alcohol is available,

- Cut a 3 × 3 to 4 × 4 cm section of tissue, chill, and submit to lab.
- Laboratory can heat-sear the tissue in-house and culture.

- Always culture the tissue rather than an effusion, and always culture near the leading edge of the lesion rather than in the central area of necrosis
- Send to the lab, chilled, ASAP. If lab facilities are not accessible within 48–72 hours, freeze the swab and tissue (the colder the better). Request bacterial culture (aerobic and anaerobic).
- Select a 5mm slice, including healthy and diseased tissue, and place in 10% formalin.
- Freeze remainder.

Swabs of affected sites in transport media or frozen (disease studies)

- Send bacteriological swabs in transport media to the lab, chilled, ASAP. If lab facilities are not accessible within 48–72 hours, freeze the swab and tissue (the colder the better). Request bacterial culture (aerobic and anaerobic).
- Keep plain swabs frozen in case needed for microbial DNA.

Ultrasound—Guidance for use of ultrasound in live or dead odontocete cetaceans[1]

Ultrasound inspection of organs and blood vessels may be useful for the detection of bubbles produced by the off-gassing of tissues. *In vivo* inspections can capitalize on both two-dimensional (2-D) imaging and Doppler modalities to assess the presence or absence of bubbles in vessels and tissues. Results from post-mortem assessments are likely to be questionable, at best, unless made immediately following euthanasia. Post-mortem inspections will be limited to 2-D imaging modalities because Doppler inspections will be useless following death.

Vascular bubbles can be detected with both 2-D imaging and Doppler modalities. The former is more easily used by the untrained technician because the bubbles appear as reflective points flowing through the vessel. The use of Doppler is more specialized and will require some training prior to application, but it permits the acoustic detection of bubbles as they pass through the sound field. Both methods have been used to detect bubbles in human divers and tissue phantoms that mimic the depth of vessels potentially observed in odontocetes. The blood vessels that can be inspected with ultrasound will be limited by the resolution of the ultrasound system and the depth of penetration provided by the transducer. There is a trade-off between depth of penetration and resolution, so the ability to detect bubbles may decline with the depth of vessel inspection.

The reflectivity of an organ should, in theory, increase as reflective substrates accumulate within the organ. For example, the accumulation of microscopic bubbles within the liver or kidney should increase the reflectivity of that organ in a manner that is proportional to the amount of microbubble accumulation. This expectation is based on physical principles and has not been demonstrated in a cetacean. However, inspection of organs should still be performed because the presence of larger bubbles should be detectable, by the trained observer, as an anomaly in the field of view.

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1 Respondents should note that, while ultrasound is a valuable diagnostic tool, it is important that, in the case of a freshly dead carcass, all ultrasound investigations take place as quickly as possible to avoid delaying the necropsy and causing further decomposition that might interfere with diagnosing the possible cause of the stranding. Respondents should use their judgment regarding the best course of action.

Following are recommendations of vessels for inspection in stranded odontocetes. These vessels are selected because of the ability to visualize or apply Doppler techniques to them via ultrasound. These vessels have been imaged in the bottlenose dolphin *(Tursiops truncatus),* and may or may not be visible in other odontocetes, depending on their depth and relationship to other tissues, organs, and air spaces. Consideration of vessel-specific blood flow should be given when applying Doppler to particular vessels. For example, blood flow within the portal vein has been shown to be sluggish in dolphins; thus, the sensitivity of the Doppler system should be set to the highest level (lowest flow) when investigating this vessel or other vessels expected to have low rates of blood flow.

Vessels and organs to inspect

- Portal vein (large vessel occurring 12–20 cm deep in dolphins; probably the easiest to visualize)
- Phrenic vein
- Brachiocephalic
- Ascending carotid artery (best observed with pulsed wave Doppler)
- Liver
- Kidney

Potential vessels and organs to inspect

- Epidural veins and retia
- Superficial veins
- Lymph nodes
- Pericardial plexus

Required Equipment

The depth of inspection and resolution of the system will depend on the design of the ultrasound head (the transducer). Similarly, Doppler capability is not available for all models of transducer. The user will need to determine specific system capabilities. The cost and summarized capability for two portable ultrasound systems are listed below. The listing is not an endorsement for any particular model of ultrasound, but rather a list of specifications for portable systems that have been used for vessel inspection in living odontocetes. When deciding on a system, consideration should not only be given to the depth of penetration, resolution, and imaging capability of the complete system, but also to the system's portability and

ruggedness. Portable systems are expensive, and the system's longevity must be taken into account. It should also be noted that the cost of transducers is in addition to the cost of the base system.

Ultrasound Base

- 2-D Imaging alone: Sonosite 180 Plus

  or

- 2-D Imaging and Doppler: Sonosite Titan, Sonosite MicroMax

Transducers

Deep penetration— C60 (obstetric/abdominal use—5-2 MHz curved array)

C15e (abdominal/thoracic use—4-2 MHz curved array)

- provide depths of penetration up to ~25 cm
- can be used with Doppler depending with which system it is coupled

Shallow penetration— L38 (vascular access/superficial imaging—10-5 MHz linear array)

- provide depths of penetration up to ~7 cm
- can be used with Doppler depending on with which system it is coupled

Video recording should be performed along with all ultrasound inspections. This provides for multiple review of recorded observations and more careful post-collection analysis. Portable recorders (DVR) that can interface with current ultrasound systems are available.

Morphology

See discussion in Chapter 2.

Genetics

Collect genetic samples in a minced 1×3 cm section of epidermis fixed in a super-saturated salt solution of 10% DMSO in water. Additional genetic samples can be collected from liver,

heart muscle, pericardium, and endometrium in DMSO if the carcass condition warrants it. Recently, researchers have been requesting frozen epidermis samples in plastic to be used to determine hormone levels, contaminants, and genetics. Collect a 10×10 cm section of epidermis, freeze it in a Ziploc bag, place on ice immediately, and transfer to a –80° C freezer as soon as possible.

Hearing Tests

Because of the concern for the potential impact of human-made sound on marine mammals and because odontocetes rely upon their hearing for communication and echolocation, hearing tests would provide useful information on potential causes of stranding as well as on the potential for release. Further, hearing tests on additional and/or new species of odontocetes would provide useful information having the potential for estimating the impact of future activities involving human-made sound in the oceans. Two methods of obtaining information on the frequency range and sensitivity of hearing exist: behavioral and auditory evoked potential.

Behavioral methods are the standard approach for audiometry in marine mammals. Several behavioral methods exist and, if an animal is determined to be suitable for behavioral audiometry, it is recommended that someone with experience in marine mammal behavioral audiometry be consulted. Behavioral hearing tests are time-consuming and can take many months to complete, particularly with a naïve subject (e.g., an animal that has never undergone behavioral training). The application of behavioral hearing tests will likely be restricted to rehabilitated animals deemed unsuitable for release to the wild.

Auditory evoked potential (AEP) methods are an electrophysiological means of testing hearing. The process involves recording electrical patterns (potentials or voltages) produced by the brain in response to a sound. AEPs are non- to minimally invasive and have been applied to odontocetes as large as the killer whale. The process is well developed in odontocetes, and methods of testing multiple frequencies simultaneously have reduced the testing time for the full range of hearing to less than 10 minutes in delphinids. Application of this technique requires the presence of an AEP system and personnel trained in the application of electrodes and sound

stimulus delivery. Multiple laboratories across the United States (Hawaii, West Coast, East Coast) have the equipment to perform these procedures as well as the experienced personnel to train stranding responders.

Tagging

Each carcass in a mass stranding should be tagged as soon as possible to manage specimens and avoid errors in recording individual case histories. Cattle ear-tags are easily applied to the trailing edge of the dorsal fin (Geraci and Lounsbury, 2005); make sure each animal has a single unique number assigned to it.

LIVE-ANIMAL RESPONSE—BRANCH B

In situations where live cetaceans are not to be released and will be removed to a rehabilitation facility for short-term rehabilitation, the following steps should be undertaken.

Prior to transport, respondents should conduct a physical exam and an ultrasound and Doppler exam in accordance with the instructions provided previously. Respondents should collect blood and begin a systematic sequential blood collection.

Once the cetacean has reached the rehabilitation facility, respondents should perform an advanced behavioral assessment and additional ultrasound measurements and continue sequential blood collection. If personnel and resources are available, staff should consider other diagnostic treatments such as Doppler, EEG/ECG, collection of samples for biotoxin, auditory evoke potential tests (behavioral hearing tests), X-rays, and MRI/CT scans (see protocol in Chapter 4).

LIVE-ANIMAL RESPONSE—BRANCH C

In those situations where live cetaceans are not candidates for rehabilitation and the animal will be euthanized, respondents should follow all of the procedures outlined under "Live Animal Response—Branch A" and as many as possible of those under "Branch B" and then take the necessary steps to euthanize the animal.

Once the animal is euthanized, urine, aqueous humor, and cerebral spinal fluid should be collected. After these samples

have been collected, perform an internal examination and necropsy using the procedure provided in Chapter 2.

FRESH DEAD OR EUTHANIZED ANIMAL RESPONSE—BRANCH D

If the animal is freshly dead (Code 2) or has been euthanized, respondents should follow the procedures outlined subsequently (in Chapter 3) for CT and MRI, the procedures outlined previously for ultrasound, and the necropsy protocol in Chapter 2. Note, however, that the necropsy of Code 2 animals should not be unduly delayed by CT, MRI, or ultrasound, especially if cold storage facilities are not available.

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Chapter 2—
Odontocete Necropsy Procedure

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INTRODUCTION

This is a description of the procedures for the necropsy of odontocete carcasses. In recent years, mass strandings associated with high-intensity underwater sounds have presented new lesions associated with fat and gas emboli. This procedure incorporates suggestions to document these lesions and provides guidelines for a more complete examination of each case.

There are three purposes for a necropsy: (1) to collect natural and life history information; (2) to scientifically determine the most probable cause(s) of death; and (3) to properly collect appropriate samples to support research, life history, and cause-of-death investigations. The necropsy is also a source of important information for determining, documenting, and mitigating human-related causes of death.

When determining cause of death, it is important to document and consider both actual cause and circumstance of mortality. One should document both immediate and any secondary conditions contributing to mortality. The final most probable cause of death determination is actually an analysis of both cause and circumstance. For example, a dolphin found dead in a trawl net may be found to have died of suffocation secondary to forced submersion (drowning)—the circumstance of death was being caught in a net.

In addition to determining the most probable cause(s) of death, one must process each carcass in order to obtain general and detailed biological information. General information collected upon primary examination must include the following:

- Morphometrics
- Total body weight (TBW)
- Body condition
- Description and measurement of wounds and scars
- Photographs and sketches for individual identification
- Epibiota

More detailed information should include the following:

- Gross appearance of organs and tissues
- Description of fat stores
- Fullness of and prey items identified in the gastrointestinal (GI) tract along its entire length
- Documentation of natural, anthropogenic, and pathognomic lesions, congenital defects, and individual organ weights

Additionally, appropriate samples for toxicology, histopathology, microbiology, virology, and parasitology may be collected on a case-by-case basis, constrained mainly by decompositional state of the carcass and by sampling logistics; these samples may provide useful insights into normal and abnormal conditions.

NECROPSY PROCEDURE—PREPARATION

This written protocol is based on procedures followed at the Florida Fish and Wildlife Conservation Commission (FWC) Marine Mammal Pathobiology Laboratory (MMPL) and those followed at the University of North Carolina Wilmington. These directions are to accompany several illustrations of the left lateral aspect of a carcass (also see the illustrations of *Tursiops* gross anatomy in the CRC Handbook by Rommel and Lowenstein 2001). Lymph node is abbreviated LN and lymph nodes, LNN. The LN descriptions used herein are modified from Rommel *et al* (2002). The gross anatomy is based on that of the bottlenose dolphin, *Tursiops truncatus*. The terminology used herein is consistent (where possible) with the Illustrated Veterinary Anatomical Nomenclature by O. Schaller (1992).

PREPARING FOR A NECROPSY

Labels

Tissue labels should be written with bold, permanent markers such as pencils or fine-point *Sharpies,* which can be found at <http://www.sharpie.com/sanford/consumer/sharpie/index.jhtml?_requestid=121078>; histology pens or pencils should be used for tissue cassettes and labels submerged in formalin. Labeling should be on both sides of good quality paper. **Do not use ballpoint pens, inkjet printouts, or other**

water-soluble inks for labels or for data sheets! Tags and field data sheets should be made of waterproof, non-reactive material—e.g., Tyvek™ or *Rite In The Rain*™. Metal tags and tag parts should be nonreactive meta— samples should be packaged so that tags are not in direct contact with sample.

Collect one tissue per container, unless otherwise specified; label tissues according to tissue and sample type (e.g., histology, microbiology, virology, blubber for archive [for retrospective analyses], reproductive tract). Tissue labels should include the following:

- Test modality or sample
- Purpose of test
- Samples required
- Storage specifications

NECROPSY PROCEDURE—INITIAL OBSERVATIONS

Lesion Description: General Guidelines

All descriptions should at least include the following listed information. Lesions should be described *after* examining the cut surfaces. The following lesion descriptions should be *non-interpretive;* description should be thought of as if conveyed to a blind person (the histologist is often blind to the gross exam).

- Lesion type (ex: laceration, mass, ulcer, effusion, etc.)
- Organ affected and the lesion's potential communication with adjacent structures
- Precise location within the organ (e.g., serosa or parenchyma; superficial, deep, or full-thickness; cranial, caudal, or middle; dorsal, ventral, or lateral; left or right)
- Distribution within the organ (e.g., focal, multifocal, diffuse to coalescing, diffuse)
- Approximate total percentage of entire organ affected (<5%, 10%, 25%, 50%, 75%, 99%)
- Amount (mL) or size range (three dimensions, in cm; or maximum diameter) of lesion
- Modifiers: color, heterogeneous or homogenous, texture, consistency, viscosity, firmness, etc.

For a mass, include the following:

- Encapsulated or unencapsulated
- Whether it deforms or infiltrates adjacent tissue
- Whether central necrosis is present
- The location of the largest mass

For description of a fracture, include the following:

- Bone affected
- Open or closed to body surface
- Communicates with another structure
- Type of fracture (simple transverse or oblique; spiral; comminuted; greenstick, etc.)
- Whether fractured ends are aligned or displaced
- Whether fracture margins are sharp or smooth and have callus formation
- Presence of foreign material, purulent material, or osseous sequestrum
- Presence of blood, edema, fibrosis, purulent material in surrounding soft tissue or structure with which it communicates.

All of this information helps determine the type of trauma sustained, whether the fracture contributed to morbidity or mortality, possible sequelae, the potential for healing, and whether the fracture occurred antemortem.

Tissue Sampling for Histology

When a lesion is present, collect a sample of the lesion (including the leading edge), AND take another sample that does not appear to have the lesion. Should the lesion have more than one appearance, take a sample from each visibly different region.

When possible, samples taken for ancillary diagnostic tests—such as microbiology—should be taken adjacent to that section submitted for histology.

NOTE: *It is important to collect as much information as possible about the carcass and its collection. For example, a bycaught animal hauled up from depth may show signs of intravascular and interstitial bubbles—yet these would be an artifact of the recovery. Information gathered before the animal is examined is essentially case history and could be the most important part of determining circumstance of death.*

INITIAL OBSERVATIONS OF THE CARCASS

If possible, the carcass should be positioned right laterally recumbent (with its left aspect up); however, each surface of the carcass should be examined, described, and photographed prior to the internal exam.

External Lesions and Non-acoustic Human Interaction Data Sheet (Appendix 2-A)

Wounds are relatively recent lesions that may have some significance in determining cause of death or in documenting events that occur between stranding and necropsy. On the wound and scar data sheet, sketch and label each substantial wound with a unique number. If there are multiple wounds from a single event (e.g., a series of similar lesions), label each lesion in the wound series sequentially (histology and frozen samples should be matched as well).

Scars are superficial skin lesions that are healed or show significant resolution and have recovered sufficiently to be unlikely as the proximate causes of death. In some cases, where a chronic condition has been established by the event that caused the scar, cause of death may be linked to that event. Healing scars may have either rough or granular margins and a red, white, or yellow color. Repigmentation of a white scar begins at the wound margins and proceeds toward the center of the lesion. Most healed scars are completely covered with darkly pigmented epidermis and blend with the other epidermis so well that only the texture of the scar can be used to distinguish it from the adjacent undamaged epidermis. If there are multiple scars from a single event (e.g., a series of similar lesions), label each lesion in the scar series sequentially.

Procedure: On the wound and scar data sheet, make a sketch of the major scar patterns. Note whether each lesion is a wound or a scar. Indicate the depth of the lesion (i.e., to epidermis, dermis, blubber, or muscle or communicates with body cavity). If prominent scars extend down the sides or are found on the ventrum, sketch them on an appropriate projection. If there are signs of human interaction (HI), follow specific procedures to document these lesions.

External Photographs

A reference scale, clearly indicating a standard length (e.g., 15 cm for large regions and 1.5 cm for close-ups), should be included in all photographs. Place the scale so that it is in the plane of the photograph. If possible, photograph each image perpendicular to the plane of the scale, this is tedious but it makes interpretation of the photographs much easier. A fresh, 15 cm–long paper scale can be printed for each necropsy (laminated scales reflect too much light). The paper scale should be printed with a laser printer or photocopied (check for copier distortion) so that the ink is fused with the paper —inkjet printer products will run when wet. Using a waterproof marker, the field ID and the date of necropsy should be written boldly and legibly on the scale. If necessary, wet the paper to stick it to the sides of the carcass when taking lateral photographs. Appendix 2-B has an example of scales used at the Marine Mammal Pathobiology Lab.

Be sure that a unique carcass identifier (field ID) is in each photograph or that the first picture of a sequence on a roll of film is uniquely identified to avoid identification errors when more than one carcass is being processed at the same time. Take high-resolution electronic (preferred, or 35 mm) whole-body photographs of the carcass. Lateral (right and left) and ventral full-body photos and close-ups of specific lesions are recommended. The whole-body photos should be taken with the photographer as perpendicular as possible to the long axis of the carcass (and of the scale) so that measurements can be made from the photos. Photographs should be taken of all scars and wounds. If the scars are poorly visible, they can be highlighted with a grease pencil (or lipstick) and photographed (two sets of photos: highlighted and not highlighted). Close-ups are hard to ID by themselves, so take a wider-view photograph for perspective first, then take the close-up(s).

Measurements on Intact Carcasses—Morphometrics and Weight (Necropsy Morphometrics Data Sheet Appendix 2-C)

If a hoist or front-end loader is available, the carcass can be lifted by straps and weighed. Alternatively, platform scales or highway scales can also be used to measure weight—make sure movable equipment and gasoline level are similar to reduce error in smaller carcasses.

Standard external measurements that characterize the carcass (e.g., species, sex, age, superficial lesions, and condition) must be recorded (Appendix 2-C). If the carcass is intact, measure total body length (TBL) from the tip of the rostrum (not the lower jaw) to the midline of the fluke. If there is decompositional or scavenger damage of the midline extremities, TBL should be estimated and noted. Partial measurements such as distance from the snout to the anus or umbilicus to tip of fluke may help compute TBL if the carcass has missing extremities. Also, the position at observation or recovery should be recorded (e.g., right lateral, left lateral). As observations are recorded, they should be checked off on the *Necropsy Morphometrics Data Sheet* (Appendix 2-C).

NECROPSY PROCEDURE—NECROPSY NARRATIVE

Each necropsy should be permanently recorded as a written narrative using a word processor (blank *Odontocete Necropsy Report* in Appendix 2-D). The necropsy report is designed to prompt the team to make specific observations of all organ systems. If the organ or tissue appears normal, record this on the sheet—no visible lesions (NVL). If an organ or tissue was not examined (N/E) note that in the report and explain why not. If the tissue or body part was missing, note it as not applicable (N/A) on the report and why. Fill in all fields on the data sheet with some type of notation. If possible, this recording process should take place during the necropsy, not afterwards when the tissues are no longer available to clarify conflicts and omissions. Noting the absence of lesions is often as important as noting the presence of lesions.

Often the sequence that occurs during a necropsy differs from that listed in the narrative, particularly when several experts are present and contributing to the procedure or when specific lesions require special attention and procedures. If there are multiple prosectors, a primary prosector and one recorder should be assigned. If there are multiple carcasses, it is helpful to keep the same prosectors grouped; this will expedite the procedure as the group becomes acquainted with each other's methodology.

The primary prosector is responsible for the external examination and initial inspection of internal organs. This person then makes decisions about culture and collection of

tissues for ancillary diagnostics. This person also assigns additional prosectors to particular tasks. Each prosector is responsible for conveying a detailed description to the recorder. The primary prosector should review the necropsy report to ensure that all information was collected and that information is not conflicting.

The person recording the narrative must pay attention at all times, *repeat all* measurements or numbers to ensure accuracy, and read back any comments that are unclear. The recorder *must* feel confident to stop the procedure at any time to clarify any point. At the end of the necropsy, the narrative should be read and edited by the prosector(s) to ensure that all statements are accurate. This final read also serves as a check to make sure all steps in the procedure have been completed.

The process for a necropsy is described in following text. The Odontocete Necropsy Report in Appendix 2-D follows the narrative and can be removed, copied, and filled out as part of a necropsy. Before beginning the narrative, label all of the pages of the necropsy data sheet with the field ID.

Carcass Condition

The details recorded for each part of the necropsy procedure depend on the decompositional state of the carcass. By necessity, the fresher the carcass, the more detailed the examination and the greater the amount of useful information that can be extracted from it. If more than one carcass has stranded, start with the freshest individual(s). Unfortunately, because of ambient temperatures and the laws of thermodynamics, many carcasses are badly decomposed and unsuitable for *reliable* histology. When deciding which tissue samples are to be collected from a carcass, decompositional state must be determined. Fortunately for forensic purposes, many features associated with traumatic death are preserved even in decomposed carcasses. If in doubt, collect as many samples as possible; later they can be discarded if unused but they cannot be collected after the carcass has been disposed. Additionally, some biochemical assays (i.e., ELISA test for HAB) can be completed reliably on even autolyzed tissues, but care must be taken to avoid cross-contamination.

The condition of an odontocete carcass cannot be evaluated solely by its outward appearance nor estimated by knowing the

time elapsed since death. The rate of decomposition is influenced more by internal temperature (resulting from proliferation of decomposing bacteria) in large or robust animals and by ambient temperature in small or lean animals. Larger, rotund carcasses retain heat more effectively than smaller, slender ones. Carcasses also have a tendency to decompose more rapidly during the summer months, if pregnant, or if diseased.

Rigor mortis is a temporary condition and thus can be a helpful indicator of the time of death. The onset of rigor is typically within 2–8 hours after death, varying with the animal's terminal condition (particularly if there is a systemic infection) and the ambient temperature and activity of the animal prior to death. The duration of the condition is also variable, but may range from 12 to 72 hours (longest under cool conditions). The presence of rigor mortis indicates a carcass in fresh or moderate condition. It is important to note that carcass rigidity can also be a result of bloating from decompositional gas, generally a sign that a carcass is not fresh (though some diseases may cause gas production in tissues even in live animals).

Cardinal signs of decomposition include a rigid or distended tongue, prolapsed penis, protruding eyes, and distension by gas, and/or sloughing of epidermis. Skin, blubber, and muscle can remain intact and may even indicate gross lesions long after death. The heart, lungs, lymph nodes (LNN, singular LN), spleen, and kidneys may maintain their integrity longer, whereas adrenal glands, brain, pancreas, liver, and mucosa of the digestive tract decompose more rapidly. Extent of scavenger damage is also an indicator of elapsed time since death.

Internal Photographs

If possible, photographically document all lesions observed during the necropsy. It is important to have a nonverbal record of all significant observations. Again, for perspective, take wider view photographs before close-ups. Whenever possible, also photograph structure, organ, or lesion after removing and placing it on a white background (often improves color in photos); also photograph cut sections. Using a color strip can also help with standardization of color perception and description. When documenting anthropogenic lesions (HI), Polaroid photo-graphs should be added to the electronic and/or film record; the Polaroid pictures help the reviewer when

editing necropsy reports. Permanent markers can write on Polaroid pictures; this provides an additional medium on which to record observations or comments.

NECROPSY PROCEDURE—NECROPSY NARRATIVE HISTORY

The history of the individual animal may be important in interpreting the results of the necropsy. Record the history of the animal since its rescue or the interval between carcass recovery and necropsy. Be sure to be clear and concise (brevity is better than loquacity, but include all important events) in recording the events leading up to the necropsy; this includes carcass position in the water and attempts to rescue, carcass orientation during transportation and refrigeration, and, in the case of mass strandings, the temporal and spatial occurrence of each carcass and of human activities (e.g., military operations, seismic testing). Environmental conditions and the presence of other sick or dead animals is important to note. Include information about trauma and any rope or line added to the carcass during postmortem manipulation.

Sample Narratives:

e.g., 30 January 2004, at 0900 hrs, MMPL-KAA received a call from fishing Captain Kenny Hyatt who was on the water near the Skyway Bridge fishing pier, Tampa Bay, Hillsborough County. Captain Hyatt informed KAA that he was observing a dead dolphin wrapped in a cast net that was secured to the pier's piling. KAA called FWC SW Dispatch–Lakeland and requested an officer to investigate and tow the carcass to the nearest boat ramp. At 0925 hrs, FWC officer Cacciurri called KAA and informed him that he was en route to recover the dolphin carcass. Meanwhile, MMPL-TDP drove to the fishing pier to photograph the carcass and the recovery. The carcass was towed to the Maximo Park boat ramp in Pinellas County. TDP arrived at the boat ramp, loaded the carcass, and transported it to MMPL where it was stored in the cold room.

e.g., The carcass was part of a mass stranding in the Florida Panhandle; details of the mass stranding are found in a summary report. Fresh when collected and had to wait several days because of carcass transfer and manatee backlog. On 12 March 2004, the carcass was in cold room for approximately 5 hours, then transferred to outside frig. It was in frig at 1.1° C for approximately 3.5 days.

A. External (wound/scar description—epibiota, lesions (bruising, tooth rakes, cookie-cutter shark bites, HI, etc.)—distention from decompositional gas—condition of the epidermis [sloughing, sloughed, shedding, intact], emaciation [peanut head, rib outlines, body folds]). Describe the external appearance of the carcass; include verbal and quantitative descriptions of wounds and scars. If human interaction (HI) is suspected (e.g., military or seismic surveys nearby) or observed (e.g., entanglement in fishing gear), an HI report should be completed (2-A). Be sure to note on which side the carcass has been lying, because it may be very important during interpretation of organ color and fluid distribution within the carcass. Describe the condition of the skin: amount of epidermis present, shedding of superficial layers of epidermis (a natural process involving loss of the superficial layers of epidermis) and/or sloughing (epidermal loss due to decomposition), and peeling due to exposure. Note the presence and types (species) of barnacles or other epibiota, and record the dimensions of the largest individual of each type of epibiota. Describe fitness and/or emaciation and general appearance. Collect samples if appropriate. Examine the body openings and describe any lesions or foreign materials (e.g., fishing gear) that are present.

Sample Narratives:

e.g., Stalked barnacles (Xenobalanus sp.) *mostly on the dorsal aspect and on the caudal margins of the flukes. No evidence of human interaction. Scavengers removed both eyes, and there was significant but superficial scavenging over the entire carcass.*

e.g., On the right side of the body, there were five scars. There were two linear scars on the left side of the body. There were patches of eroded epidermis on the dorsal fin, right shoulder, head, axillae, and flippers. There was a partial amputation of the left flipper (approximately 10 cm was missing). The carcass was found wrapped in a cast net; however, close inspection of net marks indicates that they were caused after death (this was verified in person by Bill McLellan of University of North Carolina, Wilmington). Numerous teeth were missing, especially in the upper arcade. Those teeth present were worn. There were numerous conspecific tooth rakes on the peduncle, flukes, dorsal fin, and flippers.

Initial incisions (Figure 2.1). Take care that the first few cuts are not too deep, particularly if the carcass is distended with gas, because viscera and their contents may be extruded explosively from the carcass. With the carcass right laterally recumbent (if

possible), make cuts through the blubber and superficial muscles as illustrated in Figure 2.1. Make cuts parallel to the long axis of the body along the dorsal and ventral midlines and perpendicular to the long axis at nuchal and umbilical regions (or caudal insertion of dorsal fin).

Keep any HI evidence (e.g., fishing gear, lines, bullets) in appropriate containers; if it appears that the event might be a criminal case, notify law enforcement and make every effort to maintain chain of custody and events.

Use cuts around the base of each flipper to remove it. Examine flipper joints for lesions. If the carcass is fresh enough to have intact and representative fat stores (i.e., no fats have been lost from decomposition or rendering), blubber and skin thickness measurements should be taken at mid-dorsal (slightly off-midline if there is a dorsal ridge), mid-lateral, and mid-ventral—all taken at the level of the umbilicus—and the measurements recorded on the blubber-thickness data sheet (Appendix 2-E). Examine and measure nuchal fat thickness. Be sure that the skin and blubber, when measured, are not stretched from bloating; this can be accomplished by making two parallel circumferential cuts about 3–5 cm apart to relieve the stress; measurements are made on the undistorted section. Make these circumferential transverse cuts at the level of the umbilicus to measure blubber thickness (with epidermis, note thickness of epidermis). Make transverse flensing cuts (the spacing of which is determined by the size of the pieces that are most easily handled). Check each cut surface of the blubber for parasite tracks or encysted parasites (e.g., *Crassicauda* or *Phyllobothrium,* respectively).

If the carcass is an adult female, care must be taken to delineate and measure the entire extent of the mammaries, which are located below the blubber and the superficial-most muscle fibers, just cranial and dorsal to the mammary slits at the lateral margins of the urogenital (U/G) opening.

If only a single sample is to be acquired, collect a sample of blubber at the level of the umbilicus, on the dorsal or dorso-lateral aspect of the carcass. Blubber varies at different depths and at different sites on the body; thus, if possible, multiple blubber samples should be collected; the blubber collection data sheet (Appendix 2-E) is a guide to this multi-site collection. Once the blubber is removed from the dorsal body, epaxial muscle samples can be collected (collect histology and archive

samples from the epaxial muscles near the site of the single blubber sample).

If care is taken when removing the blubber and abdominal wall muscles, the peritoneum can be left intact. This is accomplished by removing the blubber and superficial muscles of the thorax first. At the caudal margin of the ribs, slide a finger between the abdominal wall muscles and the peritoneum to separate them before proceeding with the cuts through the blubber and muscle of the abdominal wall. The hypaxial muscles dominate the caudal abdomen; examine their surfaces carefully for vascular lesions before continuing. Look for evidence of emboli and describe (include photographs) if present; the procedure for quantifying emboli is in the developmental stage. Care must be taken while making a cut on the dorsal aspect of the abdominal peritoneum (if the peritoneum was kept intact when the abdominal wall muscles were removed). Maintaining as much sterility as possible, examine the serosal surfaces of the viscera and any ascites. Abnormal (flocculent or fibrous) ascites should be sampled either with a sterile (aerobic and anaerobic) bacterial swab (from peritoneum and ascites)—see below.

Tissues to be collected. Collect samples of epibiota (in 70% ETOH) if appropriate, superficial lesions (in 10% NBF), and skin biopsies for genetics (include epidermis and dermis and store in DMSO). Collect superficial lesions such as bruises, scrapes, scars, and shark bites. Blubber and muscle samples for archive; mammaries if female.

The intestines and gonads may be exposed at this point. Before cutting anything new, examine the serosal surfaces of all organs visible at this time and describe the occurrence of any lesions (e.g., blood clots, fibrin, adhesions, lacerations, parasites, gassiness). The stomachs and liver may also be visible at this time. The condition of the liver (firmness, sharpness of margins, resistance to tearing by fingers, separation of serosa from parenchyma) is often a good indicator of the stage of decomposition of the carcass as a whole. The stomachs are typically empty in single strandings but may have contents in mass stranded individuals or anthropogenic vents.

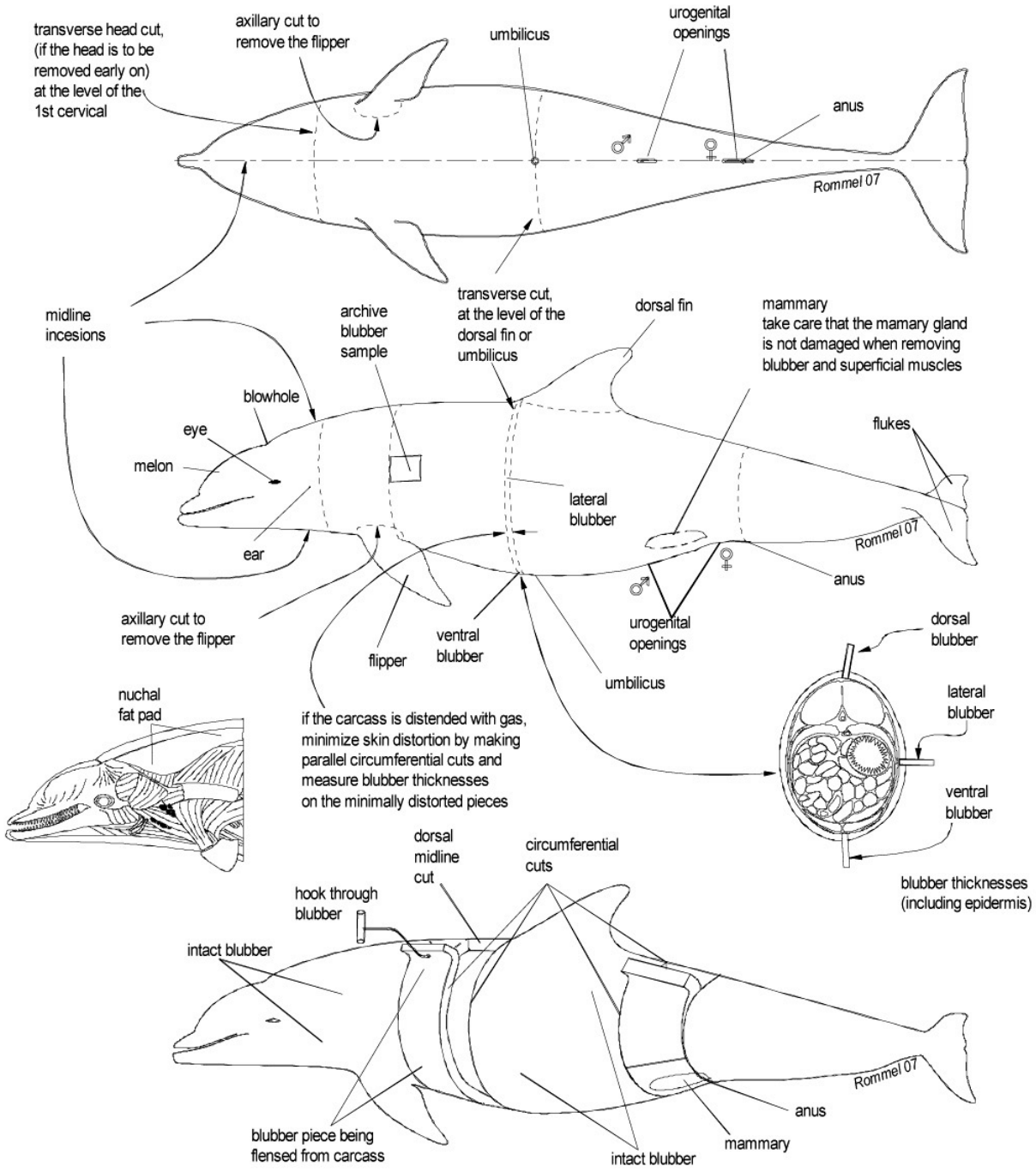

Figure 2.1 Illustration of flensing procedure; compare with Appendix 2-E.

B. Abdominal Organs and GI Tract—This section is primarily a description of the abdominal cavity, gastrointestinal (GI) tract, abdominal vascular plexuses, and the parietal peritoneum. Some or all of the ribs can be removed now or later. The left ribs can be separated at the joints between the vertebral and sternal ribs (sternal ribs are cartilaginous in beaked

whales); the sternal ribs can be left with the sternum or removed, depending on the specimen. Vertebral ribs can be disarticulated at the costo-vertebral joints starting at the caudal end. The cranial-most ribs are double headed, and these joints and the adjacent retia may be sites of sound-related lesions. Be sure to carefully examine the thoracic rete when the pleural cavity is open before the cranial ribs are removed. Examine the margins of the epidural retia at this time; when the vertebrae are separated examine retia and epridura veins at each of the sections

1. Abdominal Cavity (fluid description (i.e., color, texture, volume[1])—foreign objects (i.e., blood clots, GI tract contents) —adhesions, mesenteric and perinodal fats)

Incise the abdominal wall musculature along the caudal margin of the ribs and dorsal limit of the abdominal cavity (just ventral to the transverse processes of the lumbar vertebrae) and reflect downwards; avoid puncturing the intestines. Examine the organs in place, noting color, consistency, orientation, and any abnormalities. Normally, the abdominal cavity contains a small amount of clear serous fluid (ascites). If copious amounts of fluid are present, or the fluid has a flocculent and colored appearance, collect and freeze a sample for protein and lymphocyte analysis. If able to expose the cavity in a sterile manner, collect a swab of the surface of the peritoneum (especially where reddened or roughened) for microbiology (the fluid is less optimal for culture, because of the etiologic agent is diluted and dead). The peritoneum should be smooth and glistening, without irregularities. In fresh carcasses, the colors of the serosal surfaces of the intestines and parietal peritoneum should be light tan to pink. Note the root of the mesenteries; examine its vessels for signs of emboli and collect photos if any are present.

Sample narratives:

e.g., The abdominal cavity contains approximately 0.5 liters of clear fluid. NGVL.

e.g., There are small well-formed blood clots free within the abdominal cavity.

---

1 To simplify estimates of volume and length, you can measure the sizes of parts of your hand and arm. By submerging each part of your hand in water and observing the displacement of fluid the approximate volume of that part can be calibrated. For example, a finger on a large hand may be ~10 ml and a fist ~250 ml; in larger individuals, the distance between finger tips when both arms are extended is ~ 2m. This can be helpful in field or rushed conditions; whenever possible, however, actual measurements should be made.

Take care when cutting the parietal peritoneum, particularly if the animal is young and the gonads are small. The gonads are supported by mesenteries that are coincident with the peritoneum. Prior to removing any mesenteries or peritoneum, carefully examine them and the blood vessels of the root of the mesentery for signs of emboli (Figure 2.2) before making any additional cuts. Note if there is milky fluid in the lymph channels of the mesenteries or if the lymph channels appear unusually conspicuous.

Remove the GI tract (Figure 2.3), starting with the intestines, by cutting their mesenteries at the root; trim them a few centimeters from the intestines. Tie off the ends of the GI tract (at the esophagus a few centimeters in front of the stomach and at the rectum) with string. Remove and examine. When collecting thin-walled GI tract histology layers of the full-thickness, collect one or two sections from *each* compartment. Do not rub or scrape the mucosa to clean it. Rinse the sample with gently flowing water. Record your observations on the Odontocete Necropsy Report (Appendix 2-D) and the Abdominal Cavity Data Sheet (Appendix 2-F).

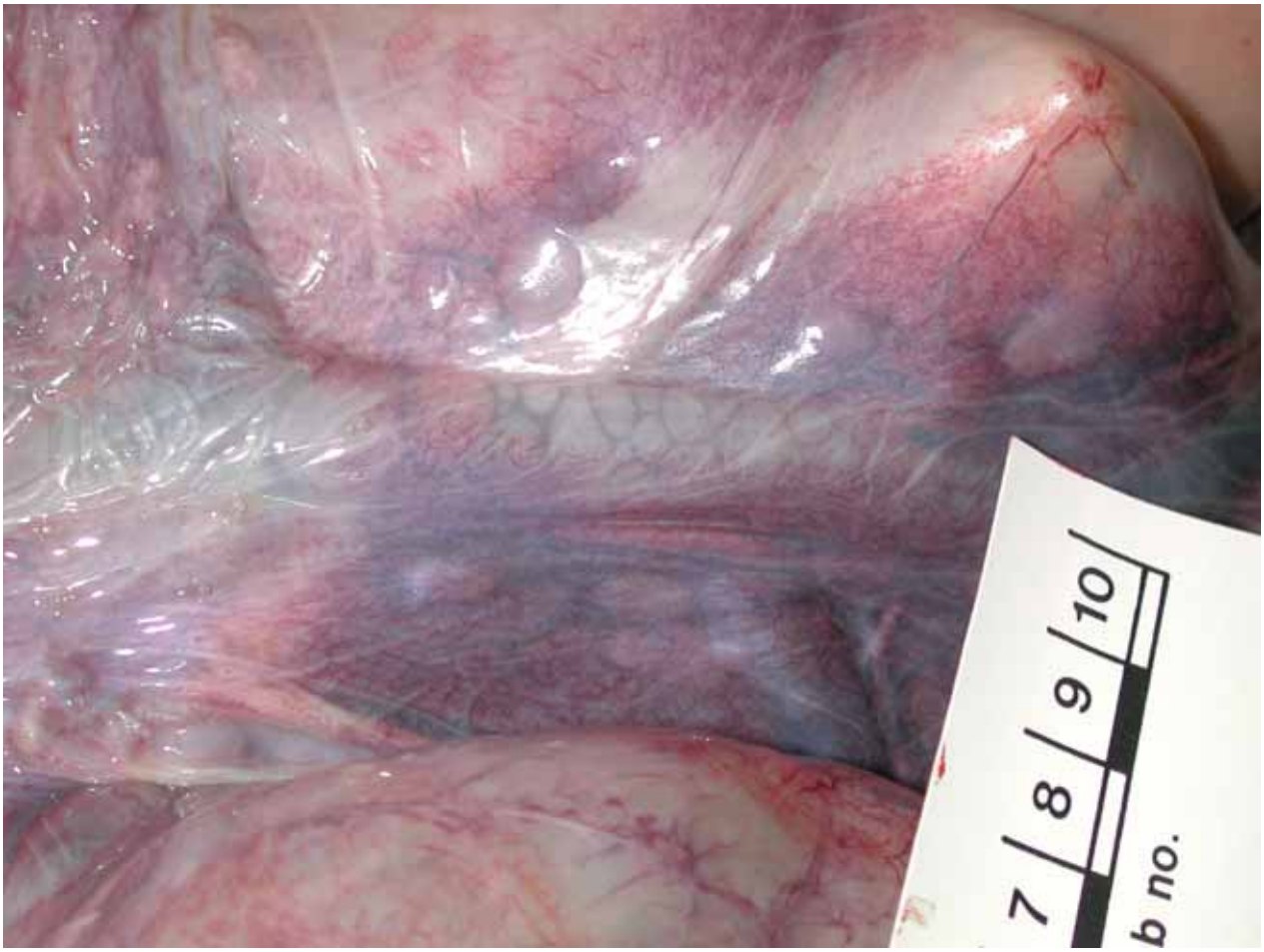

Figure 2.2 Gas bubbles in mesenteric veins. Paul Jepson

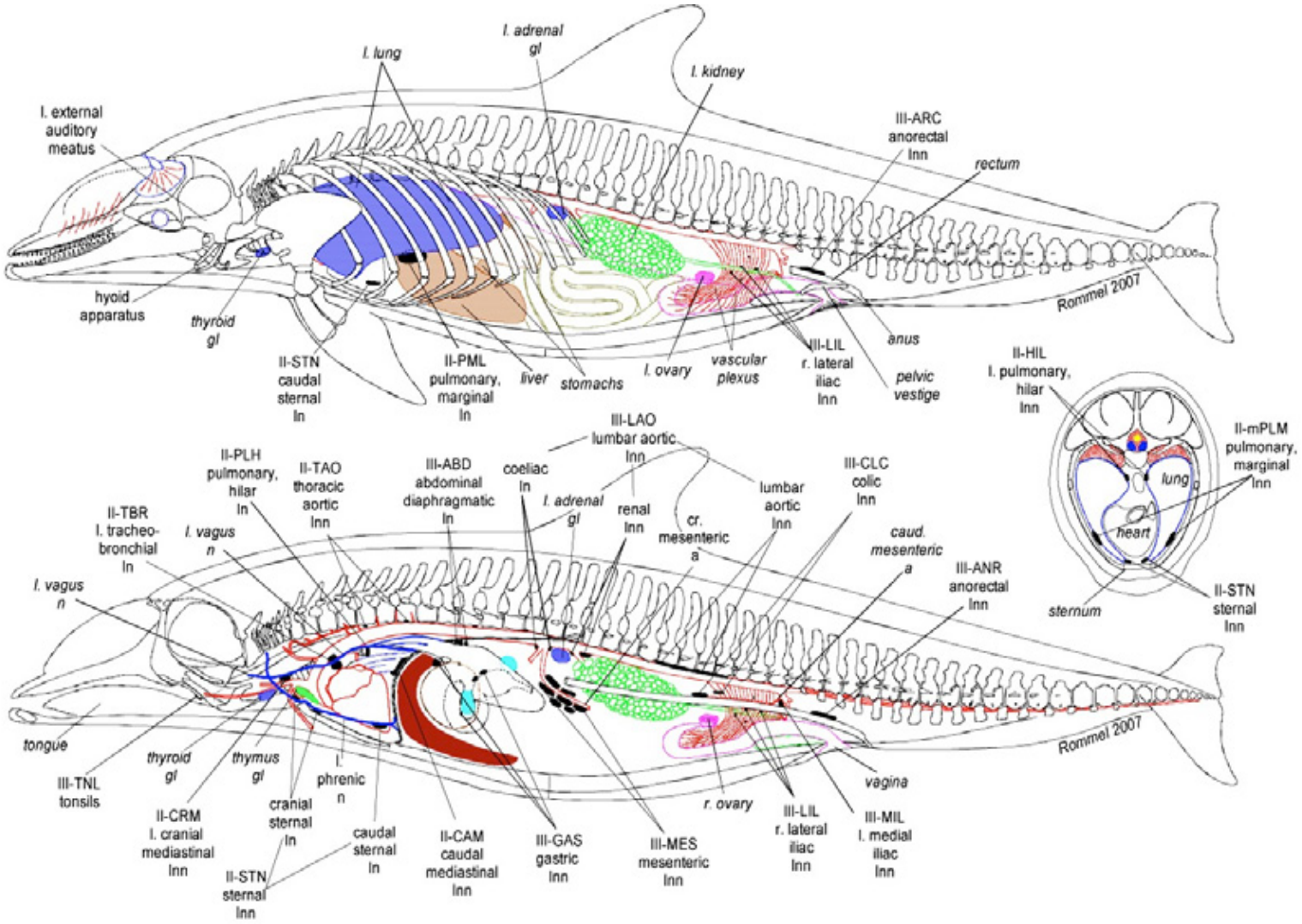

Figure 2.3 Reference drawings for lymph node identification and relative positions of thoracic and abdominal organs of *Tursiops truncates* (modified from Rommel *et al.* 2002)

2. Stomachs—Contents description (i.e., color, moisture, texture, lenses, beaks, otoliths)—mucosa description (i.e., smooth, rugose, sloughing)—parasites (i.e., type, degree of infestation, live or dead)

Normally, the stomachs are empty in most single strandings (there are cases where healthy cetaceans that have died of trauma, bycatch, etc., have stomachs full of recently ingested prey); however, in mass strandings, stomachs may contain fresh or partially digested food or remnants such as lenses, beaks, and bones. The mucosa of the first and third stomachs should be tan in color and smooth, but folded, in texture. The mucosa of the second stomach should be dark purple and smooth, but deeply folded and reticulate in texture.

Sample Narratives:

e.g., The stomach was significantly reduced in size (photos) and contained a small amount of ingesta. The stomachs were tied off and collected in entire for Nelio Barros, Mote Marine Lab. No Monorhygma and no Phyllobothrium were present. All of the abdominal organs were flaccid and slightly gassy (muscle and kidney bubbled in vacuum sealer), and the parietal peritoneum was slightly gassy.

e.g., The mucosae were unremarkable. No parasites were observed. The first stomach was full (~3 L) of moist, partially digested herring (the exact number could not be determined). Stomachs two and three were empty, and the mucosae were unremarkable.

Tissues to be collected:
Collect intact and freeze entire stomach series for examination by a specialist. Alternatively collect and freeze the contents (especially otoliths, squid beaks and lenses, spines, barbs) of each chamber. If the carcass is thought to be a HAB-suspect mortality, collect a small, finger-size or lemon-slice-sized stomach contents sample for ELISA (or RBA/LCMS). If the carcass is fresh, collect a full-thickness sample from each of the stomachs with caution to not tear mucosa from deeper layer.

Open each of the stomach chambers; if possible, collect histology prior to examination and manipulation to avoid damage to the mucosa. For each separate chamber, examine the contents and make sure to note the decomposition, degree of digestion, texture, quantity, color, and moisture as well as the presence of any inorganic objects. Freeze contents for later evaluation; incise and drain the contents into a plastic bag or bucket (if the GI track is kept intact, freeze entire). Note any ulcerations (including depth and diameter), foreign objects, or parasites, both free and embedded in the mucosa. Make a note of the quantity of any parasites present (also note if they are alive or dead), and remark on the appearance of the mucosa. In cases where a sample of the stomach wall is required, but the stomach must be saved for analysis, collect the sample and then use string to suture it. Collect a small, full-thickness sample of the stomach wall from each compartment. Take Polaroid and high-resolution electronic (preferred or 35 mm) photographs of any significant lesion.

3. Intestines

Note—the intestines of odontocetes do not have a cecum, and there are no grossly visible differences between the large and small intestines. Lay out the GI tract on the necropsy table, and fold it in a manner similar to that in Figure 2.4. Subdivide the intestines into three (equal) parts or sections. Examine the serosa for nodules, perforations, segmented discolorations, or adhesions. Open and examine the intestines for hemorrhage, parasites, obstructions, and other abnormalities—describe and then collect histology samples from the center of each section. These samples can be collected before the entire intestine is examined or as that general region of the tract is reached during exam.

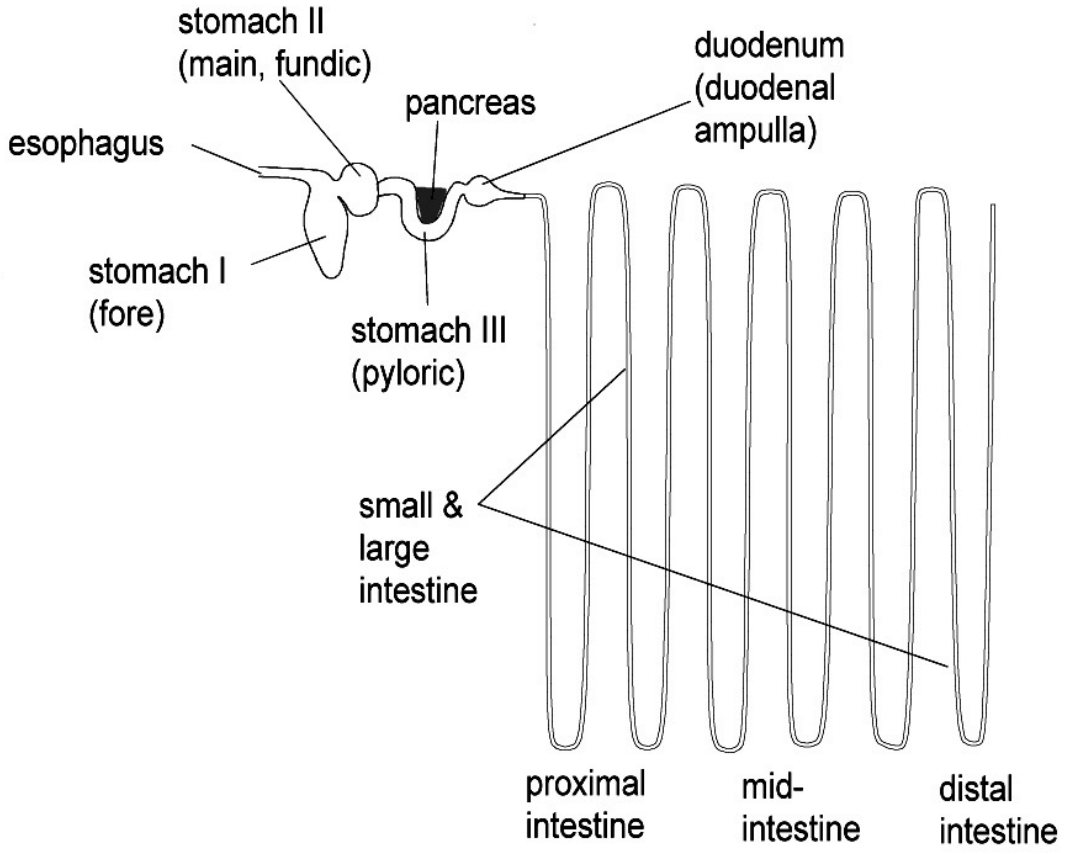

Figure 2.4 Arrangement of GI tract to differentiate and identify intestine sample sites

a. Duodenal Ampulla—contents description (i.e., color, moisture, texture, lenses, beaks, otoliths—mucosa description (i.e., ulcerated, smooth, rugose, sloughing)—parasites (i.e., type, degree of infestation, live/dead)

Tissues to be collected: **Full-thickness section of the duodenal ampulla and contents if present; caution with the mucosa.**

Normally, the duodenal ampulla is loosely filled with mucus or with bile-stained, watery ingesta and mucus. Cut open the duodenal ampulla and examine its contents. Be sure to note the quantity, color, wetness, and viscosity of the contents. Note the presence of and quantify parasites or other lesions, and remark on the appearance of the mucosa. Take Polaroid and high-resolution electronic (preferred or 35 mm) photographs of any significant lesions.

Sample Narratives:

e.g., The duodenal ampulla contained a very small amount of wet, mottled light-tan digesta. The mucosa was unremarkable. No parasites were observed.

e.g., The duodenal ampulla contained a minimum of 5 otoliths. The mucosa was unremarkable.

b. Proximal Intestine (~small intestine)—contents description (i.e., color, moisture, texture, lenses, beaks, otoliths)—mucosa description (i.e., smooth, rugose, sloughing)—parasites (i.e., type, degree of infestation, live or dead)—enteritis

Normally, this section of the intestine should be mostly empty and should contain small amounts of digesta coated with bile-stained mucus. There should be little or no intestinal gas in fresh specimens. Use ball shears to slit open the proximal intestine and examine its entire length. Note the quantity, color, moisture, and overall appearance of the contents. Note the presence and quantity of parasites or other lesions, and describe the appearance of the mucosal lining.

Tissues to be collected: **If the carcass is fresh, collect full-thickness rings (~2 cm wide) of intestine from the middle region of the proximal intestine for histology. Additional samples can be collected from specific lesions of interest.**

Take Polaroid and high-resolution electronic (preferred) or 35 mm photographs of any significant lesion. Remove and examine the mesenteric lymph nodes and sample for histology and virology (see **section F** in subsequent text).

Sample Narratives:

e.g., There were numerous stenoses along the length of the entire intestine. The proximal intestine contained watery, yellow fluid. The mid intestine was empty. The distal intestine contained watery, yellow fluid and wet, dark-green material. No parasites were observed.

e.g., The small intestine contained wet, mottled light- and dark-green material. The mucosa was roughened and rugose, with multifocal hemorrhagic lesions (enteritis). No parasites were observed.

c. Mid-Intestine (~large intestine)—contents description (i.e., color, moisture, texture)—mucosa description (i.e., smooth, rugose, sloughing)—parasites (i.e., type, degree of infestation, live or dead)

Tissues to be collected:
If the carcass is fresh, collect full-thickness rings (~2 cm wide) of intestine from the middle region of the mid-intestine. Additional samples can be collected from specific lesions of interest.

Normally, this section of the intestine should be mostly empty and should contain small amounts of material coated with bile-stained mucus. There should be little or no intestinal gas. The mucosa is typically light tan in color and smooth (with longitudinal folds) in appearance. Use ball shears to slit open the mid-intestine and examine its entire length. Note the quantity, color, moisture, and overall appearance of the contents. Note the presence and quantity of parasites or other lesions, and describe the appearance of the mucosal lining. Take Polaroid and high-resolution electronic (preferred or 35 mm) photographs of any significant lesion.

Sample narratives:

e.g., The mid-intestine was empty of contents; there was a small amount of watery mucus. The mucosa was sloughing. No parasites were observed.

e.g., NVL

d. Distal Intestine (Rectum)—contents description (i.e., color, moisture, texture)—mucosa description (i.e., smooth, rugose, sloughing), parasites (i.e., type, degree of infestation, live or dead)

Tissues to be collected: If the carcass is fresh, collect full-thickness rings (~2 cm wide) of intestine from the middle region of the distal intestine. Additional samples can be collected from specific lesions of interest.

Normally, this section of the intestine should be mostly empty and should contain small amounts of ingesta coated with bile-stained mucus. There should be little or no intestinal gas. Typically, the mucosa is light tan in color and smooth (with longitudinal folds) in appearance. Use ball shears to slit the distal intestine and examine its entire length. Comment on the quantity, color, and moisture of the digesta. Note and quantify the presence of parasites and other lesions, and examine the mucosal lining. Take Polaroid and high-resolution electronic (preferred or 35 mm) photographs of any significant lesions.

4. Pancreas—serosal surface description (i.e., color)—texture (fibrosis, extent of decomposition)

Tissues to be collected:
If the carcass is fresh, collect a histology sample.

The pancreas is located in the lesser curvature of the U-shaped third stomach (in species such as *Tursiops* that have three stomachs) proximal to the duodenal ampulla. The pancreas and

spleen can be removed before or after removal of the GI tract. Take care when separating the pancreas from the adjacent organs, and note any indications of fibrosis or parasites in this region. Normally, the pancreas has a light pinkish-gray color; it is firm but lobulated. Its enzymes make it autolyze quickly.

Examine the serosal and cut surfaces of the pancreas, and note the color and texture of the pancreas and its connective fats and tissues. Note any significant lesions.

Sample narratives:
e.g., The pancreas was soft, very gassy, and non fibrotic.
e.g., The serosa of the pancreas was light pink. Its texture was firm.

5. Spleen—serosal and cut surface description (i.e., color, texture)—lesions—number of accessory spleens

Tissues to be collected: **If the carcass is fresh, collect a sample through the midsection of the spleen for histology, virology, and biotoxicology.**

The spleen is a subspheroid located on the right side of the first stomach. Normally the spleen has a slightly mottled, dark-plum and black color, on the serosal surface; and a firm, dark reddish-plum parenchyma. If accessory spleens are present in the region, note their number and sizes. Examine the serosal surface(s) of the spleen(s) and remark on color and texture. Measure and record the weight and largest linear dimensions of the spleen(s) if fresh. Slice through the spleen(s) in a bread-loaf fashion, and examine the cut surfaces. Note the color, texture, wetness, and bloodiness of the cut surfaces.

Sample narrative:
e.g., The spleen was pale on serosal and cut surfaces.
e.g., The spleen was dark-plum on serosal and cut surfaces and was enlarged.

6. Liver—serosal and cut surface description (i.e., color, texture, margin roundness, bloody cut surfaces)—lesions

Prior to removing the liver, examine portal veins for evidence of gas bubbles. Normally the liver is a uniform or slightly mottled, shiny metallic blue on its serosal surfaces. The serosa should be uniformly and smoothly attached to the parenchyma. Some of the margins may be sharp. Roundness of the margins and separation of the serosa and mucosa from the parenchyma can reflect decompositional state of the liver and other organs, so be sure to record the integrity of these surfaces and the sharpness of the margins.

The parenchyma should be reddish-brown, firm, and moist. Bread-loaf the liver and examine the parenchyma on all cut surfaces. Record the color, texture, and bloodiness and wetness of the cut surfaces. Examine sinuses and hepatic portal and caval systems. There are large venous sinuses in the liver; palpate their margins and carefully look for evidence of gas bubble trauma (Figure 2.5).

Tissues to be collected: **If the carcass is fresh, collect four samples of liver for histology. These samples should be from right and left lobes at the cranial and caudal aspects of each (alternatively they could be collected proximal and distal to the portal circulation to reflect distribution of emboli). Collect a playing-card–sized sample for archive toxicology. If the carcass is from a harmful alagal bloom (HAB) region, a lemon-sized sample of liver should be collected each for ELISA and virology regardless of decompositional state.**

Sample narratives:
e.g., The liver was firm, with sharp margins. The serosal surfaces were metallic blue to plum, and cut surfaces were dark red-brown and bloody.
e.g., The serosal surfaces of the liver were dull metallic plum, and the cut surfaces were red-brown and wet. No sharp margins were observed.

Figure 2.5 Chronic gas bubble lesions in the liver (Delphinus delphis). Paul Jepson

Tissues to be collected: **If the carcass is fresh, collect both adrenals for histology; bread-loaf if large, and store in 10% NBF.**

7. Adrenal Glands

Examine the abdominal cavity, and locate the adrenal glands (at or near the dorsal attachment of the diaphragm cranial to the kidneys near the midline); remove, describe, and measure. Bread-loaf the adrenals, and examine the parenchyma on all cut surfaces. A central cross-section of each adrenal should be submitted for histology (a small amount from one adrenal can

be collected for virology, if appropriate). Before fixation, photograph the sections so that cortical and medullary areas can be measured. Trim fats from samples prior to weight and size measurements.

Sample narrative:
e.g., The adrenals were very firm. Periadrenal fats had serous atrophy but were abundant.

Tissues to be collected: **Separate the ovaries from the rest of the reproductive tract (uniquely label the right ovary before removal), and remove any excess connective tissue from the ovary surfaces before weighing and measuring. Collect both ovaries, bread-loaf if large, and store in 10% NBF (the ovaries may be stored separately from histology in 10% NBF for life-history evaluation). If the entire tract and/or placenta is collected, uniquely label or tie a string to the right uterine horn; attach labels to both the tract and the outside of the container. Collect genetics from fetus.**

C. Urogenital System

Reproductive—mature and/or immature—Females: multiparous, parous, or nulliparous, description of ovaries and follicles —Males: dimensions of testes, weight of testes and epididymides, seminal fluid presence, sperm presence

1. Female Reproductive Tract: Examine the serosal surfaces for any lesions. Take particular care with the nearby vascular plexuses for signs of emboli.

Uterine exam—Examine the broad ligaments for edema, parasites, extent of vascularization, opacity, and thickness. Remove the entire reproductive tract, and label one side before proceeding. Cut open the cervix and uterine horns. Examine the mucosa for evidence of placentation, regions of discoloration, or other lesions. Evaluate thickened uterine horns by measuring the dimensions of each uterine horn: length (from midline to distal tip) and outside diameter at mid-length; then examine each horn (Figure 2.6).

If the specimen is pregnant, the uterine horn in which the placenta is located is defined as the pregnant horn. Fetus gender, length, and external features (including presence of rostrum whiskers, teeth, meconium-staining of skin, fetal folds) should be recorded, and the fetus with placenta (including the amniotic sac and umbilical cord) should be preserved for additional workup. When available, collect and freeze a sample of the amniotic fluid for bioassay (seal and freeze). *See following perinatal section.*

Ovarian exam—Look for surface irregularities (e.g., corpora lutea of pregnancy and ovarian scars from past pregnancies). These qualities are used to determine reproductive maturity, history, and status.

Sample narrative:
e.g., The carcass was pregnant, and the fetus was in the left uterine horn. Left mammary dimensions: 32 × 10 × 3 cm. The male fetus's total length was 98 cm, and it did not have whiskers, teeth, or meconium-staining of the skin, but did have fetal folds.

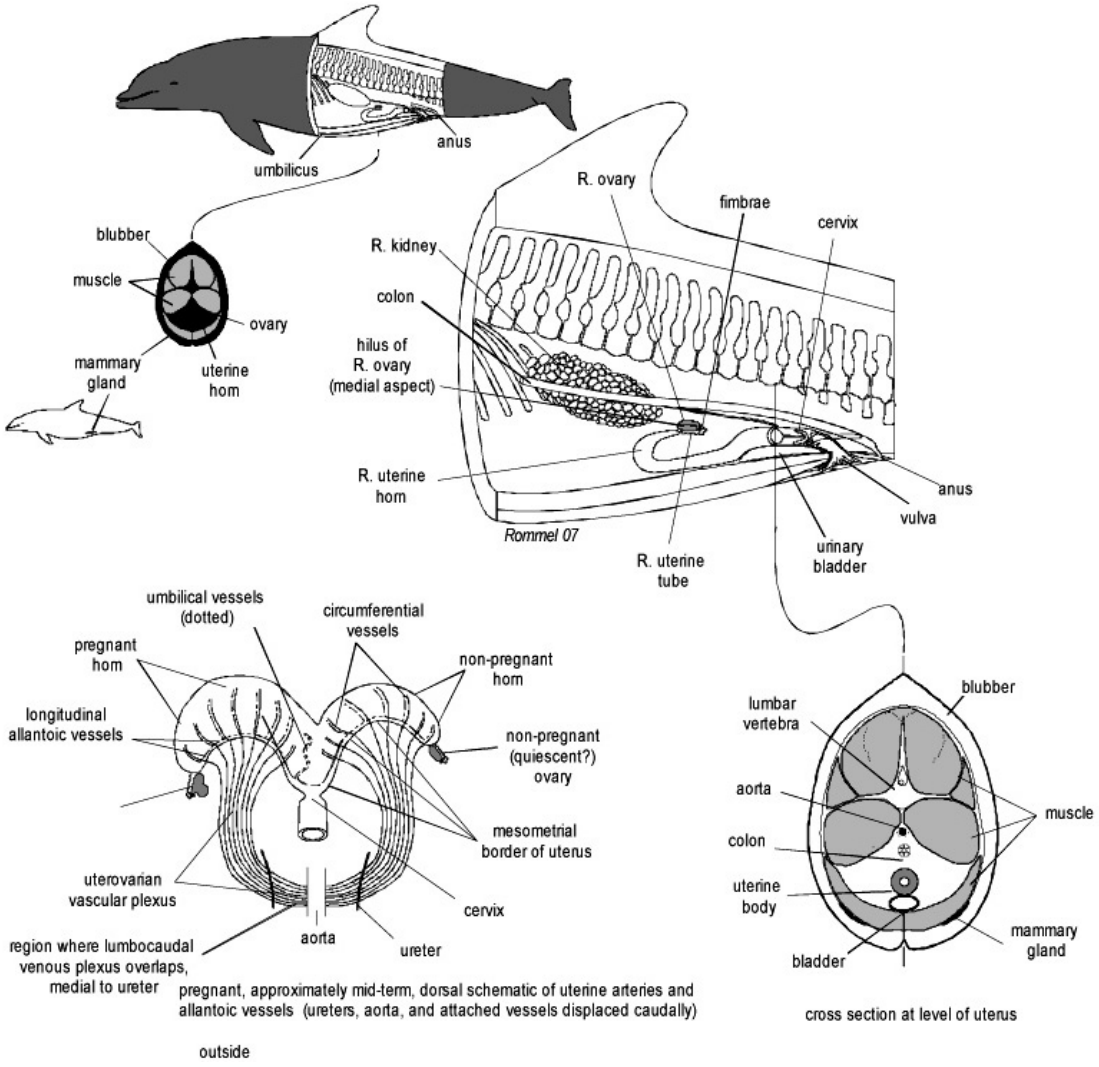

Figure 2.6 Female reproductive system and sampling sites—after Rommel *et al* (in press)

Tissues to be collected: **Collect a smear from one of the testes or epididymides (preferable) on a glass slide, and examine under a microscope (40×) for presence of sperm. If the carcass is fresh, collect a tissue sample from the head of the testes (because tendency for infection to develop first at this site), the middle of the testes for reproductive history (may be stored in 10% NBF separately from histology for life history), and from the proximal and the distal epididymides for histology.**

2. Male Reproductive System: Examine the serosal surfaces of the testes and epididymides for general appearance (color, size, shape, firmness, fullness, flaccidity) and lesions (Figure 2.7). Prior to cutting in this region, take particular care with the vascular plexuses associated with reproductive cooling to be sure there are no signs of emboli (a sample of the plexuses should be collected for histology). Cut the epididymides, and note the presence, color, and viscosity of any liquid present. Examine the cut surfaces for any lesions. Separate the testes from the rest of the reproductive tract, and remove any excess fat or connective tissue from the outside before weighing and measuring length, width, and thickness.

Sample Narrative:
e.g., Right testes had dimensions of 27.5 × 8 × 2.5 cm; left testes had dimension of 29 × 8.5 × 2 cm. The testes were flaccid and autolyzed. There was no grossly visible milky fluid.

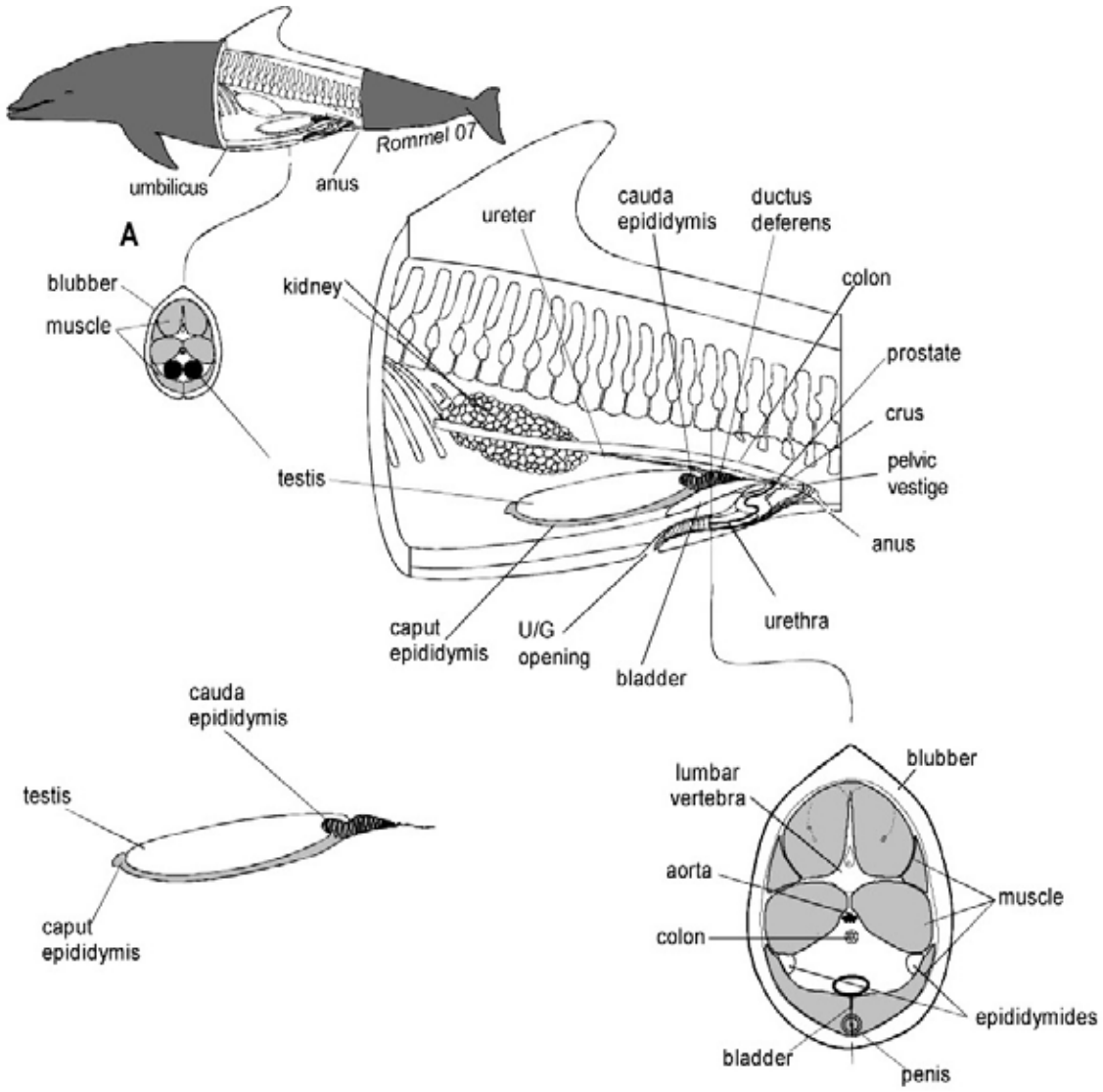

Figure 2.7 Male reproductive system—after Rommel *et al.* (in press).

Urinary bladder tissues to be collected: **If the carcass is fresh, collect a full-thickness sample of the bladder (preferably the apex where infection tends to start) for histology. If the carcass is from a HAB region then, if possible, use a syringe to collect 1-5 ml of urine for ELISA toxi-cology; to store, either tape the syringe or transfer fluid to a small seal-able container; if no urine is available, collect a finger-sized (~10 ml.) piece of kidney.**

3. Urinary Bladder—mucosa description (i.e., congested)—contracted or dilated—empty or full (i.e., volume, color, transparency)—lesions

Examine the mucosal and serosal surfaces for any lesions, and note whether or not the bladder is contracted. Make a small incision at the cranial apex of the bladder; note the total amount, color, consistency, and transparency of the urine, and collect a sample before draining the bladder. In beaked whales, examine the blood vessels and urinary ducts for nematodes or obstructions. Examine the ureters for patency, and note any lesions.

Sample narrative:
e.g., The urinary bladder contained approximately 15 cc of cream-colored fluid. The mucosa was unremarkable.

4. Kidneys—serosal or cut surface description (i.e., color, wetness and bloodiness, presence of fat—cortico-medullary boundary description (i.e., cortex and/or medulla color)—lesions (i.e., infarcts, gas bubbles)

Tissues to be collected: **If the carcass is fresh, collect a tissue sample for histology from each kidney and an addi-tional sample for archive toxi-cology. Note and collect parasites, if present. A sam-ple from a kid-ney lesion should extend from one cortex through the medulla and to the contra-lateral cortex.**

Check the region surrounding the kidneys for lesions such as hemorrhage, infarcts, and emboli (Figure 2.8), and check the ureters for dilation to suggest presence of obstruction. The serosal surfaces of each reniculus are typically plum in color, although lividity may make some surfaces darker than others. On cut surfaces, the cortices typically are darker than the medullae, and there are distinct cortico-medullary boundaries, which are often delineated by a dark-red rim of congested blood vessels. Parasites are common in some species of odontocetes. Examine the intact kidneys, and then remove them (in the case of suspected ureteral obstruction, locate the cause for obstruction prior to removing the kidneys). Closely examine all the surfaces, and remark on the color, firmness, and texture; note the presence of any blood clots or other lesions. Measure the maximum dimensions and the weight of each kidney. Cut the kidneys longitudinally along one of the blood vessels or the ureter, and note the color and bloodiness of the cut surfaces. Record the color of the cortices and medullae and any lesions present. If the carcass is from a HAB area and no urine is found in the bladder, be sure to collect a kidney sample for ELISA.

Sample narrative:
e.g., The kidneys were pale to dark-red on serosal surface, and, on cut surfaces, the cortices were diffusely pale with occasional dark-red foci and with multifocal loss of corticomedullary differentiation. The ureters were unremarkable. No parasites were observed.

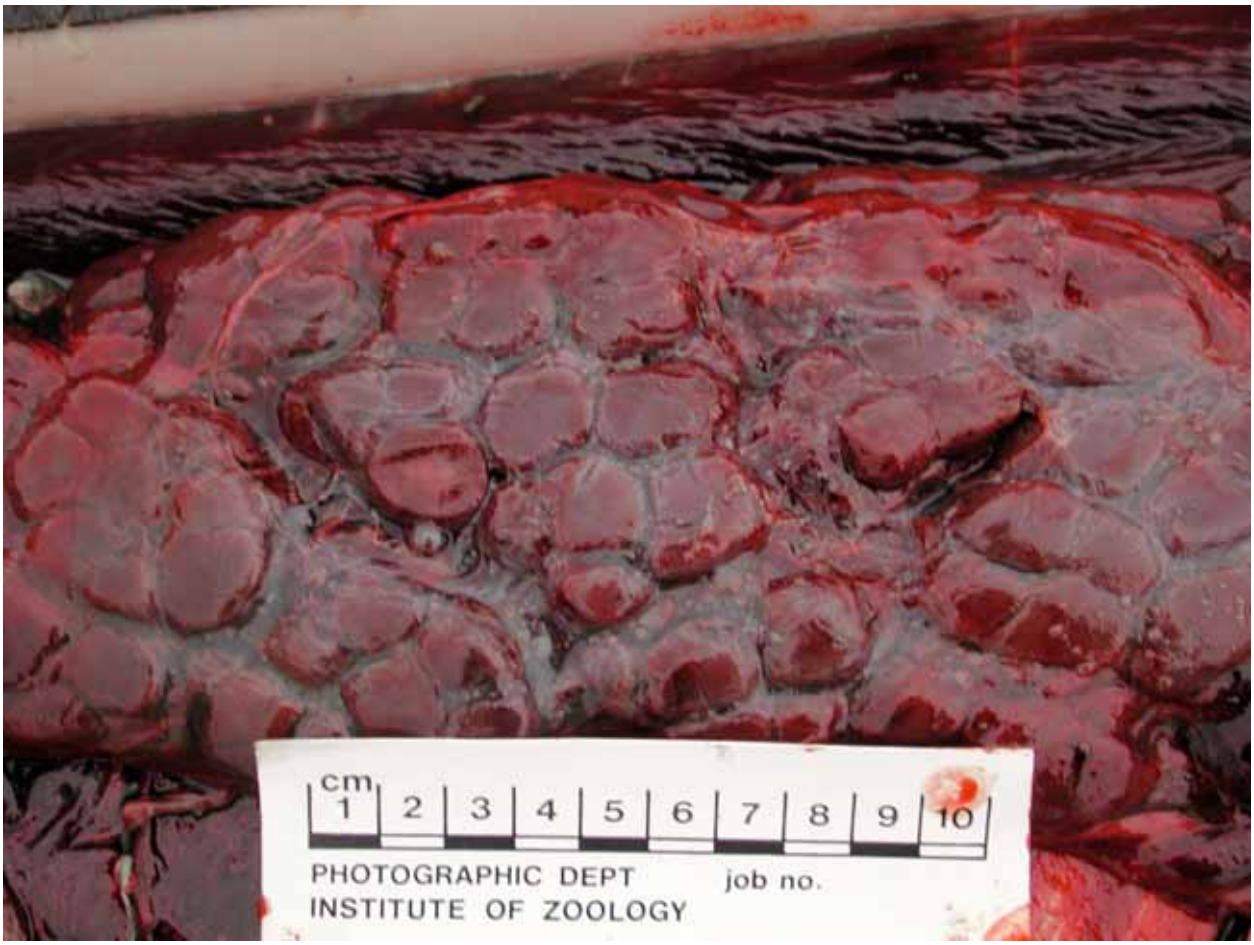

Figure 2.8 Perirenal gas emboli in *Mesoplodon bidens*. Paul Jepson

D. Thoracic Cavity

Prior to entering the pleural cavity, note the presence of tears or lesions on the diaphragm. Cut the muscular portion of the diaphragm along the ribs to expose the left lung. Note the presence, amount, color, and viscosity of any excess fluid in the pleural cavity, and note whether it is on one or both sides of the diaphragm. If able to expose the cavity in a sterile manner, collect a swab of the surface of the pleural surface (especially where it is reddened or roughened) for microbiology (the fluid is less optimal for culture, because the etiologic agent is diluted and/or dead).

E. Vascular System (see Figure 2.9 and Appendices 2-G and 2-H)

1. Heart—valves and chambers (i.e., check color, transparency, and texture of myocardium and valves), look for foci of pallor (again, necrosis is too interpretive and easily can be wrong).

Normally, the heart should be uniform in color, and the valves should be translucent and smooth in texture. Open the pericardial sac, and note the quantity and color of the pericardial fluid. Examine superficial cardiac blood vessels for presence of gas bubbles (emboli).

Tissues to be collected: **For histology, collect a sample of each atrium and ventricle and any areas with lesions. If the heart is to be weighed, be consistent; trim great vessels to margins of pericardium and remove**

Examine the proper position of the great vessels relative to the heart and for patency of the ductus arteriosus prior to removing the heart. Place the heart in your hand with the auricles facing you and note a) whether the left ventricular free wall does not curve slightly (i.e., shifting of the apex left); b) whether there is a double apex (i.e., thickening of the right ventricle) (be careful not to overinterpret should the right ventricle be filled with a large blood clot); and c) whether the left ventricle is flaccid and contains a large amount of blood (the animal should die in contraction of the left ventricle). Use a coronal approach to each valve to check for infection (enabling a sterile approach) or rupture of valves. Make a cross-sectional cut at the apex of the ventricles, and look for discoloration and/or banding or pallor in the myocardium. Cut the ventricular wall of both ventricles upward into the atria. Examine the papillary muscles and endocardium for any discoloration or scarring. Examine the interatrial septum for patency of the foramen ovale or other septal defects. Examine the atrioven-tricular (A/V) valves for signs of thickening, roughening, or other lesions. Cut into the aortic and pulmonary inlets, and examine the semilunar valves for signs of thickening, granulation, or other lesions.

Sample narrative:
e.g., Valves and chambers were unremarkable. On cut sections, there was banding on the myocardium of the left ventricle and interventricular septum, which was not noted in the right ventricle nor the atria.

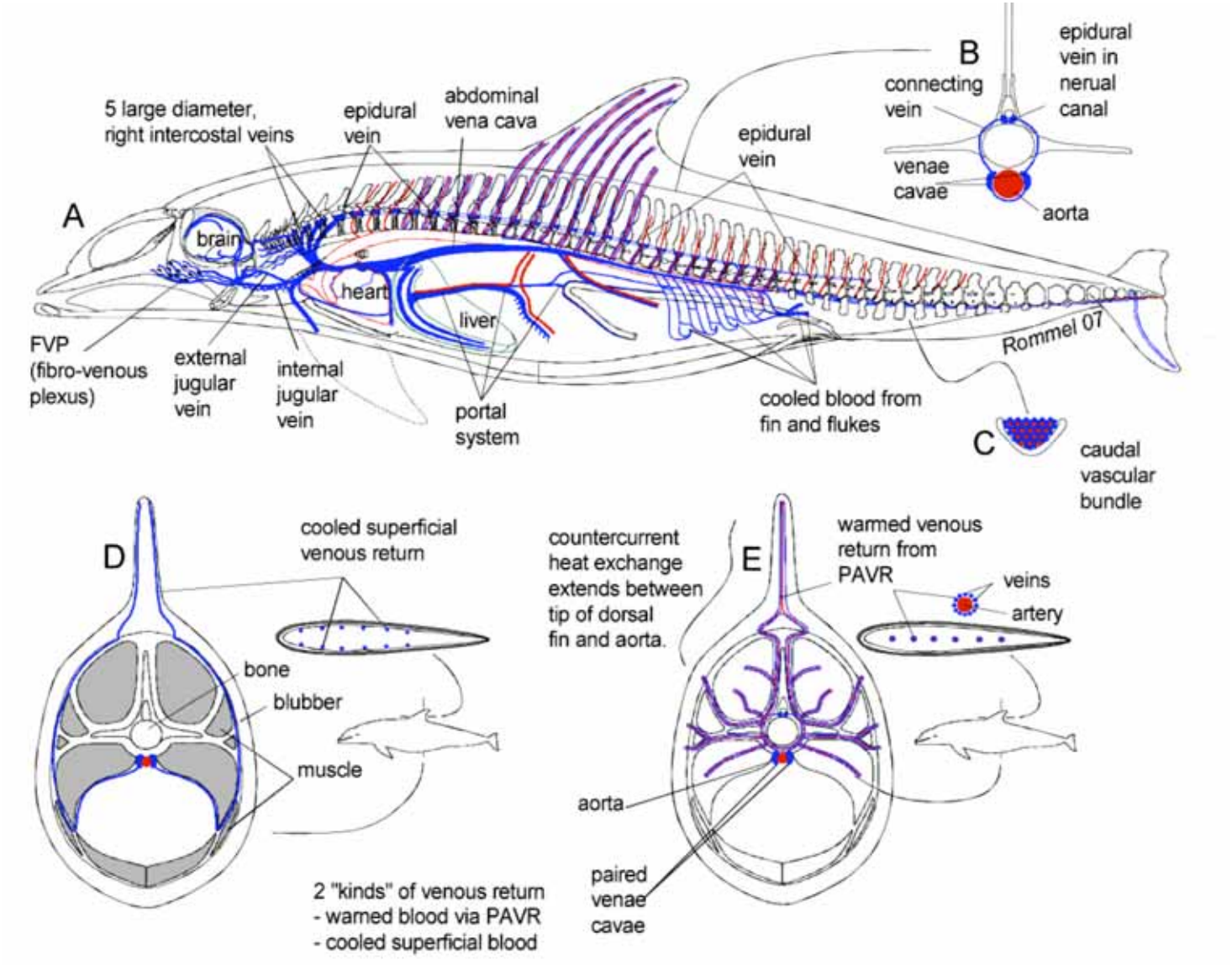

Figure. 2.9 Anatomical guide for the deep venous circulation—after Rommel *et al*. (2006).

Great vessels tissues to be collected: **For histology, collect sample of the thoracic rete from one pleural cavity and any areas with lesions. Remove two vertebrae (T2–3, if possible for double-headed rib attachment) with the proximal ribs attached; this will be sub-sampled after preservation for intervertebral and synovial joints, spinal cord, epidural, and thoracic retia.**

Perinatal indicators tissues to be collected: **For histology, collect sample of the umbilicus, the umbilical arteries at the level of the urinary bladder, umbilical vein at the level of the liver, urachus at or near the bladder tip, the ductus arteriosus with small pieces of the aorta and pulmonary trunk attached, and the foramen ovale. Sample any lesions or infections of the umbilical structures for histology.**

2. "Great" Vessels

Closely examine the serosal and luminal aspects of the aorta, vena cava, and pulmonary trunk. Look for adhesions and other lesions.

3. Perinatal Indicators

Dissect the heart, great vessels, and arterial supplies to the head and neck—for small animals, this should be done anyway to remove the thymus. In perinatal carcasses, record the presence or absence of external features (whiskers, neonatal folds, teeth, meconium staining of the skin, patency of umbilical vessels and urachus, and parts of the umbilical cord that remain attached to the perinatal carcass). Also record the patency of the umbilical arteries, umbilical vein, and the urachus (a neonate odontocete may have a patent urachus). Observe the ductus arteriosus and note if patent; record its flat (or round) diameter and the flat diameters of both pulmonary arteries (note if flat or round diameters). Examine the foramen ovale and note its overall closure, as a percentage, of the total area if multiple openings in the septum are observed. Ventricular wall thicknesses are valuable—because of the irregular internal surfaces, it is better to record the thicknesses of both walls (double-wall thickness) of each ventricle, measured with the heart intact. Be sure to examine the lungs and note whether they are consolidated. In very fresh specimens, a piece of lung can be placed in water or formalin. A floating lung is suggestive of previous inflation; however decompositional gasses in tissues that are not very fresh can cause the lung to float. When examining the lung, be sure to note the presence of lungworms in the airways, or lungworm cysts in the parenchyma (transplacental migration).

F. RESPIRATORY SYSTEM—diaphragm condition (i.e., tears, color, concavity, or cavity compromised lung serosal and/or cut surfaces (i.e., color, texture, wetness, airway contents or parasites)—lesions (i.e., lung adhesions, torn parietal pleura, abscess)

1. Diaphragm

Normally, the diaphragm is intact and—if the pleural cavity has not been compromised—tautly stretched between the midline and the lateral aspects of the abdomen, with a dorsal bulge

(concavity). When first cut, the diaphragm will spring ventrally as the negative pressure in the pleural cavities is released. There should be a scant amount of clear, watery serous fluid in the pleural cavities. The parietal pleura should be smooth, glistening, and light tan or pink.

2. Trachea and Airways

The mucosal surfaces of the major airways should be examined carefully, and their color, texture, and degree of congestion should be described. In fresh carcasses, the mucosae of the airways should be pale pink to pale red in color. The mucosal surfaces should be smooth and are often coated with a thin layer of mucus. Any coating or contents (e.g., froth, parasites, sediment) should be noted and quantified.

Sample narrative:
e.g., The tracheal mucosa is dark-red and moderately congested. There is a thick, heterogeneous coating of viscous, ropey mucus and silt-like sediment. On cut sections, the mucosa appear severely engorged.

Tissues to be collected: **If the carcass is from a HAB area, collect a finger-sized tissue sample from the cranial pole for ELISA. If the carcass is fresh, collect three tissue samples—one on the margin, one in the middle, and one along the main airways. Include a piece of bronchus in at least one of the lung sections.**

3. Lungs

The lungs should be spongy and well-inflated. The serosal surfaces of the lungs should be uniformly light in color and the pleura be clear and moist. Lung worms may occur, depending on species and feeding history; note parasites and tissue reactions to them. Look for lymph nodes and highlighted lymph channels on the surfaces of the lung. Examine the serosal surfaces of both lungs, and remark on the color, texture, and any lesions present.

Excise the primary and secondary airways with scissors, and remark on the presence, color, and viscosity of any material; also remark on any parasites or other lesions observed. Palpate the surfaces of the lung and note irregularities, then subsample any unexpected textures. Bread-loaf the entire lung, and examine the cut surfaces. Note the color, wetness, congestion, and any lesions observed. Photo-document any irregularities that are observed.

Sample narratives:
e.g., The lungs had scattered small focal nodules affecting approximately 25% of the total surface area. Both lungs were dark-red on serosal surfaces and dark-red to purple on cut surfaces. The airways are unremarkable.
e.g., The pleural cavity was distended with gas. The diaphragm was intact. There was a moderate trematode infestation in both lungs. The left lung was diffusely collapsed. There was a long laceration, approximately 2 cm, on the dorsal aspect of the left lung associated with a rib fracture. The laceration had a dark-red margin. The rib fracture was a closed, simple transverse fracture of the mid-diaphysis with displaced fragments that have sharp margins. Cut sections of the left lung sank in formalin. The right lung was diffusely dark-red, slightly wet, and heavy. There was an adhesion (approximately 2 × 3 cm) between the dorsal aspect of the mid-lobe and the parietal pleura (deep to a well-healed rib fracture). The primary and secondary airways contained a moderate amount of opaque, tan-to-red, slightly viscous fluid.

G. Lymphoid Tissues (Note: spleen was discussed previously, and tonsil will be discussed subsequently.)

1. Lymph Nodes (see Figure 2.3)

Tissues to be collected: **If the carcass is fresh, collect a sample of every major lymph node, especially lung- and GI-associated ones, for histology. If major lymph nodes are bilaterally paired, be sure to collect and label a lymph node from each side. Lymph nodes may also be frozen (preferably at –70° C) for immunological studies.**

Lymph nodes can be quite variable in appearance; however, a healthy, inactive lymph node should be pale yellowish-brown or off-white in color, should be located within a perinodal fat pad, and should be a solid structure without any cavities or hollow regions. The gross appearance of the perinodal fat can provide some clues regarding activation of the lymph node. The perinodal fat should not be gelatinous (i.e., serous atrophy), and the serosal surfaces of the lymph node should be smooth. Activated lymph nodes are typically dark-brown to black in color, but may have a heterogeneous (lumpy, nodular) external appearance; may appear wet, and the serosa may have irregularities; and the cortex may have regular, pinpoint white foci (nodular lymphoid hyperplasia). Prior to fixation, section lymph nodes, and describe cut surfaces. Describe the color, presence of serous fluid, and any structural irregularities (e.g., small pits on cut surfaces). Note that, in some species, some lymph nodes (i.e., axillary) are nearly impossible to find unless active and enlarged.

The narrative for this section should be dictated as one progresses through the carcass and each lymph node is encountered. Each lymph node should be described even though it may not be collected. Be sure to note the general appearance of the lymph nodes as well as any lesions or irregularities. Special attention should be given to the major lymph nodes draining important physiological systems (e.g., the

respiratory and digestive systems). Pulmonary and mesenteric lymph nodes are often noticeably irregular if there are respiratory or digestive complications, respectively.

a) Superficial and/or associated with extremity: Examine all superficial lymph nodes as the blubber is removed, prior to entering the abdominal cavity. Examine the axillary and superficial cervical lymph nodes while removing the axillae and scapulae. Examine the retropharyngeal and mandibular lymph nodes prior to decapitation.

b) Thoracic: Examine all pertinent thoracic lymph nodes after exposing the pleural cavities and during the lung examination.

c) Abdominal: Examine all pertinent abdominal lymph nodes after removing the gastrointestinal tract and during the abdominal organ examination.

Sample narratives:
e.g., The axillary, pulmonary marginal, and mesenteric lymph nodes were darkened and enlarged. The superficial lymph channels on the lateral aspect of the lung, joining at the pulmonary marginal lymph node, were very conspicuous. The mesenteric lymph nodes exuded serous fluid on cut sections.
e.g., The mesenteric lymph nodes were darkened and severely enlarged. The serosal surfaces of one mesenteric lymph node were nodular in texture, which on cut section was associated with regular, pinpoint, white foci in the cortex and exuded serous fluid when cut.

Tissues to be collected: **Collect section of the thoracic duct at the level of the receptaculum chyli for histology. If fat emboli are suspected, be sure to collect a second cross-section to be fixed in special adipose-tissue fixative (i.e., Pen-fix–http://www.rallansci.com/histology/histology.aspx?id=14). Collect any irregularities or lesions observed.**

Other lymph channels tissues to be collected: **If the carcass is fresh, collect a sample of every major lymph node, especially lung- and GI-asso-ciated ones, for histology. If major lymph nodes are bilaterally paired, be sure to collect and label lymph nodes from both sides. Lymph nodes may also be frozen (preferably at –70° C) for immunological studies.**

2. Thoracic Duct and Mesenteric Lymph Channels

The thoracic duct is located just dorsal to the dorsal aorta. External appearance of the thoracic duct is variable but may be important. Make a transverse cut at the distal thoracic duct, and check to see if it is significantly enlarged; open the duct cranially as far as possible. Also examine the receptaculum chili, the ampulla chyli, and the chyle cistern. Examine the lumen for signs of emboli.

Sample narrative:
e.g., The thoracic duct was unremarkable externally.

3. Other Lymph Channels

All applicable lymph channels should be examined. Most notably the lateral superficial lymph channels of the lungs should be examined and described.

Thymus tissue to be collected: **Collect a piece of thymus (near the aortic arch where least amount of involution seems to occur) for histology. A piece of thymus can also be frozen (preferably at –70˚C) for immunological studies and for virology.**

4. Thymus

In fresh carcasses, the thymus should be pale pink. The thymus involutes with age; therefore, in older animals, it can be significantly reduced in size and/or very fatty. The thymus should be examined for any irregularities. If possible, and if in good enough condition, be sure to dissect out the entire thymus and weigh it.

Sample narrative:
e.g., The thymus was pale-red on serosal and cut surfaces, but was otherwise unremarkable.
e.g., The thymus was dark-red on serosal surfaces, dark-red and wet on cut surfaces.

H. Head and Neck—lesions (i.e., bruising, abscesses, edema, clots)—description of lesions present—periotic collected, tooth collected, nuchal fat collected.

The narrative for this section should be dictated as one progresses through the blubber and muscle toward the skull. Note the nuchal fat pad located on the dorsal midline just deep to the blubber.

Sample narratives: e.g., The fats in the head and neck were very edematous. The nasopharynx was almost occluded by thick, slightly viscous, ropey, pink-to-dark-red mucous. The proximal trachea was approximately one-third to one-half occluded by the same material. This material was not observed in the lungs. The nasopharyngeal mucosa was slightly congested. The meninges of the brain and spinal cord were moderately congested. The retropharyngeal lymph nodes were darkened and enlarged

Tissues to be collected: **Collect a piece of tongue and any oral lesions present for histology. Additionally, collect a cross-section of the larynx or goosebeak and sections of the oropharynx, thyroid glands, and tonsils. Collect teeth for ageing.**

1. Oral Cavity and Throat

The tongue, lips, gums, and oropharynx should be examined for orogential ulcerations. The goosebeak and larynx should be examined for signs of human interference (HI) (e.g., monofilament, fish hooks, ingestion of marine debris) and other lesions. The tonsils should be sampled. Teeth should be examined, and the extent of fractures and/or truncation should be described.

Examine the lips, then open the mouth and examine the tongue, gums, and roof of the mouth. Describe any irregularities and lesions. If the head is not being collected intact for research, remove the ventral throat blubber and examine the hyoid bones for irregularities before dissecting out the thyroid glands. Describe the color and consistency of the thyroid glands, and note any fluid-filled follicles or thyroid cysts. Take a cross-

section of each thyroid gland for histology. Remove the dorsal blubber caudal to the blowhole, and examine and collect the external auditory meatus inn.

Sample narrative: *e.g., There were small (~0.5 cm), focal, bilateral ulcerations visible along the gum line. There were large (1.5–2.5 cm diameter), diffuse and coalescing, nodular proliferations on rostral half of the lip of the right mandible. Most of the teeth were severely truncated from wear; five teeth had oblique fractures.*

2. Sinuses and Vascularized Spaces

Special attention should be given to the mandibular fat pads, the pterygoid sinuses, and the fibrovenous plexuses between them (Appendix 2-H). This region may be susceptible to acoustic and/or barotrauma and could be a source of fat emboli (an extra section should be collected and placed in Pen-fix or other special fat fixative). The peribullar sinuses should also be examined. Be sure to note the presence of parasites, blood clots, or unusual lesions within the sinuses or mandibular fat pads.

If necessary, dissect, and examine the superficial cervical and retropharyngeal lymph nodes before decapitation. The axillary lymph nodes may be more readily located if the scapula is removed from the body starting at the dorsal border of the scapula. The axillary LNN are located at or near the brachial nerve plexus.

Brain and pituitary tissues to be collected: **If the carcass is fresh, collect a coin-sized tissue sample of pituitary, cerebrum (from occipital lobe) and cerebellum (from dorsal vermis) for ancillary diagnostic tests. Place the brain on piece of paper towel (do not wrap) and submerge in formalin (7–10 days fixation required prior to sectioning for histology). For large brains, more concentrated formalin can be used. Collect any active lymph nodes or other affected tissues according to your needs. Collect nuchal fat samples (frozen).**

3. Eyes

Remove and examine the eyes. Examine the fatty tissue (retrobulbar periobital fat) behind the eyes for hemorrhage, and sample for histopathology.

4. Brain and Pituitary

Skin the top and sides of the head in order to search for bruising or other signs of trauma. If possible, once the soft tissue surrounding the skull has been examined and removed, the brain should be extracted. Cut away at the soft tissues over the cranial vault. Make two horizontal cuts: one through the occipital condyles and the other posterior to the nuchal crest. Joint these laterally with two vertical cuts. Use a chisel to break the bony septum that separates the hemispheres. Remove the bony plate to expose the brain. Examine the brain and associated meninges for congestion and lesions. Peel the dura

matter away from the brain case and examine the skull for fractures.

5. Ears

See procedure in Chapter 3.

***I. Skeleton*—**lesions (i.e., any fractures, luxations, lacerations caused by broken or luxated bones)—remodeling—joint problems (i.e., dry and/or lumpy nucleus pulposus, osteoarthopathy)

Examine the synovial fluid of all large joints, particularly the glenohumeral and condylar joints. Additionally, the costovertebral and chevron joints have been implicated with DCS-like lesions in some divers and should therefore be examined for changes in the joint cartilage and underlying bone—osteonecrosis and osteoproliferative lesions. After exposing the ribs when flensing, the ribs can be disarticulated at the costo-vertebral joints. At least one costovertebral joint set (double-headed rib [Appendix 2-I]) should be collected for histology and the caudal vertebra of this pair could be examined for skeletal maturity. Additionally, a chevron-vertebral joint should also be collected. After removing the lungs, be sure to run your hand across all the ribs while moving the rib tips to locate any fractures. If evidence of trauma is found, remove the skin from the affected side of the carcass, look for bruising, and examine the vertebral column for fractures. Record the rib or vertebral number of the elements that are either fractured or luxated. Look for mandibular fractures (fresh or healing), particularly in adult males, and record presence and location. Photo-document any evidence of trauma or remodeling, and record the information on the skeleton and skull-lesion data sheet (Appendix 2-I and 2-J).

For description of a fracture include the following: (A) bone affected; (B) open or closed to body surface; (C) communicates with another structure; (D) type of fracture (simple transverse or oblique; spiral; comminuted; greenstick, etc.); (E) whether fractured ends are aligned or displaced; (F) whether fracture margins are sharp or smooth and have callus formation; (G) presence of foreign material, purulent material, osseous sequestrum; (H) presence of blood, edema, fibrosis, or purulent material in surrounding soft tissue or structure with which it communicates. All of this information helps determine the type

Tissues to be collected:
Collect samples of unusual fractures or unusual observations for further interpretation or for teaching.

of trauma sustained, whether the fracture contributed to morbidity or mortality, possible sequelae, the potential for healing, and whether the fracture occurred antemortem.

Sample narrative:
e.g., Left ribs #s 9 and 10 had closed, simple transverse fractures of the proximal diaphysis with slightly rounded, nondisplaced ends and early callus formation. Left rib #11 had a compound (open), comminuted fracture of the proximal diaphysis. Left rib #11 was deep to the superficial wound pattern. There were well-formed blood clots in the intercostal regions adjacent to the rib fractures.

J. Other—lesions (i.e., any comments that don't fit in previous categories)—organ weights and dimensions (i.e., of organs not in previous list)

Lesion tissues to be collected:
If the carcass is fresh, and the lesions are indicative of cause of death or are of special interest, collect a sample (see tissue checklist, Appendix 2-I).

Record any nonspecific lesions or comments such as weights of organs or tissues that have no specific category.

K. Morphological Diagnosis

A gross morphological diagnosis is a summation of the description of gross observations. It is, for most observations save for most traumatic injuries, inherently subjective because histologic examination of tissues is needed to corroborate findings. The structure of a morphological diagnosis varies among pathologists, but there are certain important elements:

- Tissue
- Modifier (if applies): Suppurative (abscess)
- Duration: Acute, subacute, chronic, etc.
- Distribution: Focal, multifocal, diffuse, etc.

Sample:

Description: The cranial surface of the left lung lobe had a focal, 2 × 4 × 2 cm, raised, white-gray, mass rimmed by a 1 mm to 3 mm erythematous line. On cut surfaces, the mass was filled with abundant cheesy-white, sticky, fetid material and was encapsulated.

Diagnosis:

Respiratory System:
Lung: abscess, focal, chronic

1. Significant Findings—important lesions and/or findings (i.e., enteritis, trauma, emboli)

Sample narrative:
e.g., Lesion in colon was associated with red mucosa; dark-red regions in each lung; nasopharynx was obstructed with thick mucus; depleted serous atrophied and edematous fats; meninges were moderately congested; numerous penetrating propeller wounds; fractured kidney; rib fractures (early healing), well-formed blood clots.

2. Most Probable Cause of Death—proximal cause of death **and suspected contributory causes of morbidity**—underlying conditions or conditions resulting from (i.e., sequelae to) a primary condition

Sample narratives:
e.g., natural; other (red tide)
e.g., watercraft

3. Necropsy Conducted by—examiners, participants, and/or observers present during necropsy (note: primary prosector and recorder)

NECROPSY PROCEDURE—SAMPLE-COLLECTION AND -CURATION

Samples from live and dead cetaceans provide valuable insights into the health of the individual and also contribute to studies to evaluate the health of the population. In collecting samples, three factors are important: (1) the development and use of standardized collection protocols; (2) the use of quality-assurance methods in specimen collection and analyses; and (3) the sharing of data and protocols among regional, national, and international groups (Rowles *et al.* 2001).

Carcasses may not be in the optimal state for all sample-collection protocols; depending on the condition code and state of decomposition, it may not be possible to collect all of the information. The best samples are obtained through careful dissection—avoiding contamination of tissues through contact with dirty instruments, other organs, or body fluids and avoiding cross-contamination during sterile sampling by resterilizing instruments between sample collections. At the beginning of the necropsy, be sure the type and quality of equipment and packaging materials are satisfactory for the task at hand; have a subset of the team dedicated only to sample-collection and processing under the direction of the primary prosector. Table 2.1 provides a guideline for the types of samples that can be collected by condition code. It is important

to note that information can be gleaned from carcasses in all states of decomposition.

In collecting information, there are three levels of data-collection:

> **Level A**—basic information primarily used to document and verify a stranding. Secondarily, this may include some (qualitative) information about cause and circumstance of stranding (and/or death of the animal). *Example:* Level A form, photographs, voucher specimens.
>
> **Level B**—Detailed, in-depth (supplemental) information about a stranding, individual, or life history. This information is generally the result of examination of samples obtained from the animal. Example: tooth age analysis, general histology, parasite identification and enumeration, herd composition.
>
> **Level C**—Detailed information about the cause and circumstance of stranding (or death). This information is generally the result of tests on samples that have been collected from the animal. *Example:* histopathology, bacteriology, virology.

Although stranding network respondents only are required to collect Level A data, this procedure's goal is to encourage the most complete necropsy possible with data-collection up to and including Level C. Chapter 1 focused on examination of and collection of important information from live animals; in this chapter and section, the discussion focused on tissue collection for freshly dead and Code 2 animals.

Table 2.1 Types of samples, by condition code, that can be collected

	Code 1 Collection Protocol	**Code 2 Collection Protocol**	**Code 3 Collection Protocol**	**Code 4 Collection Protocol**	**Code 5 Collection Protocol**
Document/Record/Report (all)					
1. Assign Field Number	×	×	×	×	×
2. Level A Data Use the National Stranding Form	×	×	×	×	×
Morphometrics	Obtain as many measurements as feasible considering human and animal safety. Total length is the priority. Use Cetacean and Pinniped Data Record.	Obtain as many measurements as possible. Use Cetacean and Pinniped Data Record. Tooth count	Obtain as many measurements as possible. Use Cetacean and Pinniped Data Record. Tooth count	Obtain as many measurements as possible. Use the Cetacean and Pinniped Data Record. Tooth count	Total length. Sex (?) Tooth and/or socket count
Sample Collection (ID verification/archive) (#1 for common; #1–3 for rare)					
1. Photographs Suggested Photographs	Whole animal, left and right sides Dorsal fin, left and right sides Close-up of the head Lesions, Abrasions, Net marks, Flukes	**Suggested Photographs (*close-up)** <u>External</u> Whole animal, left and right sides Dorsal fin, left and right sides Head, jaw, mouth* Lesions, Abrasions Ventral surface, genitalia	**Suggested Photographs (*close-up)** <u>External</u> Whole animal, left and right sides Dorsal fin, left and right sides Head, jaw, mouth* Lesions, Abrasions, Ventral surface, genitalia	**Suggested Photographs (*close-up)** <u>External</u> Whole animal, left and right sides Dorsal fin, left and right sides Head, jaw, mouth* Lesions, Abrasions, Ventral surface, genitalia	<u>External</u> Whole animal

	Code 1 Collection Protocol	Code 2 Collection Protocol	Code 3 Collection Protocol	Code 4 Collection Protocol	Code 5 Collection Protocol
		Flukes Internal Thoracic cavity Abdominal cavity Organs, if unusual Parasites * Lesions * Fetus Circumstantial (document HI) Net marks Foreign objects Possible (or reported) trauma	Flukes Internal Thoracic cavity Abdominal cavity Organs, if unusual Parasites * Lesions * Fetus Circumstantial (document HI) Net marks Foreign objects Possible (or reported) trauma	Flukes Internal Thoracic cavity Abdominal cavity Organs, if unusual Parasites * Lesions * Fetus Circumstantial (document HI) Net marks Foreign objects Possible (or reported) trauma	
2. Life History Samples		Stomach and contents, with ends tied off at the esophagus and small intestine—frozen in plastic Large intestine with fecal (seals) for otoliths—frozen in plastic Whole head, lower mandible or six teeth taken from the center of the lower mandible.	Stomach and contents, with ends tied off at the esophagus and small intestine—frozen in plastic Large intestine with fecal (seals) for otoliths—frozen in plastic Whole head, lower mandible or six teeth taken from the center of the lower mandible.	Stomach and contents, with ends tied off at the esophagus and small intestine—frozen in plastic Whole head, lower mandible, or six teeth taken from the center of the lower mandible. At least two unseparated vertebrae (include intervertebral disk, from cetaceans)	Whole head or skull, or at least jawbone with teeth. At least two unseparated vertebrae (include intervertebral disk, from cetaceans) ***Entire skeleton if rare species.***

	Code 1 Collection Protocol	Code 2 Collection Protocol	Code 3 Collection Protocol	Code 4 Collection Protocol	Code 5 Collection Protocol
		At least two unseparated vertebrae (include intervertebral disk, from cetaceans) **<u>*Entire skeleton if rare species.*</u>** **Reproductive Systems** Gonad measurements /slice of testis preserved in 10% neutral buffered formalin. Whole ovaries (preserved in 10% NBF)	At least two unseparated vertebrae (include intervertebral disk, from cetaceans) **<u>*Entire skeleton if rare species.*</u>** **Reproductive Systems** Gonad measurements /slice of testis preserved in 10% neutral buffered formalin. Whole ovaries (preserved in 10% NBF)	**<u>*Entire skeleton if rare species.*</u>**	
3. Genetic Samples	1. Sloughed skin 2. Buffy coat	"tissue" or bone Skin sample, 1" × 1" minimum. Preserved in DMSO, if possible. If not, frozen.	"tissue" or bone Skin sample, 1" × 1" minimum. Preserved in DMSO, if possible. If not, frozen.	"tissue" or bone Skin sample, 1" × 1" minimum. Preserved in DMSO, if possible. If not, frozen.	"tissue" or bone (frozen or in DMSO)
Sample Collection (health and/or cause of death)					
1. Parasites		Preserved in ETOH or 70% isopropyl (or NBformalin as substitute if neither is available)	Preserved in ETOH or 70% isopropyl (or NBformalin as substitute if neither is available)		

	Code 1 Collection Protocol	Code 2 Collection Protocol	Code 3 Collection Protocol	Code 4 Collection Protocol	Code 5 Collection Protocol
2. Histopathology		Only for special cases (i.e., if unusual species, human interaction, mass stranding, unusual mortality event, or unusual lesion present) Collect samples from all major organs and from any other tissues with lesions Lymph node samples should be labeled with collection site. *See AFIP histopathology checklist.	Early Code 3s Only for special cases (i.e., if unusual species, human interaction, mass stranding, unusual mortality event, or unusual lesion present) Collect samples from all major organs and from any other tissues with lesions Lymph node samples should be labeled with collection site. *See AFIP histopathology checklist.		
3 Virus isolation		Frozen in plastic, golf ball-to soft ball–sized sample if virus is suspected • Lung (×4) • Lung-associated lymph node (×2) • Spleen (×2) • Thymus (×2) • Brain (×2)	Frozen in plastic, golf ball–to soft ball–sized sample if virus is suspected • Lung (×4) • Lung-associated lymph node (×2) • Spleen (×2) • Thymus (×2) • Brain (×2)		

	Code 1 Collection Protocol	Code 2 Collection Protocol	Code 3 Collection Protocol	Code 4 Collection Protocol	Code 5 Collection Protocol
Preserved in 10% Neutral Buffered Formalin					
1. Toxicology (general and/or chemistry)		(biotoxi-cology) (call reference lab) Organics—50 100 g samples (baseball sized), frozen in foil (shiny side down), then wrapped in plastic. Liver, kidney, blubber, muscle Metals—same size, same tissue frozen in plastic—or hair (without skin 2 g)	Organics—50 100 g samples (baseball sized), frozen in foil (shiny side down), then wrapped in plastic. Liver, kidney, blubber, muscle Metals—same size, same tissue frozen in plastic—or hair (without skin 2 g)		

	Code 1 Collection Protocol	Code 2 Collection Protocol	Code 3 Collection Protocol	Code 4 Collection Protocol	Code 5 Collection Protocol
2. Bacteriology		Collect samples into sterile containers or transport media; transport to lab (check with lab for specific procedures for different organisms) (<24 hrs at room temperature; >24 hrs refrigerated) External openings (mouth, nose, genital) lesions, tissue surface associated with exudates discharge)			
3. Tissue Bank		Contact NIST for • Cetaceans with known time of death (24 hrs or less) • Mass strandings			
Clinical Samples (hematology and chemistry)					
1. Serology serum collected for detection of antibodies for:	•Morbilli-virus • Brucella • Herpes				

	Code 1 Collection Protocol	Code 2 Collection Protocol	Code 3 Collection Protocol	Code 4 Collection Protocol	Code 5 Collection Protocol
2. Serum Bank—save approx 1 cc serum frozen	Buffy coat (frozen white blood cells washed from green or blue top tubes—not EDTA)				

The following is a guide to help organize, prioritize, and understand procedures used to collect samples and specimens.

Code 2: Dead, fresh, intact. No bloating; as if just died. From time of death until just after rigor. No noticeable signs of autolysis.

I. Assign Field Number

II. Level A Data—

Use the National Stranding Form. See Chapter 1 for a description of Level A data

III. Morphometrics

Morphometrics provide basic biological and historical information. Obtain as many measurements as possible. Measurements are taken following the morphometric data sheet in Appendix 2-C. The procedure is straightforward, requiring one or two persons with a tape measure and, ideally, a third person to record. The measurements should be supplemented with photographic documentation. All measurements can be valuable, but standard length is consistently useful. Except for girth and other specified dimensions, **measurements are always taken in a straight line from point to point** and should not follow the contours of the animal. **Standard length** is the straight line distance from the tip of the snout (or the melon, if more anterior) to the tip of the tail or notch of the flukes. Girth measurements are useful only when there is no evidence of bloating. The girth of large whales is recorded as two times the measured distance between the mid-ventral and mid-dorsal points on one side of the body. Measurements or

weights that are estimated must be clearly indicated as such on the data sheet.

Count the number of teeth in the mouth of the carcass and record the tooth count on the data sheet.

Blubber measurements and thickness (does not include skin) are measured from a perfectly perpendicular cut; distorting the tissue distorts the results. The measurements are taken from a number of specific locations on the carcass, and multiple measurements are taken because blubber thickness varies with body region (See Appendix 2-E, blubber thickness data sheet).

IV. Sample Collection (ID verification/archive)

A. Photographs

Photographs are critical to documenting the collection of samples, Level A data, and the necropsy.

Suggested photographs with a marker of animal and ruler (*close-up):

External	Internal
Whole animal, left and right sides	Thoracic cavity
Dorsal fin, left and right sides	Abdominal cavity
Head, jaw, mouth*	Organs, if unusual
Lesions, Abrasions,	Parasites *
Ventral surface, genitalia	Lesions *
Flukes	Fetus

Circumstantial (document HI)
Net marks
Foreign objects
Possible (or reported) trauma

B. Life History Samples

Information on age, genetics, reproductive status, and feeding habits is vital to understanding the general biology of the species, developing demographic models, identifying discrete stocks, and planning conservation and management strategies (Geraci and Lounsbury 2005). Certain life-history information makes interpretation of pathologic and toxicological data more meaningful and, sometimes, essential. In general, biological data are additive; the more we can obtain on a given specimen, the more meaningful each element becomes (Geraci and Lounsbury 2005).

1. Teeth for Age Determination

In the field, teeth (or sections of skull or mandible) can be placed temporarily on ice, frozen, or packed in salt to retard tissue decomposition. Take the lower mandible or six teeth from the center of the lower mandible. In the laboratory, teeth can be extracted, cleaned in an enzyme preparation such as trypsin, labeled, and stored in 70% ethanol. It is wise to avoid drying, prolonged boiling, or use of solutions containing glycerin.

2. Other Bone Specimens

Bone specimens for aging studies can be frozen, preserved in 10% formalin or alcohol, or cleaned and dried without affecting the clarity of periosteal layers. The sample should include a bone end incorporating a cartilaginous growth plate or a scar of an old growth plate. Take at least two unseparated vertebrae (include intervertebral disk, from cetaceans) ***Entire skeleton if rare species.***

3. Genetic Samples

Skin and liver are the most commonly collected tissues for genetic analysis. White blood cells, muscle, gonads, teeth, and bone have also been collected from carcasses. Only a small sample is needed for genetic analysis, a 0.5 cm^3 or 1"×1" minimum sample (1 ml of whole blood, whole teeth, or a piece of bone). Preserve soft tissue in 5% to 20% dimethylsulfoxide (DMSO) in saturated salt solution at 1 volume to 10 to 20 volumes of preservative. The solution containing the tissue sample should then be frozen for long-term storage (Rowles *et al.* 2001). Soft tissues also may be frozen or preserved in 70% to 85% ethanol (one part sample to two to three parts preservative).

4. Stomach Contents

Collect contents from each stomach by carefully and gently flushing the contents into separate

labeled containers (plastic bag or bucket) and freeze or preserve in 70% ethanol (neutral buffered formalin will dissolve otoliths). Contents may include recognizable prey species, macerated flesh, skeletal fragments, otoliths—some so small as to be unapparent to the naked eye—numerous parasites, and a variable amount of fluid. Alternatively, after sample collection for histology, the stomach and its contents, with ends tied off at the esophagus and small intestine, can be collected as a whole and frozen in plastic. Another approach is to weigh full stomach, sieve stomach contents, dry otoliths and fish bones or spines, and place beaks and fresh remains in 70% ethanol (Rommel *et al.* 2001). There are three basic analysis: identification of prey, identification of parasites, and toxicology (biotoxin or anthropogenic). Because each type can affect the others, these analyses should be carefully coordinated. Subsamples for toxicology or biotoxins are collected from the stomach contents of fresh carcasses when they are first opened, and the subsamples are frozen for later evaluation.

Also the large intestine with fecal sample should be collected and frozen in plastic

5. Reproductive Systems

Remove, weigh, and preserve (in 10% neutral buffered formalin [NBF]) the entire reproductive tract and organs. Collect both (whole) ovaries (one uniquely identified) in glass jar with 10% NBF; if entire tract is collected, use a sealable plastic bucket to ensure ample formalin; label one horn and collect a sample of mammary tissue. Measure, weigh if possible, and preserve entire testes; repeat for epididymis. Cross-section (0.5 cm thick) from the center portion of left testis and section of vas or epididymis. Slice large testes to ensure proper fixation. Measure, weigh, and determine the sex of the fetus.

6. Core Temperatures

Postmortem temperatures (e.g., epaxial—just anterior to the dorsal fin—muscle, liver) give some indication of time since death. This is an undocumented aspect that might provide some insights, particularly if regional heterothermy plays a role in the biology of these animals (NOTE: carcass temperature roughly relates to the condition of the animal relative to environmental temperature. Temperature will go down [become uniform] then rise as animal decomposes.) To be complete, it is good to collect core temperature readings as it can help fix time of death relative to time of recovery.[2]

Temperature should be taken in deep epaxial muscle, mid-lumbar, or halfway between dorsal fin and blowhole. Drive deep temperature probe through blubber at 45º between transverse process and neural spine to the depth of the vertebral centrum. (Long stainless puncture probes and hand held readouts are available from Omega Engineering).

C. Sample Collection (health/cause of death)

1. Parasites

Collection and examination of parasites are important from both health and life-history perspectives. Collect and preserve samples of loose parasites, lifting them carefully with forceps or fine needles. Parasites should be collected intact—especially to preserve the head and mouth of the parasite—and fresh, and any associated lesions should be collected in 10% neutral buffered formalin, in 70% ethanol (preferable), or 70% isopropyl (preferable). ***Epibiota***—If you see any parasites or other epibiota collect as many as possible into a jar of fresh water, noting where they

2 NMFS observer program (in the NE anyway) has been taking temps for some years from a standard location—epaxial muscle just anterior to dorsal fin—see http://www.nefsc.noaa.gov/fsb/ Biological Sampling Manual—Marine Mammal section p. 44.

were found. If they are embedded, cut the surrounding tissue out. Put any associated lesions in 10% NBF.

2. **Histopathology**

Histology samples are most useful when collected from fresh (Code 2) carcasses; however, in many cases, tissues from moderately decomposed carcasses can provide invaluable insights into primary and even secondary pathological processes. Tissues for histopathology can vary significantly in form, texture, and location. Perhaps the most important aspects of histological sampling procedure are sampling location, proper and/or adequate labeling, and proper handling of the tissues. Tissues with important yet delicate mucosal surfaces such as the gastrointestinal tract and respiratory tree, should be handled or manipulated as little as possible, making sure that the mucosal surfaces are not scraped, rubbed, or palpated.

Care should be taken when using forceps to ensure that regions of the sample remain untouched or are not manipulated. Care should be taken with delicate organs such as the brain and lungs, to avoid squeezing or applying pressure to the tissue.

Collect samples from all major organs and from any other tissues with lesions; all tissue types, see histology check list (Appendix 2-K). Samples can be as long as desired, but must be thin enough for the formalin to penetrate and to properly fix the tissue (maximum thickness 0.5 cm), no thicker than a large wedding band. If larger tissues are collected, bread-loaf the organ by making parallel slices 0.5 cm apart[3]. Place the sample in 10% NBF, allowing for a tissue-to-formalin ratio of 1:10. Samples include all major organs, appropriate lesions, all major lymph nodes—for tissues that are not unique (e.g., lymph nodes, right and left lungs, and adrenals) and for those which it is important to

3Formalin penetrates about 1mm per hour (e.g., 0.5 cm per 5 hrs). If conditions limit the amount of formalin available, collect thinner pieces.

distinguish—identifiers such as laundry tags, spaghetti tags, or histology cassettes clipped on to an edge of the tissue should be used.

Lymph node samples should be labeled with collection site (see Appendix 2-K). Whole hearts should be sectioned (bread-loafed) according to the Kogia heart dissection protocol (e.g., Bossart *et al*.). After sampling for virology and bacteriology, whole brains should be placed in 10% NBF for at least six days in order to harden prior to sectioning. Once firm, brains should be sectioned (bread-loafed) and placed back into NBF for further fixation.

Whenever possible, livers should be sampled in multiple locations. A section should be collected from the margin as well as through the center of one of the lobes. Liver sections should include part of the hepatic portal and caval circulations. Lungs should also be sampled along the margins as well as near the center. For examination of mucosal surfaces, center samples should include primary and/or secondary airways.

Immunohistochemistry. Immunohistochemistry is a means of detecting cellular components and cell products; determining cell origin; and detecting pathogens. It can be done with formalin-fixed tissues, cytological specimens (immunocyto-histochemistry) and, less often, with frozen specimens specially embedded and preserved. Unfortunately, long-term storage in formalin may affect the quality of DNA and other cell components. It is best to paraffin-embed tissues within six to 14 days. If unable to meet this timeline, once tissues are fixed in formalin (two to three days depending on tissue thickness; four to six days for the brain), they can be transferred to ethanol for longer-term storage.

Fatty or adipose-rich tissues such as acoustic fats from the jaw or melon should be examined for gross signs of acute and/or chronic trauma, and should be sampled for histopathology and lipid constituent analysis. Fat samples for lipid/FA

analysis should be frozen. Histopathology samples of adipose tissues should be placed in 10% NBF.

3. Virus Isolation

A systematic approach to disease and viral detection is needed as part of a baseline approach to investigate the prevalence of disease and viruses in marine mammals. Most viruses are fragile and have a short life span in decomposing tissue. Viruses that persist long enough to be harvested and identified, however, are generally responsible for some infectious process. Cultures, polymerase chain reaction (PCR), serology, and electron microscopic evaluations are used for the detection of viruses.

Samples for viral culture should be collected as aseptically as possible. Tissue specimens or swabs are placed in viral transport media or in 1 to 2 ml of physiological saline with 5% bovine serum albumin containing approximately 50 μg/ml of gentamicin, and shipped immediately. If immediate shipment is not possible, tissues may be frozen at –70 to –80°C or colder for later shipment. PCR or nucleic acid hybridization can be performed on either frozen or fixed samples, although fresh-frozen samples are preferred (–70 to –80°C) The samples should be golf-ball to softball–sized sample if virus is suspected. Following are the tissues to be collected:

a. Lung (×4)
b. Lung-associated lymph node (×2)
c. Spleen(×2)
d. Thymus(×2)
e. Brain(×2)

4. Contaminants and Biotoxins (Toxicology and biotoxicology)

Marine mammals are the potential ultimate repository for oceanic contaminants passed through the food chain. Cetaceans may reveal the influence of contaminants and toxins on health. To be effective, the collection and preparation of

contaminant and biotoxin specimens must be impeccable and the samples matched with reliable life-history information. Deterioration of tissues after death leads to change in contaminant load, with the extent of change depending on the tissue and the analyte involved. Samples for the National Biomonitoring Specimen Bank must be taken within six hours of documented death.

A rigorous sampling protocol has been developed by the National Biomonitoring Specimen Bank (U.S. National Institute of Standards and Technology) and the Alaska Marine Mammal Tissue Project.[4] Following is the sample collection for contaminant and biotoxin analyses for non-NIST sample:

a. Blubber and other body fats concentrate lipid-soluble organic contaminants. Blubber is always accessible and may be the only practical tissue to collect. Samples (300–400 g, about 10 cm square) include the full thickness of the layer, without skin or muscle. Standardization of sampling sites is suggested for more accurate cross-comparison purposes. Two sites are recommended for cetaceans: one about 10 cm caudal to the blowhole and the other directly below the dorsal fin on the mid-lateral line.

b. Liver accumulates all known organic and inorganic contaminants and some biotoxins. Collect the entire liver from small carcasses. For

4 The NIST National Biomonitoring Specimen Bank protocol includes a target species list and specific criteria for each animal that must be met before samples can be collected for banking. Persons collecting samples specifically for the NMMTB must first be approved and trained by NIST personnel. The National Biomonitoring Specimen Bank protocol requires that the carcass be opened with clean stainless-steel instruments and that personnel wear talcum-powder–free vinyl gloves. Samples should be taken with a clean stainless steel or titanium knife (washed, rinsed with high-purity water followed by ethanol, and air dried). Each sample should then be trimmed using a clean titanium knife, washed with high-purity water, and cut into subsamples of suitable size for storage. Each subsample is placed in a pre-weighed Teflon® jar with a Teflon-lined lid; the jar is labeled and weighed again. Containers are then refrigerated for immediate analysis or frozen at –70 ° C or in liquid nitrogen for shipping or storage. At every stage of the procedure, care is taken to avoid chemical contamination of the tissues. All chemicals must be pesticide-free grade.

large ones, slice 300–400 g samples from the distal end of each side of the medial indentation.

c. Kidneys concentrate metals. Take both kidneys from small animals; from cetaceans, take the entire left kidney or a 300–400 g slice from the caudal end.

d. Brain and muscle are of questionable value. Brain decomposes quickly, and its removal from small animals requires skill and is often difficult. It is possible to measure changes in acetylcholinesterase resulting from exposure to short-lived pesticides and herbicides in brain tissue; for this purpose, it may be worthwhile to collect (and immediately freeze) the brain from small Code 2 animals. Indicate location in the brain from which any sample was taken.

e. Liver and blubber samples of about 100 g (about one-quarter pound or hamburger–sized) and lung (cranial pole), urine (if not available, then kidney), contents from stomachs or duodenal ampulla, feces, and blood are taken for biotoxin analysis such as Harmful Algal Bloom (HAB) Toxicology for ELISA (thumb-size samples) placed in whirl-packs and frozen.

f. Organics samples—collect 50 100 g samples (baseball-sized) using a clean (solvent washed, if possible) stainless-steel knife. Tissues are collected in clean glass jars, Teflon bags or jars, or placed in foil (shiny side down), then in plastic. The samples should be stored at temperatures less than –80°C.

g. Metals samples—collect the same size, same tissue, and freeze in plastic.

5. Bacteriology

Cetaceans harbor a variety of microorganisms, some of which are pathogenic. Even under ideal conditions, it is often difficult to associate bacteria isolated from a carcass with specific lesions.

Bacteria that are part of the normal flora may proliferate rapidly after death and may interfere with successful isolation of an offending pathogen (Geraci and Lounsbury 2005). Any study on normal flora requires that only the freshest, uncontaminated specimens be used. Bacteria associated with active infectious processes tend to endure longer in viable concentrations, and certain species may be isolated from more deteriorated carcasses, even frozen stored specimens (Geraci and Lounsbury 2005).

Sample selection for bacteriology is determined largely by the nature of the gross pathologic findings. Samples should be taken aseptically, first from external surfaces and then from body cavities and internal organs as soon as they are exposed. The core of a fleshy or hollow organ or fluid-filled lesion (e.g., abscess) is sampled by inserting a swab in an incision made through the sterile surface, prepared by searing or disinfected with 10% formalin or 70% ethanol and allowed to dry (Geraci and Lounsbury 2005). Fluid samples from a cavity are taken by aspiration (through a sterile surface) before or moments after opening (Geraci and Lounsbury 2005). *Note:* Bacteria within effusions or exudates are more diluted and often dead; therefore, sampling the associated surface often is more rewarding. Tissues destined for laboratory sampling (sear-sampling, cultures, impression smears) should be large enough (about 6 × 6 × 6 cm) to allow for trimming and must have one capsular or serosal surface intact (Geraci and Lounsbury 2005). Take separate samples and freeze additional tissues for later use.

Large lesions are sampled from two or three distinct regions; collect any lymph nodes in the vicinity. If intestinal infection is suspected, tie off a 10 cm loop and place in a sterile container (Geraci and Lounsbury 2005). Place swabs in the appropriate transport medium (generally available from diagnostic laboratories). Aspirated pus from abscesses and other lesions where anaerobic

organisms are suspected should be transported in anaerobic vessels (Geraci and Lounsbury 2005). *Note:* Bacteria within effusions or exudates are more diluted and often dead; therefore, sampling the associated surface often is more rewarding. Package tissues in sealed, sterile, leak-proof bags or jars (Geraci and Lounsbury 2005). Label, cool, and transport to the laboratory immediately. Avoid freezing samples for bacteriology; however, if long delays are unavoidable, freezing at –70° C is preferable to decomposition. Record conditions of collection and storage.

Fecal samples that cannot be cultured immediately can be placed in a stool preservative (equal volumes of 0.033 M phosphate buffer and glycerol) to prevent changes in pH.

Material taken for examination of mycotic agents (usually from the skin) is obtained by scraping with a scalpel blade and/or by removing a number of hairs from the affected area. The samples should be refrigerated until they can be inoculated onto a suitable growth medium (Geraci and Lounsbury 2005).

6. Entire head—If not scanning the head, remove the head intact, examine the oral cavity for abnormalities, and hold chilled.

7. Eyes—Collect aqueous humor, collect eyes entire (sans fluid from one). Remove the anterior segments and the globes, and immerse in either a modified Davidson's solution (available commercially) or in phosphate-buffered formalin (3%, pH 7.4) for 24–48 hours, and then transfer to formalin or alcohol (longer fixation in Davidson's solution will reverse the effect and harden the eye).

8. Immunology—Tissues taken for microbiology and virology (described previously) can be use to assess immune system function. Samples swabs should be taken from the blowhole, anus, U/G opening, eye, and mouth are in descending order of importance and placed in viral or bacterial transport media. These swabs can be stored frozen at –70° to –80°C. Samples should be taken of the liver, spleen, lymph nodes, kidneys, and

abdominal cavity when first opened and should be approximately 6 cm^3. Place tissue sample in a whirl-pack bag and store frozen at –70° to –80°C until shipped. Serum should also be collected, when possible, for evaluation of serum antibody titers.

9. Cerebro-spinal Fluid (CSF)—CSF may be collected by two methods: dorsal or ventral once the pluck has been removed. A sterile 16- to 20-gauge needle on a 3 to 6 ml syringe is inserted into the vertebral canal at the junction between the head (occipital condyles) and cervical vertebrae. When the syringe is inserted, a "pop" may be felt at the dura is breached. CSF fluid can be utilized for serology (morbillivirus), chemistry (alkaline phosphatase, creatinine, and total protein), and cytologic examination. If cytology and clinical chemistry cannot be completed within a few hours, freeze a portion (at least 1.0 ml) at -–80°C and place a small aliquot (0.5 to 1.0 ml) of CSF with a few drops of formalin. CSF degrades quickly, so freezing some and putting some with formalin may help preserve cells if they can't be analyzed within a few hours. Similarly samples of CSF should be taken for alkaline phosphatase, creatinine, total protein analysis.

10. Skeleton—Flense, dry, and store (frozen if practical) for final osteoprep.

11. Tympano-periotic Bones—See Chapter 3.

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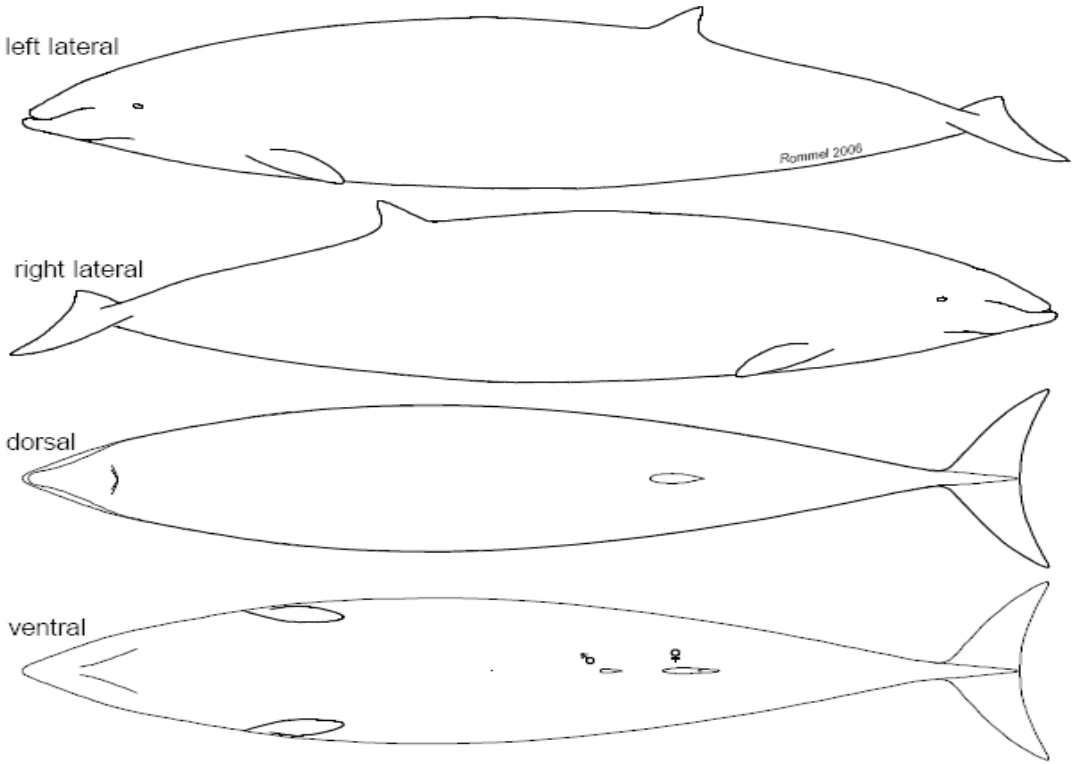
Appendix 2-A
External Lesions and Non-acoustic Human Interaction Data Sheet

BEAKED WHALE EXTERNAL LESIONS & NON-ACOUSTIC HI

Field # _____ Date_____ Observer_____



Left Side Observations:

_____
_____
_____
_____
_____

Right Side Observations:

_____
_____
_____
_____
_____

Dorsal Observations:

_____
_____
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_____

Ventral Observations:

_____
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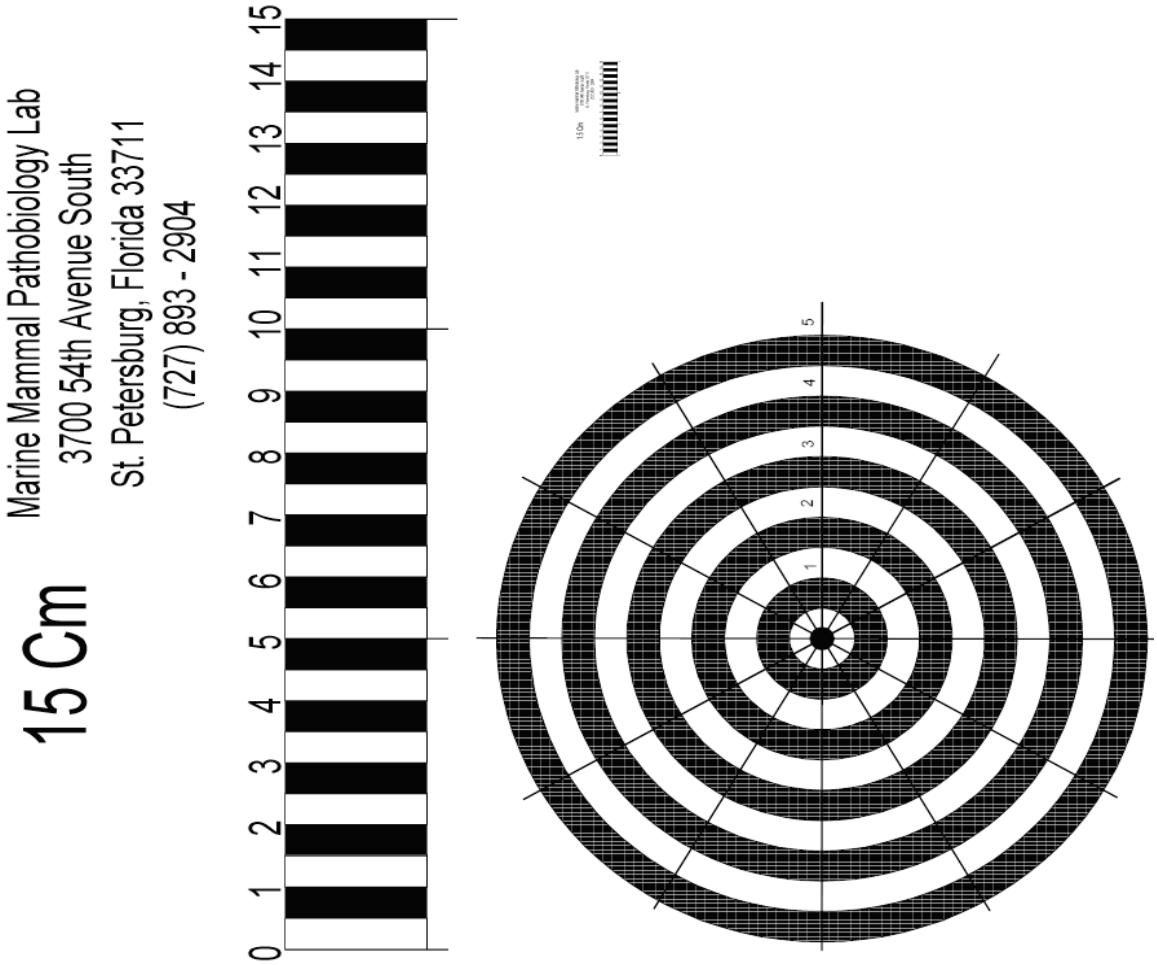
_____

Appendix 2-B
Paper Scale Templates



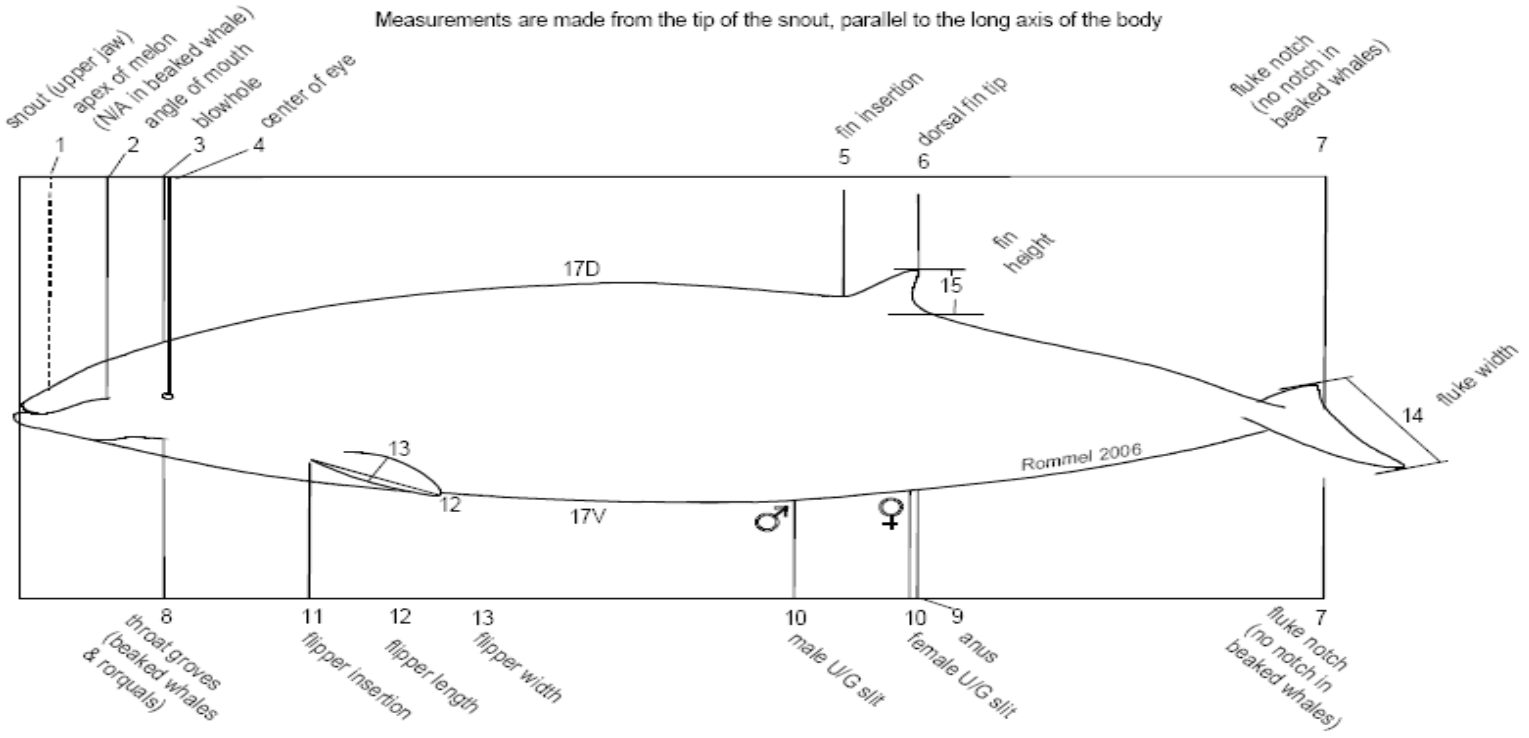
Periodically check scale for copying errors

Appendix 2-C
Necropsy Morphometrics Data Sheet

BEAKED WHALE MORPHOMETRICS

Field # _____ Date_____ Observer_____

Measurements are made from the tip of the snout, parallel to the long axis of the body



1. Snout to melon _____
2. Snout to angle of mouth _____
3. Snout to blow hole _____
4. Snout to center of eye _____
5. Snout to ant. insertion of fin _____
6. Snout to fin tip _____
7. Snout to fluke tip (notch if present) _____
8. Snout to caudal end of ventral grooves _____
9. Snout to center of anus _____
10. Snout to center of genital aperture _____
11. Snout to ant. insertion of flipper _____
12. Flipper length _____
13. Flipper width _____
14. Fluke width _____
15. Fin height _____

Appendix 2-D
Odotocete Necropsy Report

ODONTOCETE NECROPSY REPORT

SPECIES _____ FIELD NUMBER _____

LOCATION _____LAT_____/LONG_____

DATE _____ GENDER _____ TOTAL LENGTH _____

HISTORY:

EXTERNAL EXAM:

INTERNAL EXAM:

Field Number _____

Reminder for lesion description: general guidelines.

All descriptions should include at least the information listed below. Lesions should be described AFTER examining the cut surface. Lesions should be noninterpretative. The description should be thought of as if conveyed to a blind person (the histologist is often blind to the gross exam).

- Lesion type (e.g., laceration, mass, ulcer, effusion).
- Organ affected and the lesion's potential communication with adjacent structures
- Precise location within the organ (i.e., serosa/parenchyma; superficial/deep/full-thickness; cranial/caudal/middle; dorsal/ventral/lateral; left/right)
- Distribution within the organ (i.e., focal, multifocal, multifocal to coalescing, diffuse).
- Approximate total percentage of entire organ affected (<5%, 10%, 25%, 50%, 75%, 99%)
- Amount (mL) or size range (three dimensions, in cm) of lesion
- Modifiers: color, heterogenous/homogenous of appearance, texture, etc.

For a mass, include the following: encapsulated/unencapsulated, whether pushes or infiltrates adjacent tissue, whether central necrosis is present, and the location of the largest mass

For description of a fracture include: A) bone affected; B) open/closed to body surface; C) communicates with another structure; D) type of fracture (simple transverse or oblique; spiral; comminuted; greenstick, etc); E) whether fractured ends are aligned or displaced; F) whether fracture margins are sharp/smooth and have callus formation; G) presence of foreign material/purulent material/osseous sequestrum; H) presence of blood/edema/fibrosis/purulent material in surrounding soft tissue or structure with which it communicates. All of this information helps determine the type of trauma sustained, whether the fracture contributed to morbidity/mortality, possible sequelae, the potential for healing, and whether the fracture occurred antemortem.

Tissue sampling for histology:
When a lesion is present, take a sample of the lesion (including the leading edge), AND take a sample that does not appear to have the lesion. Should the lesion have more than one appearance, take a sample from each different appearing region. A sample from a bone lesion should extend from one cortex through the medulla and to the opposite cortex.

When possible, samples taken for ancillary diagnostic tests, such as microbiology, should be taken adjacent to that section submitted for histology.

a. Digestive System

Mouth:

Tongue:

Esophagus:

Fore Stomach:

Field Number _____

Main Stomach:

Pyloric Chambers:

Duodenum:

Intestine:

Colon:

Anus:

Describe content found in GI tract per each segment:

Describe parasites found in GI tract:

Liver:

Pancreas:

Mesenteric lymph nodes:

b. Blubber:

Field Number _____

c. Musculoskeleton

Axial Muscle:

d. Axial Skeleton

Thoracic Vertebrae:

Lumbar Vertebrae:

Caudal Vertebrae:

Ribs and Sternum:

Flippers:

Flukes:

Cranium:

Field Number _____

e. Circulatory System

Heart:

Great Vessels:

Cranial Circulation:

Spinal Circulation:

f. Respiratory System:

Blowholes:

Nares:

Larynx:

Trachea:

Lungs:

Bronchi:

Alveoli:

Field Number _____

g. Urinary System:

Kidneys:

Ureters:

Bladder:

Urethra:

h. Endocrine and Hemolymyphatic Systems:

Thymus:

Thyroid:

Adrenals:

Pituitary:

Spleen:

Lymph Nodes:

Field Number _____

i. Reproductive System

FEMALE
Ovaries:

Uterine Horns:

Uterus:

Cervix:

Vagina:

Mammaries:

MALE
Testes:

Epididymis:

Vas Deferens:

Prostate:

Seminal Vesicles:

Penis:

Field Number _____

j. Central Nervous System

Brain:

Spinal Cord:

k. Sensory Organs

Eyes:

Ears:

l. Conclusions:

m. Cause of Death from Field Determination:

n. Contributory Conditions from Field Determination:

Field Number _____

This report was generated by:

Field Number _____

o. Additional Observations:

1. Evaluation of Human Interaction

Field Number _____ Species_____

Date_____ Location_____

Code 1 2 3 4 5

External Examination

A. Body Condition: Emaciated specimens often exhibit sunken epaxial musculature and neck

Emaciated_____ Not Emaciated_____ CBD_____ N/E_____

B. Net or Line Marks: Indicate Y/N/CBD/NE for each area and carefully describe net or line marks:

Head _____ D.Fin_____ L.Flipper_____ R.Flipper_____ Peduncle_____Other__

C. Fishing Gear Present on Animal (Yes) or (No)

D. Gear Retained (Yes) or (No)

E. Penetrating Wounds: Yes____ No____ CBD____ N/E____

F. Mutilations: Body Slit or Mutilated? Yes____ No____ CBD____ N/E____

G. Hemorrhaging / Bruising: Yes____ No____ CBD____ N/E____

Describe extent and area:

_____

_____

Internal Examination

A. Sub-Dermal Hemorrhaging: Yes____ No____ CBD____ N/E____

Describe extent and area:

_____

_____

B. Broken Bones: Yes____ No____ CBD____ N/E____

Describe:

_____

_____

D. Stomach Contents Retained: Yes ____ No____

E. Histopathology Samples Retained: Yes ____ No____

F. Gross Pathology: Yes____ No____ CBD____ N/E____

Describe:

_____

_____

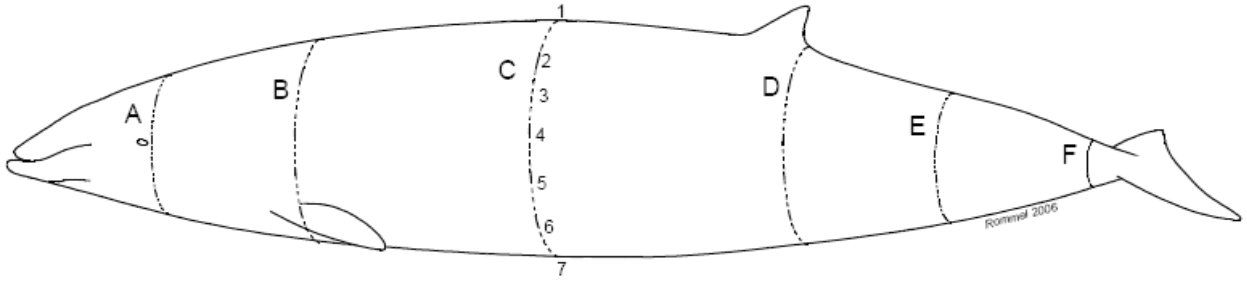
*CBD—Cannot be determined

*N/E—Not examined

Appendix 2-E
Blubber Thickness Data Sheet

BEAKED WHALE BLUBBER THICKNESS

Field # _____ Side Examined _____ Observer _____ Date _____

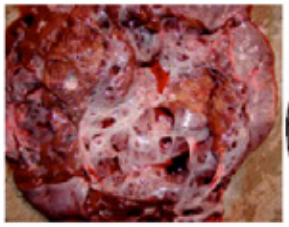


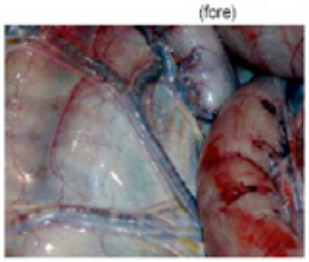
	A Nuchal Crest	B Axilla	C Umbilicus	D Anus	E Mid peduncle	F Fluke insertion
Dorsal						
1	_____	_____	_____	_____	_____	_____
2	_____	_____	_____	_____	_____	_____
3	_____	_____	_____	_____	_____	_____
4	_____	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____	_____
6	_____	_____	_____	_____	_____	_____
7	_____	_____	_____	_____	_____	_____
Ventral						
Circumference with blubber	_____	_____	_____	_____	_____	_____
Circumference w/out blubber	_____	_____	_____	_____	_____	_____

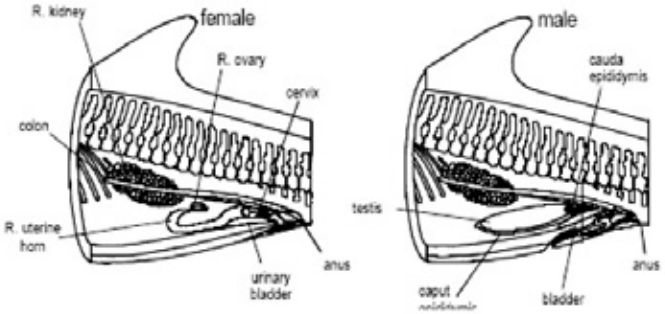
Appendix 2-F
Abdominal Cavity Data Sheet

BEAKED WHALE ABDOMINALCAVITY illustrations of *Tursiops*

Field # _____ Date_____ Observer_____







Liver Observations:

_____
_____
_____
_____
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_____
_____

GI Tract Observations:

_____
_____
_____
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_____
_____
_____

Kidney & Repro Observations:

_____
_____
_____
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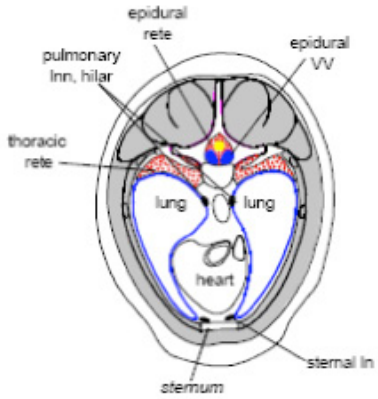
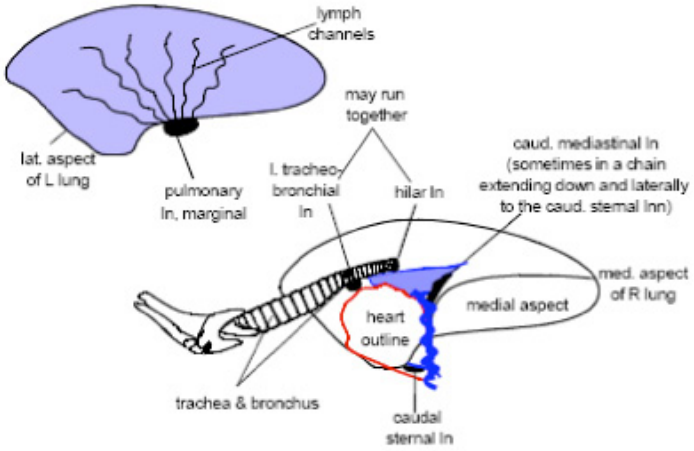
_____
_____
_____

Appendix 2-G
Thoracic Cavity Data Sheet

Field # _____ Date_____ Observer_____





Left Side Observations:

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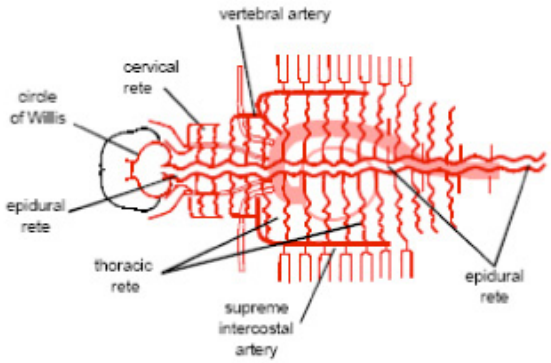
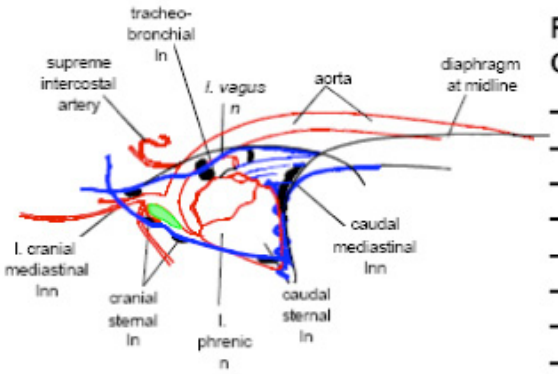
_____



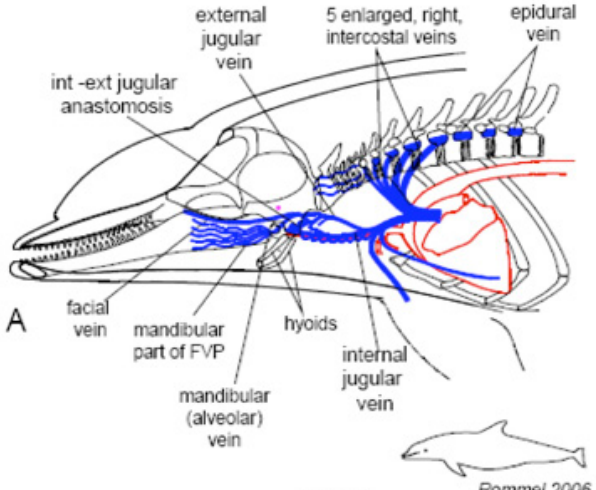


Right Side Observations:

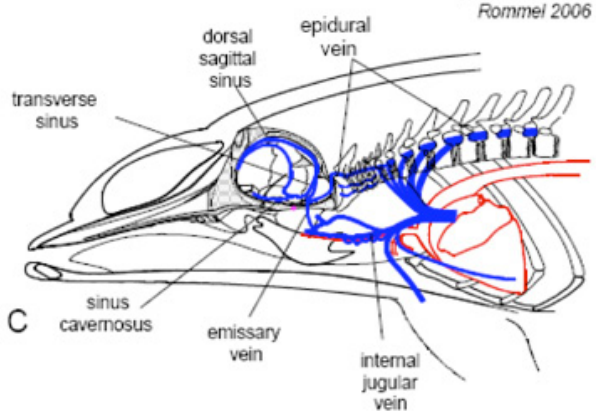
_____
_____
_____
_____
_____
_____
_____
_____

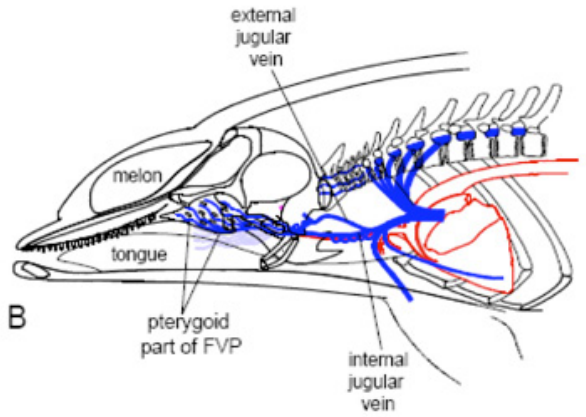
Appendix 2-H
Reference Drawings of Head

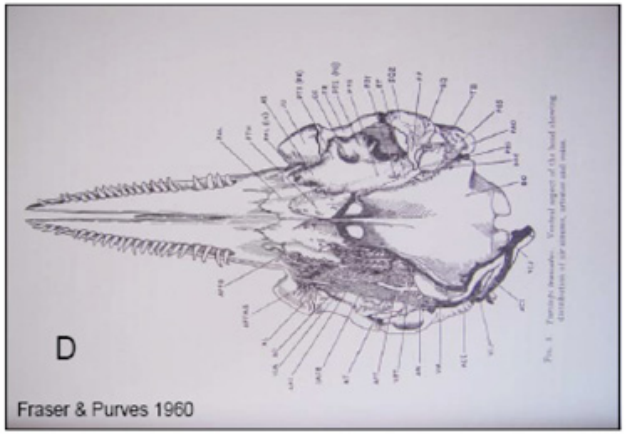
REFERENCE DRAWINGS FOR HEAD from *Tursiops* after Rommel *et al* 2006; mark lesion sites



Rommel 2006



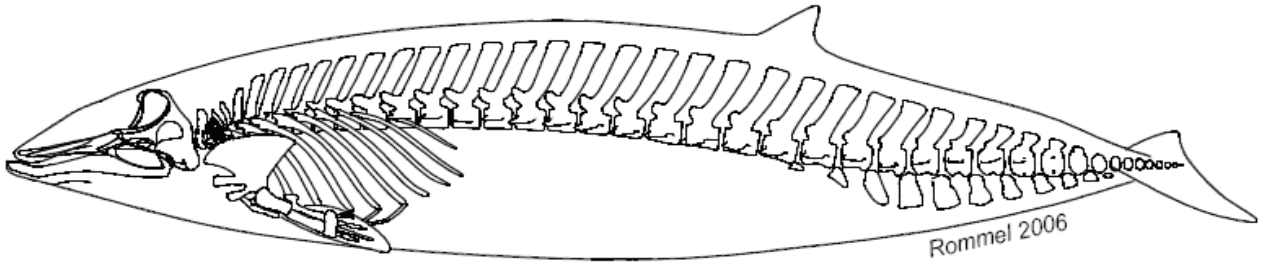




Appendix 2-I
Skeleton Lesions Data Sheet

BEAKED WHALE SKELETON LESIONS

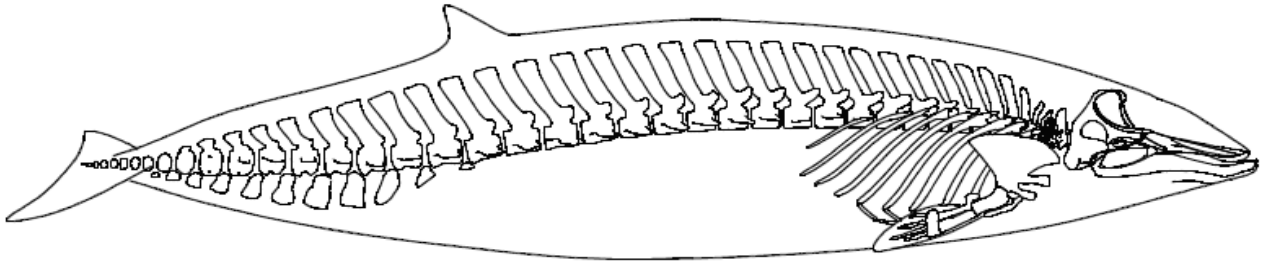
Field # _____ Date_____ Observer_____



Left Side Observations: _____

_____

_____



Right Side Observations: _____

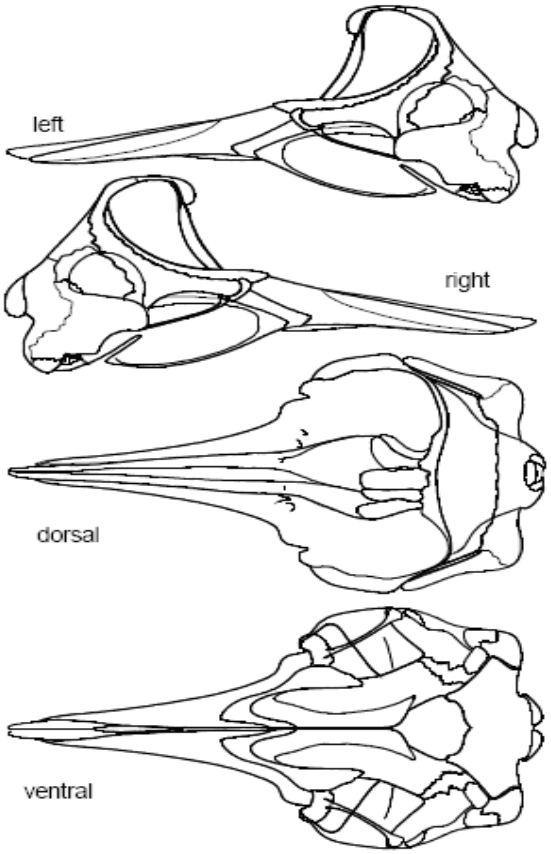
_____

_____

Appendix 2-J
Skull Lesions Data Sheet

BEAKED WHALE SKULL LESIONS

Field # _____ Date_____ Observer_____



Left Side Observations:

_____
_____
_____
_____
_____

Right Side Observations:

_____
_____
_____
_____
_____

Dorsal Observations:

_____
_____
_____
_____
_____

Ventral Observations:

_____
_____
_____
_____
_____

Appendix 2-K
Tissue Collection Check List

A= archive, F=Formalin, C = culture, T = toxicology, FT=fat protocols (signature & stains), TH= thoracic, AB= abdominal, lnn = lymph nodes

Collected Y/N	*Tissue*	*Site*			*A*	*F*	*C*	*T*	*FT*	*Comments*
Head & Neck		**Left**	**Right**							
	Conjuctiva of the Eye (CNJ)					F	C?			culture of subconjunctival region if evident ocular lesions (ulcers, discharge)
	Cerebrospinal fluid (CSF)				A		C			
	Melon					F			FT	
	Larynx					F				
	Eustachian tube					F	C			
	Peribullar plexus					F	C		FT	
	Mandibular fat					F			FT	include vascular tissue
	Fibrovenous plexus (FVP)					F			FT	
	Pterygoid sinus (PTS)					F				collect both medial & lateral walls of cavity (labeled)
	Tonsils w/ pharynx (TNL)				A	F				
	Superficial cervical lnn (SPC)				A	F				
	Larynx (LRX)					F				
	Thymus (THM)					F				
	Thyroid (THR)					F				
	Brain case w / dura @ hiatus (BC@H)					F				When brain is exposed collect bone sample
	CNS									examine both sides of brain, sub-sample for encephalitis/infection, sample 1/2 brain 1/2 frozen @ -80°F -- if CT'd or MRI'd & abnormality detected then section accordingly, if not or if results are negative then sample as protocol
	Cerebrum (CBR)				A	F	C	T	FT	
	Cerebellum (CBL)					F				
	Brainstem (BST)					F				
	Pituitary (PIT)					F				
	Cranial nerves					F	C			swab and collect sample of CNVIII

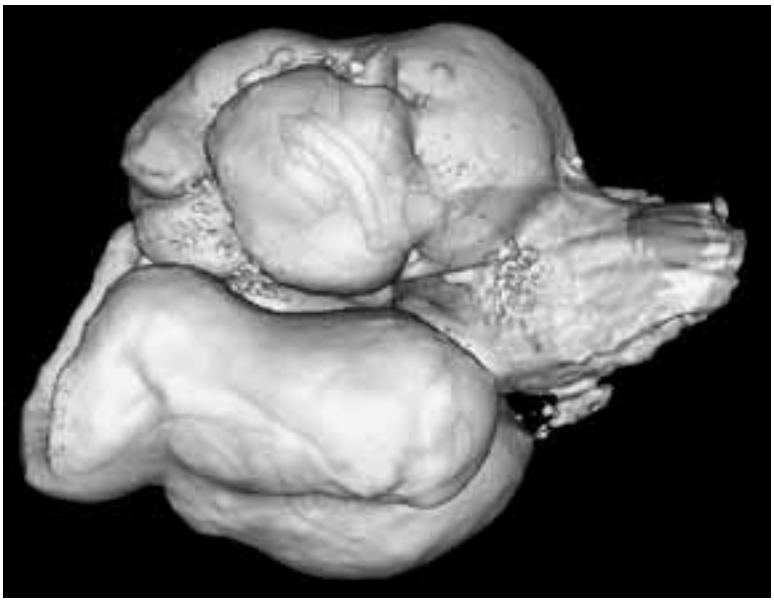
Thorax		*Left*	*Right*							
	Thoracic cavity						C			swab pleura
	Trachea (TRA)					F				
	Lung (LNG)	cranial	cranial		A	F		T	F	
		middle	middle			F				
		caudle	caudle			F				
	Heart	Atria (HTA)				F				use Kogia protocol if applicable
		Ventricles (HTV)				F				
		Valves (HVA)				F	C?			culture if high profile case
	Vascular Structures	Aorta				F				
		Epidural rete			A	F			FT	include spinal cord in histo sample
		Thoracic rete			A	F			FT	
	Tongue	Tip (TGT)	Root (TGR)			F				
	Mediastinal lnn (MDS)					F				
	Tracheobronchial lnn (TRB)				A	F				
	Pulmonary lnn (PLM)	Marginal	Hilar			F				
Abdomen		*Left*	*Right*							
	Abdominal cavity						C			swab peritoneum
	Liver (LVR)	Proximal	Distal			F		T		
	Stomach walls (STM)	1st	2nd	3rd		F				additional chambers in beaked whales
	Stomach contents					A				seal in bag, & and freeze
	Duodenal ampulla					F				
	Intestine	Proximal (PIN)				F				
		Mid (MIN)				F				
		Distal (DIN)				F				

	Feces				A			T		
	Kidneys (KID)				A	F		T	FT	
	Gonads (GND)					F				
	Uterine Horns (UTH)	Mucosa, mid-length on both horns				F				if present, collect scar and adjacent region
	Vas Deferens (VDF)					F				
	Epididymis	Proximal (PED)	Distal (DED)			F				
	Urinary Bladder (BLD)				A	F		T		cranial pole of bladder for histo
	Colic lnn (CLC)									
	Anorectal lnn (ANR)					F				
	Spleen (SPL)				A	F	C	T		
	Thoracic spinal cord epidural rete (TSC)				A	F			FT	sample both spinal cord and rete for histo ~@ mid-thoracic level
	Mesenteric lnn (MES)				A	F				
	Adrenal (ADR)				A	F				
	Pancreas (PAN)					F				
	Mammary (MAM)				A	F				
Other Tissues		***Left***	***Right***			F				
	Diaphragm (DIA)					F				
	Rib marrow				A	F			FT	
	Skin	Lip (LIP)	Urogenital (UGN)	Mediolateral (MLT)	A	F				
	Synovial joints	Occipital condyle (OCC)	glenohumeral joint (GHJ)			F				
		Costo-vertebral joint @ T-2,3	Chevron-vertebral joint			F				

		(CVJ)	@ anus (CHJ@A)							
	Blood				A		C	T		from thoracic vena cava
	Urine				A			T		
	Muscle?									
Other Lymph Nodes		**Left**	**Right**							In general for abnormal lymph node(s) culture node(s), freeze a piece @ -80^{0}F, and send a swab for routine culture. Be sure to label all individual lnn!.
	Axillary (AXL)									
	Ext. Auditory Meatal (EAM)									may be important in trapping FE
	Retropharyngeal (RPH)									
	Sternal (STN)	Cranial	Caudal (L/R)							
	Aortic (ART)	Thoracic	Lumbar							
	Gastric (GTR)	Hepatic	Pancreatic	Gastric						
	Diaphragmatic Abd. (ABD)									
	Iliac (ILC)	Medial	Lateral							
	Head parasites	middle ears								only if extruding from it; 70% ETOH
		pterygoid sinus								70% ETOHl
	Other									

General Rules:
1) If lesion: culture (bactT / Fungal); archive
2) If suspect sepsis: culture liver, spleen, lung, lymph node, kidney, heart valve
3) If suspect virus: LN, Lung, spleen, liver, tonsil + other
4) Abnormal lymph node(s) culture node(s), freeze a piece @ -80^{0}F, and send a swab for routine culture
5) Fat protocol must define appropriate storge – formalin?, freezing? etc.

Chapter 3
Procedure for the Removal, Fixation, and Preservation of Cetacean Ears



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Scott Cramer
Julie Arruda

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July 2007

PREFACE

This chapter is intended as an instructional guide for the removal, fixation, and preservation of auditory system tissues of marine mammals. Each section describes procedures for a major ear type for marine mammals. The main intention is to provide both inexperienced and seasoned stranding responders with sufficient instructions to locate, document, and remove all structures related to the ears and hearing in order to optimize the fixation and preservation of these tissues for later, more-extensive examination. It is strongly recommended that examination be performed collaboratively with auditory system experts, but careful documentation and preservation are the critical first steps that will allow accurate diagnoses.

Key Terms: inner ear; cochlea; ossicles; vestibular system; auditory bulla, temporal bones, peribullar tissue, round window, oval window, hearing, auditory system

CONTRIBUTORS

Text	Photographs	CT Imaging	Illustration
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Scott Cramer	Scott Cramer	Julie Arruda	Inge Milde
		Scott Cramer	

EQUIPMENT

Animal size, condition, and location influence the equipment available for any necropsy. Items cited in bold and italics are useful in all necropsies. Items cited in plain text are helpful, but not critical. **At a minimum, for most ear extractions, you will need one small, thin-bladed knife and one large heavy-bladed knife**.

—Surgical Tools—	
Knives—multiple lengths, serrated and plain edged	***Hammer***
Scalpels—handles and blades	Chain Saw
Clamps—forceps and hemostats	***Chisels—narrow to broad blade***
Saws—hand and electric	Rope
Narrow flexible tubing or catheters	Twine
Probe Sharp	Plastic Ties
Probe Blunt	***Duct® Tape***
Metzenbaum Scissors Straight *	***Measuring Tape Nylon or Plastic (metric)***
Metzenbaum Scissors Curved *	***Ruler (metric)***
Syringe (1, 5, 10 and 50 cc)	***Thermometer—electronic probe type or conventional***
Suture Kits	***Headlamp***
Calipers	***Flashlight***
Femoral Disarticulator	***Screwdriver—Flathead; Long Blade***
Ronguers and Bone Shears	***Crow Bar***
Meat Hooks (with handles and/or hooks with attached chain)	**Hack Saw and Blades**
Cutting Board or Sheet (plastic)	***SawzAll® and Blades***
Scalpel Blade Remover	Cordless Drill
Sharpening Stone	

—Safety and First Aid—		
Safety Glasses	Wet Suits	Elastic Bandage
Survival Suits	***Ice Packs***	QuickClot® *
First Aid Kit—Professional	***Sunscreen***	***Disinfectant Soap and/or Hand Cleaner***
Hand Warmers	***Soap and Shampoo***	***DermaBond® or generic super glue***
Ear Plugs	Dry Suits	

—Bag, Containers, Labels, Pens, and Pencils—		
***Whirl-paks*®**	***Permanent Markers***	Histology Cassettes
***Sealable Plastic Bags* (e.g., ZipLoc®)**	Cooler	Duffel Bag
Plastic Bags	***Labels and Tags***	***Lidded buckets***
Garbage Bags	***Pencils***	
Body Bags	***Plastic Containers (25 to 500 mL)***	
—Miscellaneous—		
Necropsy Forms	Cloth Towels	**Compact Discs for archiving images**
***Formalin*®**	Microscope Slides	
Expanding Foam	Ethanol	
—Audio and Video Equipment—		
Digital Video Camera and Tapes	**Waterproof Housing for Camera(s)**	
35mm Digital Camera	**Tripod Stand and Case**	
35mm SLR Camera	**Storage Media**	
—Clothing and Gloves—		
Disposable Latex® Gloves	Rain Suit	
Sterile Gloves	***Rubber Boots***	
Nitrile Gloves	Surgical Gowns	
Dish Gloves	Scrubs	
Plastic/Rubber Aprons		

***** Available through medical or veterinary supply outlets.

FIELD AND LABORATORY NECROPSY KIT CHECKLIST—SAMPLE

Date:_____ Location:_____Description:_____

Item	S	M	L	Other	Item	S	M	L	Other
Surgical Instruments and Tools									
Autopsy Handle, Grey					Needles (Gauge 22 or smaller—25, 28)				
Scalpel Handle, No. 4					Sutures				
Scalpel Handle, No. 5					Calipers				
Scalpel Handle, No. 6					Femoral Disarticulator				
Scalpel Handle, No. 7					Bone Shears				
Scalpel Handle, Long Straight					Spreaders				
Scalpel Handle, Long Curved					Meat Hooks				
Autopsy Blade, No. 60 (pointed tip)					Rib Cutters				
Autopsy Blade, No. 70 (rounded tip)					Endoscope				
Scalpel Blade, No. 10					Methyl Blue				
Scalpel Blade, No. 11					Contrast				
Scalpel Blade, No. 12					Scalpel Blade Remover				
Scalpel Blade, No. 15					Sharpening Stone				
Scalpel Blade, No. 20					Sharpening Steel				
Scalpel Blade, No. 21					Crow Bar				
Scalpel Blade, No. 22					Hack Saw and Blades				
Scalpel Blade, No. 23					SawzAll and Blades				
Hemostats Straight					Cordless Drill				
Hemostats, Curved					Dremmel Tool				
Forceps					Hammer				
Gigli Saw and Wires					Electric Chain Saw				
Stryker Saw and Blades (with cord)					Chisels				
Stryker Saw and Blades (portable)					Rope				
Knives					Twine				
Probe, Sharp					Wire Ties				
Probe, Blunt					Duct Tape				
Histology Cassettes					Measuring Tape Metal				

Item	S	M	L	Other	Item	S	M	L	Other
Surgical Instruments and Tools *(continued)*									
Metzenbaum Scissors Straight					Measuring Tape Nylon				
Metzenbaum Scissors Curved					Ruler (cm)				
Balloon Catheters					Thermometer				
Catheter Sheaths					Thermocouple and T-wires				
Vacuum Catheters					Flashlight				
Syringe Barrels (any size—1 cc needed for injection)									
Safety and First Aid									
Safety Glasses					Wet Suits				
Survival Suits					Ice Packs				
First Aid Kit					Sunscreen				
Hand Warmers					Soap and Shampoo				
Ear Plugs					Hand Cleaner				
Dry Suits									
Bags, containers, Labels, Pens, and Pencils									
Whirl Paks					Sharpie Markers				
Ziploc Bags					Cooler				
Plastic Bags					Plastic Containers				
Garbage Bags					Glass Containers				
Body Bags					Vials				
Labels and Tags					Histo Cassettes				
Pencil					Duffel Bag				
Miscellaneous									
Necropsy Forms					Cloth Towels				
Formalin					Gauze				
MO Discs					ETOH				
Microscope Slides					**Compact Discs for archiving images**				
Great Stuff Expanding Foam Insulation									

Item	S	M	L	Other	Item	S	M	L	Other
Audio and Video Equipment									
Sony Digital Video Camera and Tapes					Olympus SLR 35mm Camera				
Sony Digital 35mm Camera					Waterproof Housing for Sony Digital 35mm Camera				
Olympus Dig ital 35mm Camera					Waterproof Housing for Sony Digital Video Camera				
Nikon Digital 35mm Camera					Tripod Stand and Case				
Clothing and Gloves									
Latex Gloves					Rain Suit				
Sterile Gloves					Rubber Boots				
Nitrile Gloves					Surgical Gowns				
Dish Gloves					Scrubs				
Plastic Aprons									

ODONTOCETE EAR REMOVAL

EXTRACTION

Documenting the procedures and tissue condition as you proceed with ear extraction is crucial. Photograph the area you are working on before and after each stage of the procedures, being sure to add a scale and a marker indicating any abnormal area. Take wide-area and close-up shots of such areas. Label areas on the photograph consistent with the labeling of tissues subsampled from each area.

Approach odontocete ears from the side of the animal unless the lower jaw has been removed. Each ear consists of two joined spheres of dense bone: one hollow (tympanic), which forms the middle ear cavity; and one nearly solid (periotic), which contains the inner ear. These paired bones sit in a cavity (peribullar sinus) below the brain case, bounded by the squamosal (lateral and dorsal) and the exoccipital (posterior and medial).

Figure 3.1a—Bottlenose Dolphin (Tursiops truncates) with a marker for lateral ear extraction incisions.

Figure 3.1b—Lateral view of a harbor porpoise (Phocoena phocoena) head heavily flensed to show the right tympanic ear bone and anterior region of the peribullar sinus.

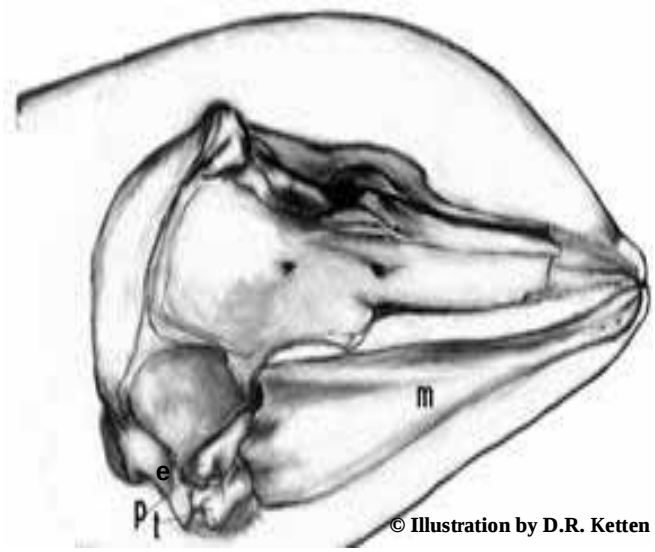


Figure 3.1c—Harbor porpoise *(Phocoena phocoena)* head showing the ear position in relation to the lower jaw (mandible). Periotic (p); Tympanic (t); Mandible (m); Exocciptial (e).

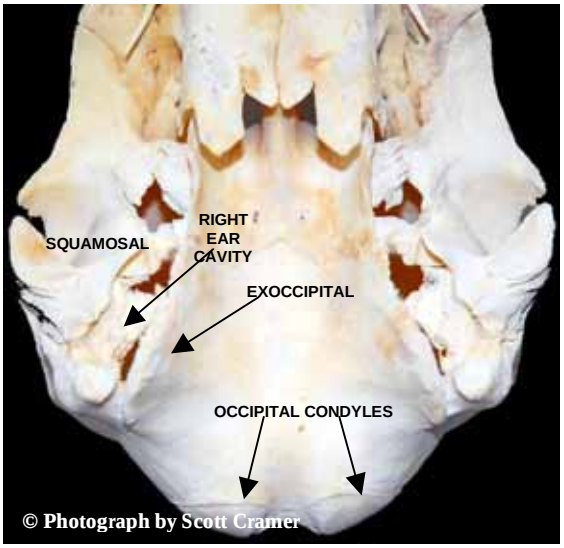


Figure 3.1d—Ventral view of a harbor porpoise *(Phocoena phocoena)* skull. Ear bones have been removed.

The ears are located just behind and deep to the lower jaw, on a line about midway between the eye and the insertion of the pectoral fin (Figures 3.1a through 3.1d). To extract the ears from a lateral approach, first make an ×-shaped incision about midway between the eye and the pectoral fin, with the midpoint of the × in line with the lower jaw. Pull the flaps back and down, cutting through the blubber and muscle. There is considerable soft tissue filling the cavity around the ear bones, and you will probably not see either the tympanic or periotic at this point. Pushing a probe straight in, you will feel a hard surface, which is the posterior section of the tympanic bone. If possible, photograph the area to document its appearance before cutting further. Then, gently cut away the tissue with a scalpel or knife until you find the tympanic bone. In a typical delphinid, the tympanic will be about 40 mm long and 25 mm wide. It resembles a conch shell, with a hollow interior that contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the eardrum. The periotic bone contains the inner ear. It is slightly smaller and is located just dorsal and medial to the tympanic (Figure 3.2). The hyoid bones are generally attached to the posterior/lateral edge of the tympanic by a cartilaginous cap. Cut this juncture with bone shears or a scalpel.

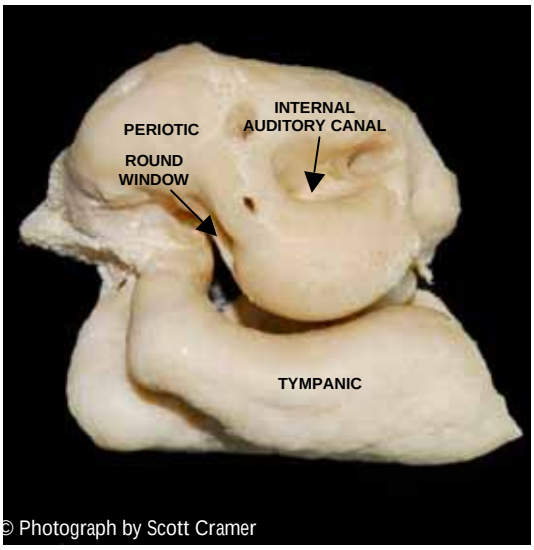


Figure 3.2a—Medial view of a delphinid left ear; tympanic, periotic, and neural canals. This ear is slightly rotated downward from the image at the right.

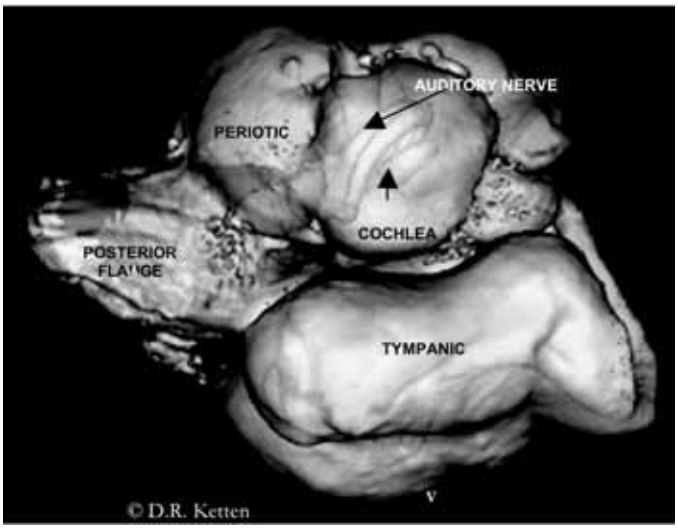


Figure 3.2b. Medial view of a 3-D reconstruction of a pygmy sperm whale *(Kogia breviceps)* right ear from CT scans. The periotic is rendered transparent to show the actual position of the cochlea and auditory nerve (VIII) in the periotic.

The tympanic and periotic are partly fused to each other at the rear edge by a semi-fused (synosteotic) joint and at the lateral edge by a curved or sigmoid process. These joints are relatively weak. Try to keep the two halves together and remove them as a unit. If the tympanic separates from the periotic during removal or is loose, be sure to extract both, and check for ossicles that may have fallen from the middle ear.

Having cleared enough tissue to identify the two bones, you will now need to cut a set of five to eight suspensory ligaments and the facial and auditory nerves located on the medial surface of the ear (Figure 3.2.). Gently rock the ear while cutting the attached soft tissue on the medial, anterior, and posterior surfaces with a narrow, sharp knife or scalpel. If the ear is difficult to move, the periotic may be attached to the skull by a short bony process or the ligaments may be calcified. This is particularly common in older animals. (*Note:* Some groups such as Ziphiids [beaked whales] and Physeterids [sperm whales] have substantial bony connections. Separate protocols are given for these ears).

Any bony attachments that are resisting removal should be cut with bone shears or pried loose with a small chisel, screwdriver,

or flat-bladed instrument. Scrape the posterior area where the periotic joins the exoccipital, and try to locate suture margins. Insert your screwdriver or chisel into these lines and gently tap it into the bone, periodically wiggling the blade to see if the flange can be levered free of the skull. Do not use a scalpel blade for this procedure; it will snap. Some soft tissue will be attached to the ear. Simply leave that in place.

FIXATION AND PRESERVATION

After removal, place the ears in fixative immediately. The best fixative is 10% buffered formalin. If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of distilled water or a chemical buffer provides sufficient buffering. If formalin is not available, 70% ethanol may be used. If the specimen is very fresh, and if you are comfortable with the anatomy, it is best to inject formalin through the round window with a 22–25 gauge needle. The round window is located at the posterior/medial edge of the periotic, ventral to the stapes (Figure 3.2a). *If you are not familiar with the anatomy, please do not attempt this injection. Also, please do not inject if only large bore needles are available.* **If trauma is suspected, do not inject** at the round window; instead, insert the needle into the center of the VIIIth nerve, and slowly inject formalin. If you are injecting the round window, insert the needle in the middle of the membrane approximately 3 mm and **slowly** inject a small quantity of formalin. Be certain to record the location, needle size, and fluid quantity injected in all cases, and send with the other data for the animal's ears to the examiner.

In the field, getting the tissues into any quantity of formalin that surrounds them is acceptable; ideally, however, place the ears in a fixative volume five times that of the specimen as soon as possible. After one week, move them to half that volume, changing to fresh formalin once or twice weekly until they are well fixed. Once fixed, the soft tissues will be moderately stiff and brown and the formalin clear or light tan in color. The ears can be held for several months as they are, moved to another preservative, or shipped at this point.

Freezing is acceptable only if fluid fixation is not possible. If possible, try to obtain fixative later, and place the frozen specimens in the fixative to thaw. Do not thaw in water or in air. If the ears are frozen, do not thaw before shipping. Hold

them without thawing, and contact the receiving lab to discuss shipping methods.

SPERM WHALE (*PHYSETER MACROCEPHALUS*) EAR REMOVAL

EXTRACTION

As is most important in any necropsy procedure, document photographically the tissue condition at each major step, external to final removal. Label all images consistent with any cassette labels of tissues sampled from the region photographed. Include in the picture a label with the animal ID; indication for dorsal, ventral, anterior, and posterior directions; and a metric or other scale. Take both wide and close-up views of suspected abnormalities.

Sperm whale ears can be approached from the ventral or lateral side. Ventral approaches require the removal of the lower jaw. The ears sit in cavities below the brain case, located either side of the occipital condyles and behind a large squamosal shield (Figure 3.3). If you are taking a ventral approach, part of the ears will be visible as two large, white, egg-shaped bones (Figure 3.4).

From the side, the ears are located just behind and deep to the lower jaw, about midway between the eye and the posterior insertion of the pectoral fin (Figure 3.5)[1]. On a newborn or very young sperm whale, they are located approximately 17 cm behind the rear edge of the lower jaw on a head that is 120 cm long. The distances should be proportional on an adult

For a lateral approach, make an ×-shaped incision about mid-way between the eye and the pectoral fin, with the midpoint of the X in line with the lower jaw. Pull the flaps back and down, cutting through the blubber and muscle. Your incision should be just posterior to the jaw. As you probe towards the center of the head, the next bone you will come to is the squamosal, which in this species is a large, lateral wing or shelf extending from the skull. Because of this "squamosal shield", it is easier in this species to approach the ears ventrally or to remove the head and work from the posterior face than to attempt a lateral approach.

Figure 3.3—Ventral view of an adult sperm whale (Physeter macrocephalus) skull.

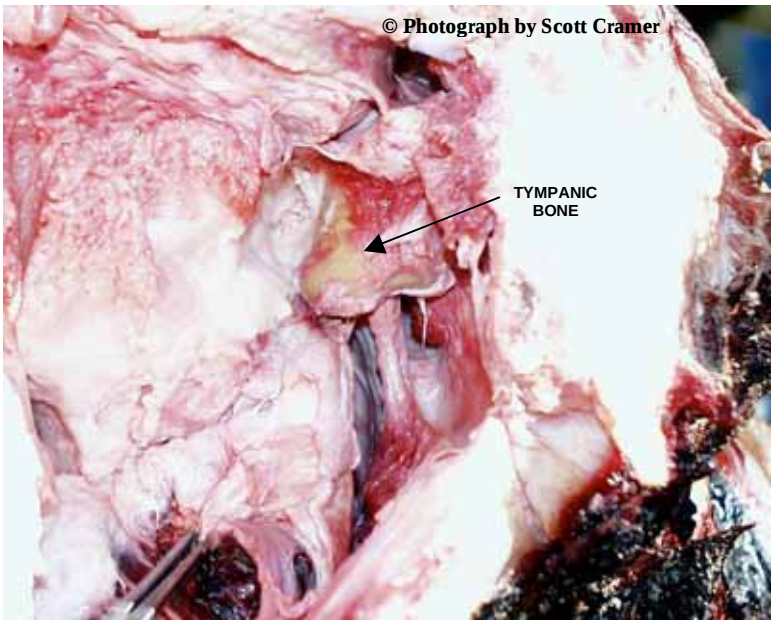


Figure 3.4—Posterior view of the right ear of a young sperm whale (Physeter macrocephalus) head**.**

Figure 3.4—Incisions for a lateral approach to remove the ear of a sperm whale *(Physeter macrocephalus)*. Photos by D. Ketten and S. Cramer

Depending upon the animal's position and the need to preserve the skull parts, you can either reach under the squamosal flange or cut through its narrow neck, which is just above the two bones (tympanic and periotic) that make up the ear. You may also cut away a block of tissue using a SawzAll or chain saw. The block should be approximately 20 cm on a side to include both ear bones, but please be certain that you have included both parts of the ear, described in the following text, in the block.

Each ear consists of two dense, joined bones that sit in the cavity below the brain case adjacent to the squamosal(s) and the

exoccipital(s). Each of the bones is about the size of a tightly closed fist (Figure 3.5).

There is considerable soft tissue surrounding the ear bones. Remove this tissue with a scalpel or knife until you find the tympanic, the lower and more ventral and lateral of the two bones.

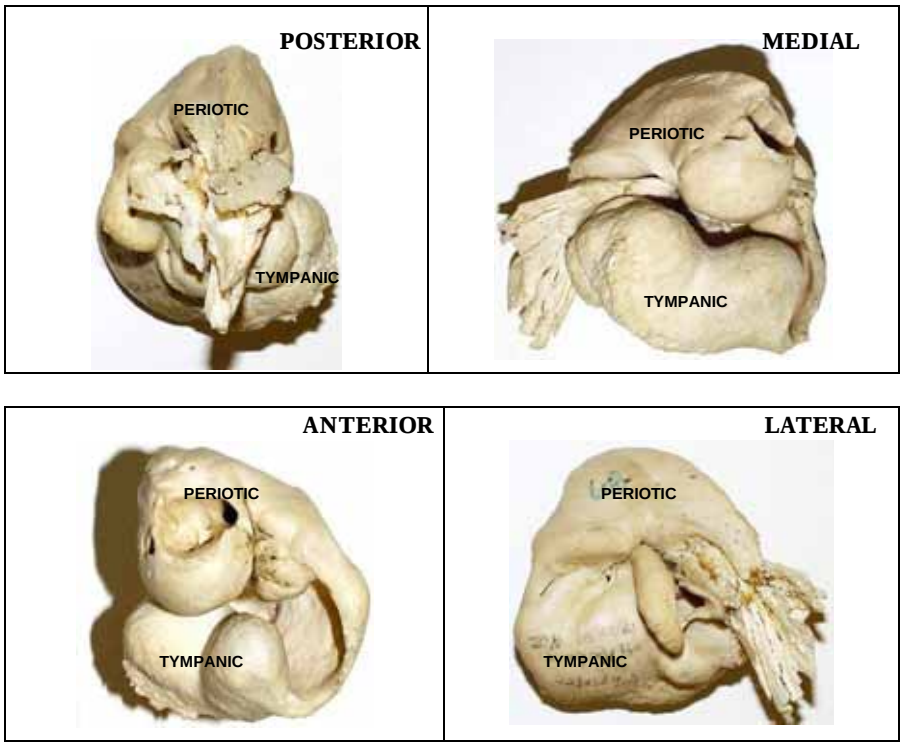


Figure 3.5—Left ear of a sperm whale *(Physeter macrocephalus)* (© Photographs by Scott Cramer)

The tympanic resembles a dense conch shell, with a hollow interior that contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the eardrum. With the jaw removed, the ventral tympanic is readily visible. The periotic contains the inner ear and is just above and medial to the tympanic. The tympanic and periotic are fused, but the joint may be weak. Remove the ears as a unit if at all possible. If the tympanic separates from the periotic during removal or is loose, be sure to preserve the ossicles and any soft tissue from the middle ear.

Once you locate both bones, you will be able to locate five to eight ligaments as well as the auditory nerve on the medial and posterior faces of the periotic. In the sperm whale, there are also substantial flanges protruding from the posterior edge of

the periotic. The ear will likely be difficult to move, and the periotic flanges will need to be cut or levered out of the skull. Chisel any bony attachments that are resisting removal using a screwdriver, narrow chisel, or other stiff, flat-bladed instrument. Do not use a scalpel blade to pry the ear; the blade will snap, and it is difficult to remove from the ear cavity. Do not attempt to chisel into the dense periotic. Instead, probe until you find softer, spongy bone on its posterior margin. Wedge your chisel or screwdriver into this flange or into the skull at its juncture. Pry gently until the suture separates or the flange breaks. If this specimen is to be used for osteologic studies, try to maintain the flange.

Once the ear bones can be moved, try to locate the ligaments and nerves retrobullar (behind and medial to the ear bones). Cut these with a sharp knife or scalpel. Grasping the two ear parts, rock the ears gently until they can be cut. Do not pull soft tissue to free them or they may evulse the nerve (i.e., rip the nerve out of the ear).

FIXATION AND PRESERVATION

After removal, place the ears in fixative immediately. The best fixative is 10% buffered formalin. If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of distilled water or a chemical buffer provides sufficient buffering. If formalin is not available, 70% ethanol may be used. If the specimen is very fresh, and if you are comfortable with the anatomy, it is best to inject formalin through the round window with a 22–25 gauge needle. The round window is located at the posterior/medial edge of the periotic, ventral to the stapes (Figure 3.2a). *If you are not familiar with the anatomy, please do not attempt this injection.* Also, please do *not* inject if only large bore needles are available. **If trauma is suspected, do not inject** at the round window; instead, insert the needle into the center of the VIIIth nerve and slowly inject formalin. If you are injecting the round window, insert the needle in the middle of the membrane approximately 3 mm and **slowly** inject a small quantity of formalin. Be certain to record the location, needle size, and fluid quantity injected in all cases and send with the other data for the animal's ears to the examiner.

In the field, getting the tissues into any quantity of formalin that surrounds them is acceptable, but, ideally, place the ears in a

fixative volume five times that of the specimen as soon as possible. After one week, move them to half that volume changing to fresh formalin once or twice weekly until they are well fixed. Once fixed, the soft tissues will be moderately stiff and brown and the formalin clear or light tan in color. The ears can be held for several months as they are, moved to another preservative, or shipped at this point.

Freezing is acceptable only if fluid fixation is not possible. If possible, try to obtain fixative later, and place the frozen specimens in the fixative to thaw. Do not thaw in water or in air. If the ears are frozen, do not thaw before shipping. Hold them without thawing, and contact the receiving lab to discuss shipping methods.

EXTRACTION

Documenting the procedures and tissue condition as you proceed with ear extraction is crucial. Photograph the area you are working on before and after each stage of the procedures, being sure to add a scale and a marker indicating any abnormal area. Take wide-area and close-up shots of such areas. Label areas on the photograph consistent with the labeling of tissues subsampled from each area.

Approach beaked whale ears from the side of the animal, or ventrally, if the lower jaw has been removed. Each ear consists of two joined, dense bones—one hollow (tympanic) and one spherical (periotic)—that sit in the cavity lateral to the brain case and are bordered by the squamosal laterally and the exoccipital posteriorly (Figure 3.6 and Figure 3.7).

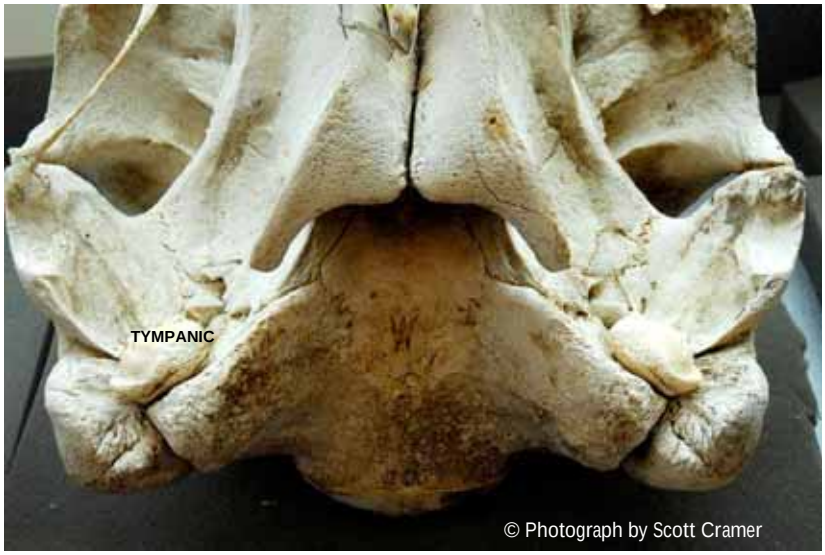


Figure 3.6—Ventral aspect of a Cuvier's beaked whale *(Ziphius cavirostris)* skull. The tympanic bones are ovoid. The periotics are not visible in this photograph and are located dorsal and medial to the tympanic bones.

Figure 3.7—Left ear of a Cuvier's beaked whale *(Ziphius cavirostris)*

The ears are located just behind and deep to the posterior edge of the lower jaw. To extract the ears using a lateral approach, locate by palpation the posterior edge of the jaw. Make an ×-shaped incision about mid-way between the eye and the pectoral fin, with the midpoint of the × in line with the jaw (Figure 3.8)[2].

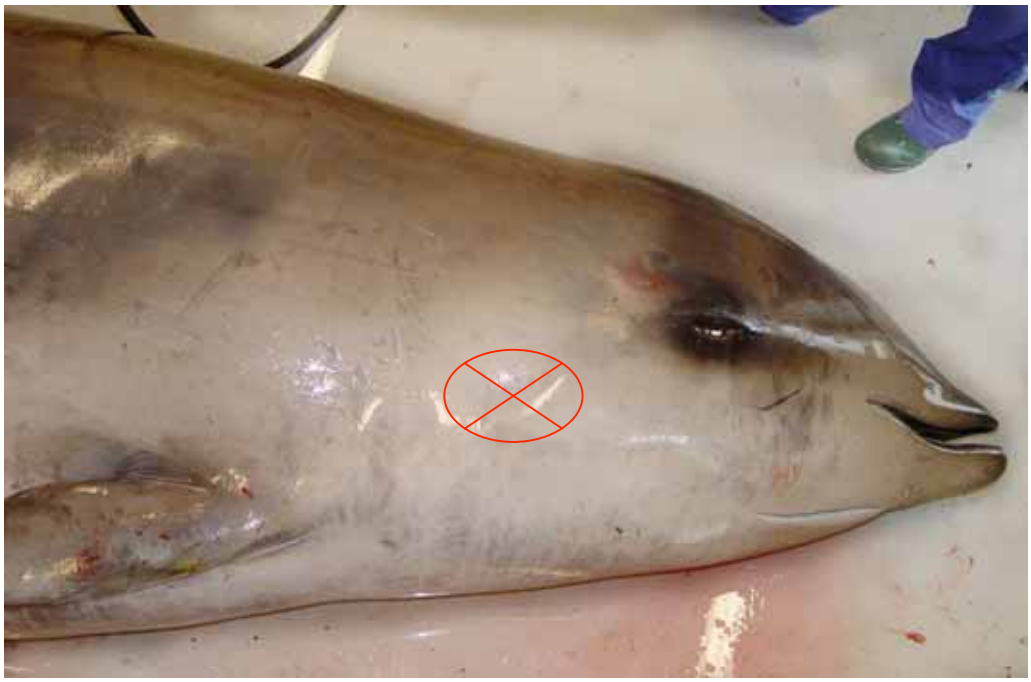


Figure 3.8—Incision location for a lateral ear extraction (Photo by S. Cramer)

Bend the tissue flaps back and down. Probe straight in from the center of your incision until you feel a hard surface. That is the tympanic bone of the ear.

There is considerable soft tissue filling the cavity around the ear bones. Gently cut away the tissue with a scalpel or knife until you expose the tympanic. In a typical ziphiid, the tympanic will

be approximately 40–50 mm long and 30 mm wide. It resembles a conch shell, with a hollow interior that contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the eardrum. The periotic bone, which contains the inner ear, is slightly smaller and is dorsal and medial to the tympanic. One of the hyoid bones generally attaches to the posterior/lateral edge of the tympanic by a cartilaginous cap. Cut this juncture with bone shears or a scalpel. On beaked whales, there is also a thick slice of bone loosely attached to the anterior margin of the tympanic.

The tympanic and periotic are partly fused to each other at the rear edge by a semifused (synosteotic) joint and at the lateral edge by a curved or sigmoid process, but the joints may be weak. Try to extract tympanic and periotic as a unit. If the tympanic separates from the periotic during removal or is loose, please be sure to extract both bones and be certain to get any ossicles that may have fallen from the middle ear.

Having cleared enough tissue to identify the two bones, you will need to free them by cutting or levering a posterior flange attached to the skull. Try to move the ear bones, looking for motion in the sutures of the skull posterior to the ear. It may help to scrape the soft tissue from the skull in this area. Place a chisel or flat-bladed screwdriver in these sutures and gently pound the wedge in with a hammer or mallet until you can lever the periotic and tympanic out of their cavity with an approximately 2 cm chunk of softer skull material attached at the posterior edge. You will now cut five to eight suspensory ligaments and the facial and auditory nerves located on the medial surface of the ear. Gently, rock the ear while cutting the attached soft tissue on the medial, anterior, and posterior surfaces with a narrow, sharp knife or scalpel. This is the most difficult part because it is not easy to cut the tissues blindly. A narrow or curved scalpel helps. Any attachments that are resisting removal should be cut with bone shears or pried loose with a small chisel, screwdriver, or flat-bladed instrument. Do not use a scalpel blade for this procedure; it will snap. Leave any soft tissue attached to the ear.

FIXATION AND PRESERVATION

After removal, place the ears in fixative immediately. The best fixative is 10% buffered formalin. If concentrated formaldehyde

liquid or formaldehyde powder is used, mixing it with sea water in lieu of distilled water or a chemical buffer provides sufficient buffering. If formalin is not available, 70% ethanol may be used. If the specimen is very fresh, and if you are comfortable with the anatomy, it is best to inject formalin through the round window with a 22–25 gauge needle. The round window is located at the posterior/medial edge of the periotic, ventral to the stapes (Figure 3.2a). *If you are not familiar with the anatomy, please do not attempt this injection.* Also, please do *not* inject if only large bore needles are available. **If trauma is suspected, do not inject** at the round window; instead, insert the needle into the center of the VIIIth nerve and slowly inject formalin. If you are injecting at the round window, insert the needle in the middle of the membrane approximately 3 mm and **slowly** inject a small quantity of formalin. Be certain to record the location, needle size, and fluid quantity injected in all cases and send with the other data for the animal's ears to the examiner.

In the field, getting the tissues into any quantity of formalin that surrounds them is acceptable; ideally, however, place the ears in a fixative volume five times that of the specimen as soon as possible. After one week, move them to half that volume changing to fresh formalin once or twice weekly until they are well-fixed. Once fixed, the soft tissues will be moderately stiff and brown and the formalin clear or light tan in color. The ears can be held for several months as they are, moved to another preservative, or shipped at this point.

Freezing is acceptable only if fluid fixation is not possible. If possible, try to obtain fixative later, and place the frozen specimens in the fixative to thaw. Do not thaw in water or in air. If the ears are frozen, do not thaw before shipping. Hold them without thawing, and contact the receiving lab to discuss shipping methods.

SHIPPING

Once the specimen appears well-fixed, call the lab receiving the specimen to confirm that you are ready to ship and the day for shipment. If shipping to this lab (Woods Hole Oceanographic Institute), contact us by phone first at the numbers listed below.

On the day of shipping, wrap the specimen in several layers of formalin-soaked gauze and place in three or more sealed plastic bags with an absorbent material such as diapers inside each bag

to prevent leakage. If you use a jar, seal the jar with wax or waterproof tape, and place it in a sealed plastic bag. Do not use glass containers. The important point is to preserve moisture around the ears without a large fluid volume and to have several leak-proof seals. Place the packaged samples inside a cooler or reinforced box. The shipping container should be capable of withstanding a drop of at least three feet without damage.

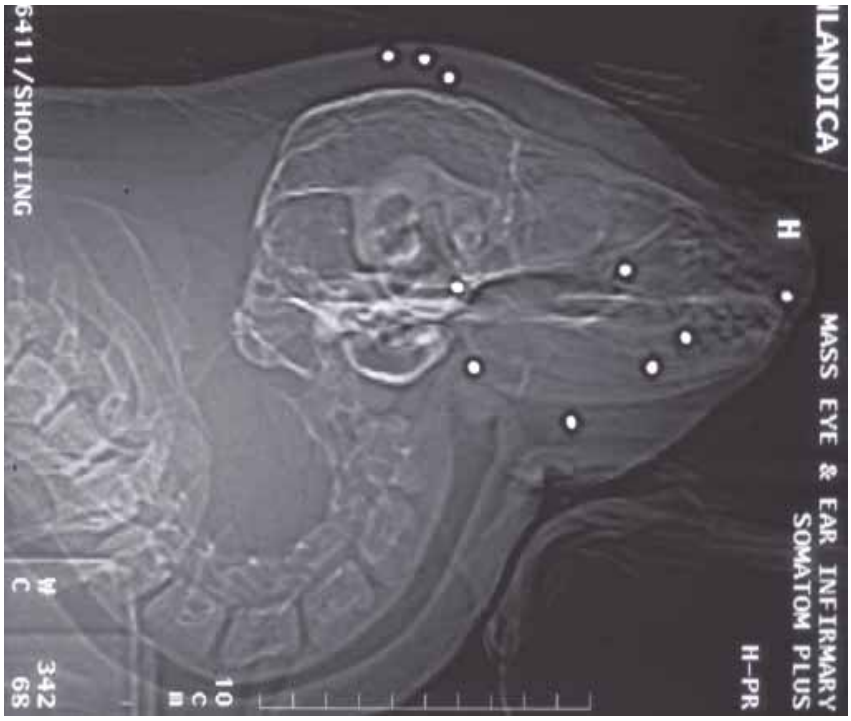
Within the United States, ship by Federal Express or other expedited service for one- or two-day delivery. If sending from overseas, please use a method that will deliver within seven days. You will also need to confirm with us any permit numbers that are required for domestic or international shipping for some species. Check with your shipper that formalin fixed, nonliquid samples are allowable and considered nonhazardous. If so, mark the container: Scientific Specimen—No Medical Hazard—Deliver Immediately.

COMMENTS OR QUESTIONS ON EXTRACTION PROCEDURES

We welcome your comments on this manual and will be happy to answer additional questions.

Contacts—For further questions please contact the following:	
Dr. Darlene Ketten or Scott Cramer Laboratory: 508-289-3582 Office: 508-289-2731 or 508-289-2832 Mobile: 774-836-5012 Fax: 508-457-2041 Email: dketten@whoi.edu scramer@whoi.edu	Biology Department Woods Hole Oceanographic Institution 266 Woods Hole Road MS #50 Woods Hole, MA 02543 USA

Chapter 4—
Imaging Procedure for Stranded Marine Mammals



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June 2007

INTRODUCTION

This section provides an introduction to biomedical imaging techniques and guidelines for diagnostic imaging of marine mammals to assist with both live examination and necropsy procedures. The procedures described are based on imaging equipment and techniques relatively common in human and veterinary facilities; thus, the procedures will provide the majority of stranding-response groups with the most likely options to assist their efforts. The imaging techniques described include basic radiography, computed tomography (CT), and magnetic resonance imaging (MRI); these techniques are applicable to both live and postmortem cases.

Special emphasis has been placed on whole-body–, airway-, head-, and ear-imaging procedures. Subsections cover basic information on the following:

- Basic principles and appropriate applications for radiography versus CT versus MRI;
- Handling and preparation of live and dead animals in clinical settings; and
- Image and data formats that may be encountered.

The protocols are also listed in outline form to provide a rapid overview. The introductory discussion of principles behind techniques is not required to employ the protocols, but does provide additional information that can aid in deciding which techniques are most efficacious as well as the limitations for interpretation of imaging data. Examples of some pathologies imaged with these procedures are also provided.

BIOMEDICAL IMAGING—AN INTRODUCTION TO TECHNIQUES

RADIOGRAPHY

Plain-film radiographs, commonly called ×-rays, are the most common noninvasive imaging method used by physicians and veterinarians. Most veterinary clinics and some stranding networks will have ×-ray machines. The equipment for plain films is relatively inexpensive and broadly available, and the exams are relatively rapid. Depending on the size of the animal

versus the tissue type and pathology present, plain-film ×-rays may be sufficient for diagnosing some conditions, including fractures, hemorrhages, tumors, and foreign object. They are particularly useful for determining the presence of any metallic objects; thus, they may be an important rapid-screening device to use prior to attempting MRI on any animal.

Radiographs are essentially a shadow picture obtained by exposing a photosensitive film with ×-ray energy that has passed through an object or animal. Differences in the density of the tissues or objects in the animal being imaged determine how much of the ×-ray beam will be attenuated or passed through to the film. The denser a structure is, the fewer ×-rays that pass through it. These differences are encoded as grades of exposure ranging from black (e.g., air and therefore full pass of ×-ray energy, fully exposed film) to white (fully attenuated or absorbed ×-rays). Areas of very low density such as normal, air-filled lungs will be dark gray to black with mottlings of dark to light gray where there are thicker alveolar walls, bronchioles, etc. The cartilage and soft tissue areas of the trachea will be a lighter gray, and all of it will be overshadowed by the brighter, white bone of the ribs. Very dense objects such as bones, teeth, calcium deposits in tumors, and plastic or metallic foreign bodies absorb or reflect the ×-ray beam and appear bright white. Muscles and organs (heart, liver, and spleen) are shades of grey.

In a plain film or radiograph, we are actually looking at a two-dimensional (2-D) composite of all the overlying tissue. Therefore, ×-rays can be difficult to interpret because of the multiple layers of tissues, especially if the animal is very large or if the tissues to be visualized are surrounded by several denser or complex structures. In most cases, on simple radiographs, pathologies are observed as inappropriate darker areas if there is a collection of fluid (henatoma), an air pocket (pneumothorax or emphysema), or bone separation (fractures); they may also appear as denser and whiter areas, sometimes called "clouding," as in the case of advanced pneumonia, consolidated lungs, fibrous tissues, or inflammatory regions. Resolution of a plain film depends on balancing many factors. The most important are voltages used and length of exposure which determines radiation received. These may be increased or decreased depending on the mass of the animal, the depth and type of tissue that must be imaged, the density of tissues overlying the

target areas, and other technical factors such as screens used, film cassette types, and emulsion quality. The latter can help reduce ×-ray beam scatter and improve definition. All of these factors should be discussed with the technician or radiologist conducting the exam in order to determine how to obtain the best images in the shortest time for the animal and the pathology being investigated.

COMPUTED TOMOGRAPHY

Computed tomography refers to the production of sectional images of an animal or specimen. Tomography derives from the Greek *tomos* meaning slice as opposed to *topos* meaning surface (Figure 4.1). Computerized refers, of course, to the use of computers rather than film to acquire the data and reconstruct it as images of sections of the body.

Sectional imaging is analogous to slicing a loaf of bread and acquiring an image of whatever is in each slice. In standard ×-rays, the entire loaf would be visible with all internal structures as overlapping shadows. In one sense, a CT image is a tomogram, and a plain film or standard ×-ray is a topogram. In comparison to plain film ×-rays, tomograms provide far greater detail but they are more time-consuming and generally more expensive. Sectional CT images amount to a digital dissection, allowing us to view internal structures within individual sections in far greater detail because we do not have the interference of the surrounding tissues. However, sectional CT images require somewhat longer examination times and may involve more exposure to ×-rays or use of tranquilizers than do plain films. Most hospitals and an increasing number of veterinary facilities have one or more tomographic machines.

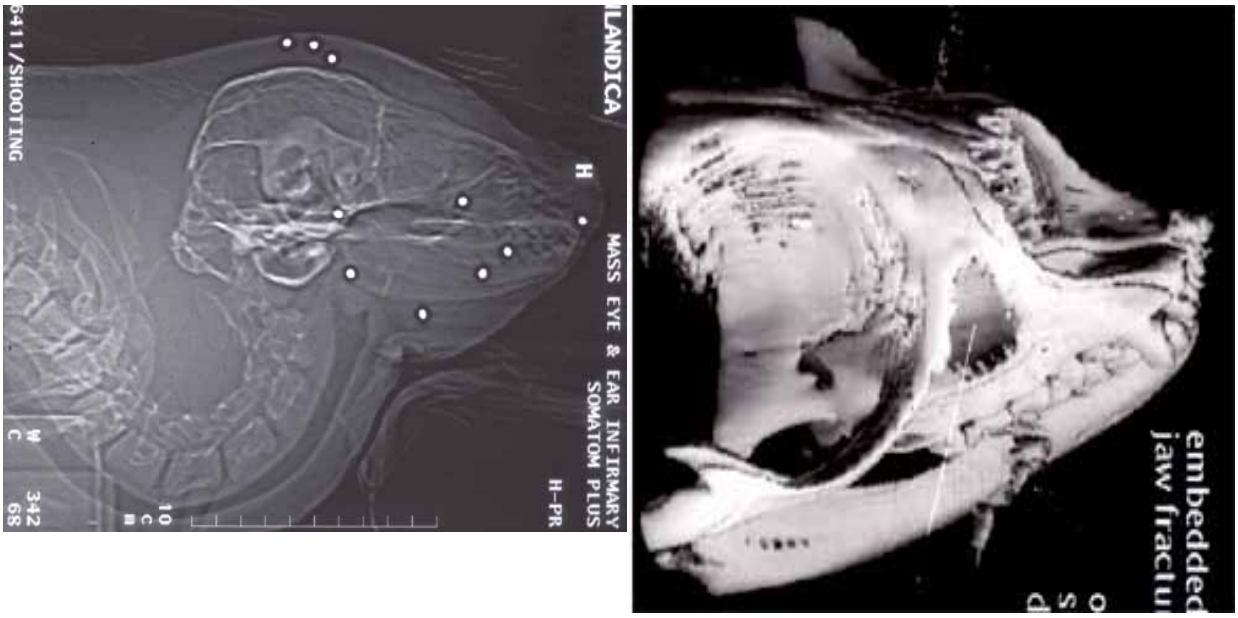

Figure 4.1. A CT topogram or scout image (left), which is similar to a plain film, of a live seal that had been shot in the head, showing multiple metal fragments. On the right is a three-dimensional (3-D) reconstruction from the CT images of the animal demonstrating the extent and pattern of the fractures that resulted in relation to the fragments.

The two most common forms of sectional imaging are based on ×-ray and on magnetic resonance techniques. Technically, the term "computed tomography" applies to both, but the phrase and its abbreviation, CT, are most often used to mean sectional imaging from ×-rays; magnetic resonance or nuclear magnetic resonance imaging is called MRI or NMR. These two techniques are complementary. CT can image soft and hard tissues, but is especially well-suited for any exams requiring information on bones, foreign objects, or air filled spaces. MRI provides superior images for soft tissues; however, because the technique depends upon having hydrated tissues, it cannot accurately represent bony or heavily aerated tissues, and because it employs shifting magnetic fields, it is precluded from use in any animal that may have any metal fragments.

CT: ×-RAY COMPUTERIZED TOMOGRAPHY

Particular indications for CT imaging include pathologies that involve any form of bone lesion, aerated tissues, and mixed bone and soft tissue evaluations(e.g., trauma cases; intracranial hemorrhage; fracture evaluation; dislocations of any bony structures; foreign bodies; and diagnosis of primary and secondary neoplasms of the liver, kidney, brain, lung, bone; and tumor staging).

As noted previously, as with plain films, CT images are based on ×-ray attenuation, but the image data is acquired by means of an array of detectors linked to a computer rather than by film. A computer-controlled table (gurney) moves the patient through a doughnut-shaped opening in the housing (gantry) that contains the ×-ray tube. As the tube rotates along the arch of the housing and the table moves through the gantry, pulses of ×-rays are emitted at multiple positions and recorded at the detectors beneath the patient.

A grid or collimator overlying the detectors assists in reducing the recoding of scattered beams. Analogous to the process in plain films, the resolution of the digital images produced is based on a complex combination of the kilovoltage (kV), exposure time, number of pulses, collimators, detectors, and table speed. In addition, modern scanners have two basic modes: single slice and spiral. In the former, the table moves in increments from 1 mm to 10 mm, and images are produced at matching thicknesses with one travers of the tube per slice. In the spiral mode, the table moves at rates of 0.5 mm to 10 mm per second, with the tube moving and the data being collected continuously. This method is far more rapid and allows slice reconstruction at thicknesses from 0.1 mm to 10 mm from one data set.

Both forms of acquisition can also be used for imaging soft or hard tissues. “Acquisition kernel” is the term used to refer to the reconstruction algorithm that is used to transform the attenuation values into gray-scale values. The kernel used will provide an optimal gray-scale distribution for detail of different tissue types. Thus, there are many components that go into the exact protocol used for any exam: spiral or single slice exposures, table speed, kV, level of detail needed, tissue type to be imaged, time the animal can be on the table, body area to be examined (e.g., head or whole chest or abdomen). For example, for CT imaging of the thorax and abdomen, a protocol using a 10-mm slice thickness and soft tissue kernels is common because these structures are large. Lung scans use a 3 mm and specialized protocol to emphasize fine differences in the aerated passages; exams of the middle and inner ear use an even smaller slice protocol of 0.5 mm acquisitions with 0.1 mm image reconstruction and high-resolution bone kernels. Some of these numbers will vary according to manufacturer of the scanner; in most facilities, however, at least some spiral imaging will be available, and in all CT facilities, bone and soft tissue kernels for

most organ types are available. In addition, CT images can now be reconstructed at virtually any angle required even though they are acquired in all cases in a standard transaxial plane.

As in a standard radiograph, the CT images are shown as grades of gray that relate to the amount of x-ray beam that was attenuated by tissues of the specimen. Attenuation is affected by many factors, but is largely determined by density of the exposed tissues. In the images, the higher the attenuation, the denser the object, and the whiter the appearance of the structure. In addition to the 256 gray scale used to tint each pixel, a second value—the Hounsfield units (HU)—is also available from the CT data. Hounsfield, named after one of the inventors of CT methodology, is a measure of attenuation standardized to water. Air is therefore –1000 HU; water is 0. Higher numbers indicate increasing density. Mammalian tissues typically range between –100 (fats) to +3100 (very dense bone), but even metals can be measured up to approximately 41000 HU on an extended scale. Hounsfield units therefore offer a far greater range of information about the density of and physical characteristics on a per-pixel basis than does the 256 gray scale used for imaging alone. Hounsfield units are especially useful in diagnosing hypermineralization, the quality or age of a blood deposit (clots are denser and have higher HU values than fresh, fluid blood), etc.

This brings us to a rather technical area about how images are formed using kernels and look-up tables. It is not necessary to understand these fully, but it is important to be aware of them. The attenuation values or coefficients are the raw data of the CT scan. These values are processed into image files by applying a convolution algorithm, which briefly amounts to combining multiple values that are obtained from each x-ray projection, weighting them according to the tissue characteristics that are of interest, and then providing a third output that results after this "filtering" has been applied. At the user interface, this operation amounts to choosing first a protocol that weights the data for better definition of bone compared to soft tissues and then adding a second weighting that emphasizes a range of Hounsfield values, commonly called "windows" and "centers."

Therefore, for any scan session, you must indicate to the technician whether you need primarily images of the soft tissue or of the bony structures or of both. Applying the proper kernel

is particularly important if you are planning to make measurements of tissue dimensions. Reformats of any CT data set must be done from the raw data set for accurate information. Reformats should not be from the image files, because these are already set by the kernel previously chosen; although the appearance may change, the representation of the anatomy may be distorted. In most facilities, only the image files are archived because the raw data files are large and time-consuming to copy. It is important, however, to archive both raw data and the basic image files whenever possible, especially if the animal is rare, has an unusual condition, is a legal case, or the reformats for other tissue types may be needed later.

Contrast agents may be employed in live cases by injecting an agent that is radio-opaque and that assists with tracking fluid accumulations or flow rates. These are most commonly used to determine presence of tumors or for cardiac assessments. Contrast agents require veterinary oversight; although reactions are rare in nonhumans, precautions to treat a reaction should be in place before the agents are administered.

CT Summary

In general, CT provides very high-resolution imaging in a relatively short time frame. It is the primary choice for live animals that require quick exams, and it provides the best images for diagnoses of bone, airway, or foreign-body pathologies.

Tranquilization may be required, but for very short spiral CT exams, it may be sufficient to simply restrain the animal or in, the case of a captive animal, to train it to hold a position for five minutes or less. The procedure requires exposure to ×-rays, but, for most exams, this does not represent a significant increase over plain film procedures. CT exams are limited to animals weighing less than 250 kg, less than 1.7 m long, and less than 70 cm in diameter. Some larger gantry machines that accommodate greater weights are available but are rare.

Because CT scanners are generally located in clinical settings, proper precautions for both live and postmortem specimens are required to prevent contamination, as described in the subsequent outlines. The first scan will be a “topogram” or “scout” of the whole animal or specimen (Figure 4.1). This image resembles a regular ×-ray and is used to determine the regions of the animal that will be examined. Following the scout

and the designation of scan sequences, the raw data for generating section images are acquired by either spiral or single section scan sets. The typical whole-body exam will require approximately 20 minutes of table time to acquire the data, at which point the animal can be removed from the scanner. Image reconstruction typically may require 30 minutes to two hours to obtain complete sets of all image sets depending on the number of types of tissues and reconstructions required. Both 2-D and 3-D images are possible, as are multi-tissue displays and measurements of significant features. Bone- or soft-tissue kernels or both should be used as appropriate for each case rather than attempting to convert one series to the other by re-windowing from just the image files. Contrast agents may assist with diagnosis of hemorrhage, tumors, or heart conditions, but they require careful administration under veterinary supervision. Images are generally exportable as hard-copy films or digital DICOM (digital imaging and communications in medicine) format images (described in subsequent text) on CD or other electronic medium.

MRI: MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) is another increasingly available method for sectional anatomy imaging and is extremely valuable for many soft-tissue exams. The strength of MRI is the ability to detect differences in soft tissue; radiography and CT, which provide excellent examinations of bone and air-filled spaces, provides less resolution than MRI for most soft tissues. For example, MRI is an excellent tool to view the brain and gastro-intestinal or reproductive systems, because it provides good resolution of subtle differences in soft-tissue hydration such as between white matter and grey matter. On the other hand, magnetic resonance cannot be used to image most bony structures or highly pneumatized areas. It also is precluded for subjects that have any metal in or on them; plus, because of longer imaging times, generally MRI will require tranquilizers or anesthesia for most veterinary cases.

Magnetic resonance (MR) images are acquired by generating a magnetic field across the subject. Hydrogen nuclei (protons) present in the tissue align with this field. Pulses of radio waves are then broadcast into the sample, energizing the hydrogen protons, causing them to resonate and deflect away from the magnetic field. Once the radio pulse stops, the protons decay (i.e., return, or “relax”) to their original alignment. A receiver

coil detects the number of resonating protons and the changes in their spin, which is then translated into a signal-intensity value. Tissues with high levels of fluids (e.g., blood, cerebrospinal fluid) have high proton content and produce high signal intensities; those with low fluid content (e.g., lungs or dense bone) produce low signal intensities.

Analogous to the process of converting ×-ray attenuations in CT, the MR signal intensities are transformed by a convolution process also into gray scales for imaging. High-intensity, high-fluid content areas are generally imaged as bright white while lower fluid-content structures are in graded shades of gray. Bone and air have virtually no fluid content; therefore they produce little or no signal and are both black. These parameters can be adjusted, however, to preferentially enhance imaging of some tissue types. Typically, two types of images can be produced, depending on what stage of decay is being measured (i.e., how long after the radio pulse transmission is stopped): T1-weighted images are taken early in the decay process, while T2-weighted images are taken later (shorter and longer relaxation times). T1 images, which have greater resolution, are most useful for examining anatomic detail. By comparison, T2-weighted images have greater contrast and are most useful in diagnosing gross pathologies such as cysts or some tumors because these pathologies typically contain relatively large amounts of water, which results in high signal intensities that appear bright white.

As with CT, MR images are usually provided in sections in coronal, sagittal and axial planes, and at 1–10 mm thicknesses. The absolute level of resolution depends partly on the number of signal sequences that are employed and, therefore, on the total time required for the exam. The more pulse sequences measured and compared, the higher the resolution and the longer the exam for each case study. The average head or abdominal MRI exam will usually require about 30–45 minutes of table time and have a resolution of 2–8 mm per pixel. Resolutions of less than 2 mm will normally require upwards of one hour of signal acquisitions and are therefore practical generally for postmortem cases.

As with the kernels in CT, most MRI units have multiple sequence modes that can be employed, and this should be discussed with the technician prior to the start of the exam. In addition to the T1 and T2 sequences described previously, some

of the more commonly available protocols include Constructive Imaging via Steady State (CISS) and fat-suppressions as well as some newer techniques for viewing lungs or cardiac functions.

Contrast agents are also available for use in MRI; as with CT agents, they should be employed only with veterinary approval and oversight, and precautions to treat a reaction should be in place before the agents are administered.

MRI SUMMARY

MRI provides exceptional soft-tissue imaging but requires longer table time for most exams than does CT. It is the primary choice for animals or specimens that requires good soft-tissue differentiation; MRI provides the best images for diagnoses of brain and abdominal soft-tissue pathologies.

Tranquilization is likely to be required in all live cases. Size limitations depend on the unit and on whether a body, head, or surface coil will be used. In general, animals must weigh less than 200 kg, and the exam area must be less than 1 m long and less than 55 cm in diameter.

Because these units are generally located in clinical settings, proper precautions for both live and postmortem specimens are required to prevent contamination, as described in the outlines that follow. As with CT, MRI requires a scout acquisition to determine orientation and regions to be imaged. Following this process, the full MR sequences are applied. The typical whole body exam will require approximately 40 minutes of table time for each sequence or mode chosen and 30 minutes to two hours for obtaining complete sets of images, depending on the number of types of tissues and reconstructions required. Both 2-D and 3-D images are possible. Contrast agents may assist with diagnosis of hemorrhage, tumors, or heart conditions, but require careful administration under veterinary supervision. Images are generally exportable as hard-copy films or digital DICOM format images (described subsequently) on compact disc (CD) or other electronic medium.

PRACTICAL GUIDELINES

CHOOSING THE RIGHT IMAGING MODALITY

There are numerous factors to consider when determining which imaging technique(s) to use for a stranded marine mammal. These include equipment availability, amount of time available, size of the animal, specimen condition, and suspected pathology type. Following are guidelines for each modality and procedures for preparing the animal or specimen. It should be noted that the times listed do not include specimen preparation or positioning prior to scanning. Dimension and weight tolerances vary by scanner manufacturer; those listed are typical for most recent units. For postmortem material, limits include all containers, packaging, and fluids surrounding the specimen when scanned.

Procedural Outlines and Size Limits

Species size categories employed in the protocols below assume the following limits:

- Small

  Weight < 250 kg
  Maximum diameter < 58 cm
  Maximum girth < 80
- Medium

  Weight < 400 lb
  Maximum height < 70 cm
  Maximum girth > 80 cm
- Large
  - Weight >400 lb
  - Maximum height >70 cm
  - Maximum girth >80 cm

Plain Film x-rays

- Time required: Seconds per exposure for each x-ray
- Size restriction: NONE
- Weight: No weight limits; resolution decreases with body mass in the field of view
- Specimen condition

  - Live
  - Fresh dead
  - Moderate to advanced decomposition
- Particular applications
  - Fractures
  - Hemorrhages
  - Pneumothorax
  - Tumors
  - Foreign bodies, especially metallic objects
  - Prescreening for MRI

CT

- Time required: 0.5 to 10 minutes, depending on body region examined
- Size restrictions: small to medium species for whole animals or parts of small, medium, and large species that fit within the following limits
  - Weight: <250 kg
  - Maximum dimension: <70 cm (diameter)
  - Maximum length: 1.7 m
- Specimen condition
  - Live
  - Fresh dead
  - Moderate to advanced decomposition
- Particular applications
  - Trauma
  - Gunshot wound
  - Fractures
  - Middle and inner ear pathology
  - Skull and spinal column pathology
  - Osteolytic disease
  - Joint pathology
  - Air emboli
  - Foreign bodies
  - Abdominal organ injury
  - Hemorrhage
  - Tumors

MRI

- Time required: 15–45 minutes per scan
- Size restrictions: small species for whole animals or parts of small, medium, and large species that fit within the following limits
  - Weight: <200 kg
  - Maximum dimensions
  - Head coil: 80 cm circumference (girth) or ~20 cm diameter
  - Tunnel opening: 58 cm diameter
  - Maximum length: 1 m
- Specimen condition
  - Live
  - Fresh dead
- Particular applications
  - Trauma
  - Neurological symptoms (aberrant behavior, seizures, tremors, circling)
  - Soft tissue damage
  - Brain lesions
  - Hemorrhage
  - Tumors
  - Circulatory system function
  - Cysts

BIOMEDICAL IMAGING PROCEDURES FOR STRANDED ANIMALS

Applicable imaging approaches differ depending on many factors, including whether the animal is alive or dead, whether sedation is feasible, the specimen size and weight, and the available imaging options. As outlined previously, both CT and MRI have weight and aperture limits that may vary by manufacturer but, in general, limit the exams to small to mid-sized cetaceans and pinnipeds or to postmortem exams of parts of larger animals

If all imaging options are available and there is time, the optimal scenario would be to perform a screening radiograph of the head and body of most specimens and then pursue CT or MRI scans

of the body and head for high-resolution imaging of any areas of interest. The following recommendations therefore provide procedures for screening with plain films followed by CT and MRI. If CT is available, the plain films may be eliminated. If MRI is available, either plain films or CT should be used first to screen the specimen or animal to be certain no metallic components are present in the gut or embedded in any tissues.

We recommend employing CT rather than MRI for full-body scanning because MR imaging of the whole body requires extensive table time and has imaging length limits that make it impractical for all but the smallest postmortem specimens. It also will not provide adequate resolution of many structures in the head, thorax, or abdomen (e.g., ears, lungs or other bony or gas-filled regions) that are important for assessing strandings. When possible, we do recommend performing MRI of the head, because this modality provides the best resolution of the brain.

Prior to the examination, it is advised that you contact the facility, inform them of the species, size category, and exact gross condition of the animal. Basic details they will require are live or dead, intact or with external trauma, and, if dead, the state of decomposition. Handling for each of the stages in postmortems is discussed in following text.

ANIMAL OR SPECIMEN PREPARATION:

All Live Animals

Transport Preparations

a. Document the condition of the animal with photographs and notes.

b. Clean animal to the extent feasible to remove superficial sand and mud because the minerals in these particles create image artifacts and may cause injury in MRI exams. It is advisable to photograph again after cleaning and prior to transport.

c. Transport to radiography facility.

Any carrier normally used for animal transport is acceptable, but care should be taken to consider the animal's health and that of other patients in the facility. It is recommended that an arrival time be agreed upon; and, if it is a clinical facility, that entrance location and access to a transport gurney or cart be arranged in advance. It may also be advisable to alert the security services in larger hospitals in order to arrange parking for any large transport vehicles.

NOTE: Photography, in the radiography facility, of the procedure is also recommended however, ***it is imperative to get permission from any clinical facility, particularly a human radiology unit, before any photography is done.*** *Although most facilities will allow photographs, they may be understandably limited or forbidden for reasons of protecting patient privacy.*

d. Prepare the animal for placement on the imaging table and for examination.

Anesthetize or tranquilize the animal based on best veterinary practice, anticipating up to 10 minutes of sedation needed for handling for plain films and 30 minutes minimum for one CT or MRI procedure. During the exam, an attendant or veterinarian should be in the room and wearing proper shielding for CT or MRI during the exam in order to observe the condition of the sedated animal and to notify the scanner technician of any movement by the animal while scanning is in progress.

All Postmortem Animals and Extracted Tissue Preparation:

Most of the preparation of postmortem material is the same as for live animals described previously, with the exception, of course, of sedation and the added need for proper sealed containers for any decomposing material.

Chilling, Freezing, Fixation

Preparation and scanning of the animal or tissues should be done as soon as possible postmortem or postextraction, preferably without the need to hold for more than a few hours.

In most cases, there will not be a preparation area connected to the scanner facility; therefore, preparation will need to be done on the beach or in the necropsy facility.

All bodies should be cooled as soon as possible. If several hours' holding are required, it is reasonable to consider placing dry ice, if available, in the mouth cavity and blowhole to cool the brain and in the rectum to chill the abdominal area. The body may also have ice packs or cold packs applied to the abdomen and head regions.

If the animal or tissues must be held longer, they should be packaged (described in the next section) in a container with as little air surrounding the specimen as possible and placed in a chiller at approximately +4 degrees C. If a chiller is not available, the bagged animal can be placed in a vat of ice or in a second bag with ice between the layers. To prevent artifactual water deposits or bloating and dilution by osmotic processes, the unpackaged animal should not be immersed directly into water or an ice bath. Animals may be held in a chiller for several days if necessary. If chilling is not possible, freezing is a reasonable alternative but can create artifacts. As with chilling, the animal should be sealed in a bag with little air surrounding it and placed in the freezer by suspending or on cushioning to avoid compression of major organs during the chilling and freezing process. If frozen, the animal may be ×-rayed or CT-scanned without thawing. However, because MRI depends upon fluid molecular mobility, all material must be fully thawed before MRI scanning.

Formalin or other fixative materials should be handled with the usual procedures for fixation and then packaged with the same precautions against leakage or contamination as outlined subsequently. The imaging facilities should also be contacted to ascertain if they have any regulations related to fixatives. Above all, care should be taken to prevent leaks or fumes. Small specimens can generally be scanned in their fixative in all modalities and may not require removal from containers.

Packaging and Transport Preparations

a. Photo-document and clean the animal (see live in earlier text) to the extent feasible to remove superficial sand, sediments, and mud

b. Package the animal or specimen and transport to the imaging facility

Proper packaging to prevent odor or fluid leaks during transport and imaging is the most important step in this process. Fortunately, most animals can be placed in plastic bags or body bags that may be used in a scanner and therefore can be prepared and sealed prior to entering the clinic or scanner area.

The basic rule is transport in one or more bags and examine in a triple bag. The following is an ideal situation, modify as needed:

Place the clean animal in a clear or translucent, thick plastic bag with disposable diapers or other absorbent material and seal the bag securely with nonmetallic tape or ties. Then place it in a second sealed bag for transport, again placing some absorbent material between the bags. If available, place the double-bagged animal in a body bag or other sturdy container, with sealed icepacks surrounding the animal, for transport. The main idea is to keep the animal cool and to avoid leaks from the inner or outer bags. Loose ice should be avoided so that pooling does not occur that will interfere with MRI and make the specimen harder to handle. Tear-resistant paper bags, sometimes called Grainger bags, are also available from some industrial suppliers and may be used for MRI. Be sure to check, however, that they do not contain any ferrous staples or other metallic elements.

As noted previously, it is recommended that an arrival time be agreed upon, and, if it is a clinical facility, that entrance location and access to a transport gurney or cart be arranged in advance. It may also be advisable to alert the security services in larger hospitals to arrange parking for any large transport vehicles. It is also helpful to assure the facility that you are aware of the need for proper containment of the specimen and for consideration of their patients.

c. Prepare the animal for placement on the imaging table and for examination.

In general, the animal can be examined inside the double bagging. Some body bags have nonmetal zippers that do not interfere with either CT or MRI and therefore will not have to be removed. In most cases, however, the animal will have to be removed from the outer packaging, but can be left in the two plastic bags. It is advisable to have clean bags to use at the scanner if needed. If performing MRI, the absorbent materials

may need to be removed to reduce artifact; therefore, come prepared also with gloves and trash bags to handle waste removal and to minimize odor.

Scanning and Imaging Protocols:

The following procedural outlines apply to imaging both live and postmortem material for most odontocetes, pinnipeds, and extracted mysticete and sirenian tissues. Longer sequences and higher ×-ray dosages are, of course, possible in postmortem or extracted tissues. Whenever time and costs allow, multiple sequences for different tissue types should be employed for optimizing images, particularly of the head areas or ears.

Plain-Film Radiography

Exposures depend heavily on the thickness of the area being imaged and the relative densities of material within the image field. In the absence of prior experience with marine mammals, it is recommended that, for small to medium animals, an initial image be obtained using parameters for human abdomen or for large dogs if imaging is being done in a veterinary facility. For large marine mammals, substantially greater voltages and times will be required for the head and lower abdominal regions. Projections to employ are as follows:

- Head—dorsal-ventral and oblique, orbit to occiput; lateral for skull base trauma
- Thorax—dorsal-ventral
- Abdomen—dorsal-ventral and lateral for genitourinary track and for any suspicious areas.

CT

Scouts should be obtained to cover the regions of interest in as few sequences as possible. Transaxial scans should be obtained using a spiral-scan protocol, particularly for live animals, and images should be formatted in both soft and bone windows. Rapid reconstruction images obtained during scanning can be used to determine whether the proper areas and detail were obtained; the animal can then be removed from the scan area while full resolution 2-D and 3-D images are produced and reviewed.

The following values should be considered as general suggestions and modified as necessary to provide best image

quality. More than one spiral set may be required to cover the entire body. Overlap sequential spiral sets by one full section. The following are suggested values for image slice thickness for proper diagnostic surveys of each region:

- Head:
  - 3 mm increments through the entire head
  - 3 mm feed (pitch of 1)
  - 1 to 1.5 sec rotation time
  - 180 mAs, 120 kV
  - Bone reconstruction: 1 to 3 mm U90s, w4000 c1000
  - Soft-tissue reconstruction: 1 to 3 mm H30 or H40, w500 c50
- Brain: 1 mm increments, reconstructed as for soft tissue
- Ears:
  - 0.5 mm slice increments
  - 0.5 mm feed (pitch of 1)
  - 1.0 sec rotation time
  - 180 mAs, 120 kV
  - Bone reconstruction: 0.1 to 0.3 mm U90s, w4000 c1000, smallest FOV (field of view) possible
- Thorax and abdomen:
  - 3–5 mm increments through the entire head
  - 5 mm feed
  - 1 to 1.5 sec rotation time
  - 180 mAs, 120 kV
  - Bone reconstruction: 1 to 3 mm U90s, w4000 c1000
- Soft-tissue reconstruction: 1 to 3 mm H30 or H40, w500 c50
- Lung image reconstruction:

If available, use specialized lung kernel or reconstruct with windows near +1200 and centers near –200.

If 180 eff mAs is not available, request exposures to produce ~160 mAs. Lower voltages may decrease image quality, particularly in odontocetes, and increase artifacts in the images, especially in sections with dense bony elements. In addition to the image parameters noted previously, which are typical for

odontocetes, the following values should be tried to optimize imaging of any animal:

- Bone window reconstructions:
  - High-resolution 80-95 kernel, w 2800 to 4000; c 600 to 1000
- Soft-tissue reconstructions:
  - Mid to soft tissue 30–40 kernel, w 200 to 500; c 50 to 150
- Lung reconstruction:
  - Mid tissue 50 kernel, w 1000 to 1500; c –100 to –500

Note: If any tissues are extracted and fixed for further examination it is recommended that they be rescanned as isolated tissues prior to further dissection or histologic processing in order to optimize imaging of the substructure of the tissues of interest. The same parameters as listed previously apply, but the field smallest FOV possible (generally one-tenth the maximum image field) that will encompass the specimen should be used.

MRI

MRI is recommended for the head if brain lesions or hemorrhage are suspected and for suspected abdominal lesions. If there is suspicion of metallic fragments or contaminants present in the animal, MRI should not be attempted, regardless of whether the exam is on live or dead tissue. Injury to a live animal or attending personnel or to the equipment can occur if ferrous material is present in the magnetic field. Any specimen larger than 80 cm in circumference cannot be imaged in most MRI units. To optimize imaging of the brain, head coils if available should be used. In some cases, larger coils may be available by special order from the scanner manufacturer but may require a special purchase.

Before scanning the head, particularly if it is large and the brain images are the focus of the exam, the blubber, nuchal fat, and semispinalis muscle may need to be removed to decrease the circumference to fit into the head coil. If possible, scan the head outside the plastic bag and in a Grainger bag as noted previously, but be certain to provide a waterproof table cover and to avoid contamination of the head coil. Excess fluid inside the bag and/or pooling around the head can cause signal artifacts, so it is

important to remove standing liquid prior to scanning. Rapid reconstruction images obtained just after the acquisitions are complete can be used to determine whether the proper areas and detail were obtained; the animal or specimen can then be removed from the scan area while full resolution 2-D and 3-D images are produced and reviewed.

The following values should be considered as general suggestions and modified as necessary to provide best image quality. A scout should be completed to ensure that the entire region of interest—e.g., the brain or lungs—will be captured in the planned MRI scan sequence. Initial images should be acquired, at a minimum, in the sagittal and coronal planes. The following are suggested values for image-slice thickness for proper diagnostic surveys of the brain:

- Two-dimensional proton density (PD) and T2-weighted images acquired using a fast spin-echo sequence
- Parameters:
  - TE = 15/106 ms for PD and T2, respectively
  - TR = 8000 to 9000 ms
  - slice thickness = 2 mm
  - flip angle = 180^{o}
  - FOV = 240 × 240 mm
  - matrix = 256 × 256
  - voxel size = 0.9 × 0.9 × 2.0 mm

For thorax and abdomen, similar acquisitions may be used, but slice thickness may be increased to 5–8 mm, and the field of view (FOV) and voxel size increased as necessary.

Viewing, Copying, Exporting, and Archiving Images and Exam Data

Plain film radiographs may be printed on ×-ray film; in larger facilities, they are now available as on-screen, digital images in the same formats as MRI and CT images. The digital-image form most commonly encountered is a DICOM format. DICOM stands for digital imaging and communications in medicine, and is a standard developed by the American College of Radiology Manufacturers Association to define the connectivity and communication protocols of medical imaging devices. It is the current, open architecture standard for

biomedical images. CT and MR images in both 2-D and 3-D reconstructions are now generally exportable directly from the scanner as DICOM copies on CD or magneto-optical disks and as hard-copies on film. In some cases, TIFF or JPG formats may also be available for export or available through post-processing at the facility or through third-party software now available for both Mac and Windows computer platforms. Some of these programs will work to a limited degree with the standard RAM in most computers; in general. however, 1 GB or more is recommended for full functionality of these programs.

It is strongly recommended that, at a minimum, all images produced during an exam be archived in DICOM formats by the facility onto CDs, external hard drive, and magneto-optical disks at the imaging facility. At present, there is no common archive for marine mammal images, but several have been proposed and may become available in the near future. Raw-scan data are not usually retained or archived by the original scan facility, but it is highly recommended that raw data copies be requested in addition to the image files for all significant cases and particularly for all research or legal cases. The raw-scan data are critical for any reformats, data-based magnifications, or accurate HU analyses that may be required at a future date. Some facilities may be unable to archive raw data or may charge additional costs for this archiving.

Interpretation of Images

Radiographic, CT, and MR images should be interpreted by a veterinary radiologist or other professional who specializes in specific anatomical structures (e.g., head and neck trauma, ears, brain, airways, lungs, abdomen, extremities, genitor-urinary). We advise avoiding any interpretation without specific training or consultation because many conditions will produce similar grayscale appearances, and the proper interpretation depends heavily on a comprehensive, integrated analysis of multiple features, including shape, position, attenuation characteristics, and HU values. In addition, marine mammal anatomy is sufficiently different from that of humans and most common veterinary cases that even well-trained conventional radiologists may find these images challenging.

In following text, we provide contact information for a list of individuals who have extensive experience in interpreting marine mammal radiographs. The listings of these individuals is an

unsolicited recommendation by the authors of this manual and does not indicate an agreement for consultation by any of the listed individuals.

- Dr. Darlene Ketten, Woods Hole Oceanographic Institution, Woods Hole, MA, (508) 289-2731, dketten@whoi.edu

  Head and ear structures, lungs
  Blast trauma, fractures, bone lesions
  Image processing, 3-D reconstructions

- Dr. Eric Montie, Woods Hole Oceanographic Institution, Woods Hole, MA, (508) 289-3501, emontie@whoi.edu

  Brain, volume analyses
  Anthropogenic chemicals, biotoxins, parasites
  Image processing, 3-D reconstructions, measurements of brain regions

- Dr. Lori Marino, Neuroscience and Behavioral Biology Program, Emory University, Atlanta, GA, (404) 727-7582, lmarino@emory.edu

  Brain, volume analyses

- Dr. Sam Ridgway, Department of Pathology, University of California, San Diego, CA, (858) 534-0455, ridgway@spawar.navy.mil

  Brain Pathologies

- Dr. Ted Cranford, Department of Biology, San Diego State University, San Diego, CA, tcranfor@mail.sdsu.edu

  Airways and Melon

- Dr. Joy Reidenberg, Mount Sinai School of Medicine, New York City, NY, (212) 241-7563, joy.reidenberg@mssm.edu

  Larynx, airways

- Dr. Frank Fish, Department of Biology, West Chester University, West Chester, PA, (610) 436-2460, ffish@wcupa.edu

  Limb and propulsive structures

Data Analysis

Sectional anatomy imaging (CT and MRI) offers many advantages. It creates a permanent archive of images of anatomical structures, without the disruption of dissection, and allows us to view rapidly the anatomy of freshly dead, stranded specimens at a much finer scale. CT and MR images also allow us to produce three-dimensional models of anatomical structures to examine spatial relationships and provide accurate volumetric details. Furthermore, the images can be used to guide dissections for sampling smaller pathologies that may have been missed in conventional necropsy.

CT and MRI Scans Completed in Cetaceans

Appendices 4-1 and 4-2 list species for which CT or MRI scans have been completed at WHOI and for which images are available for review by researchers or veterinarians. Access to these images can be obtained via the WHOI CSI website (http://www.whoi.edu/csi). Numbers in the tables reflect tissue scan sets, not numbers of individual animals. In some cases, multiple tissues from one animal or multiple protocol scan sets were obtained from one tissue. Many other examples may be available through other laboratories or other facilities, and we encourage the formation of a centralized data base and the exchange of cross-listings of available image data sets.

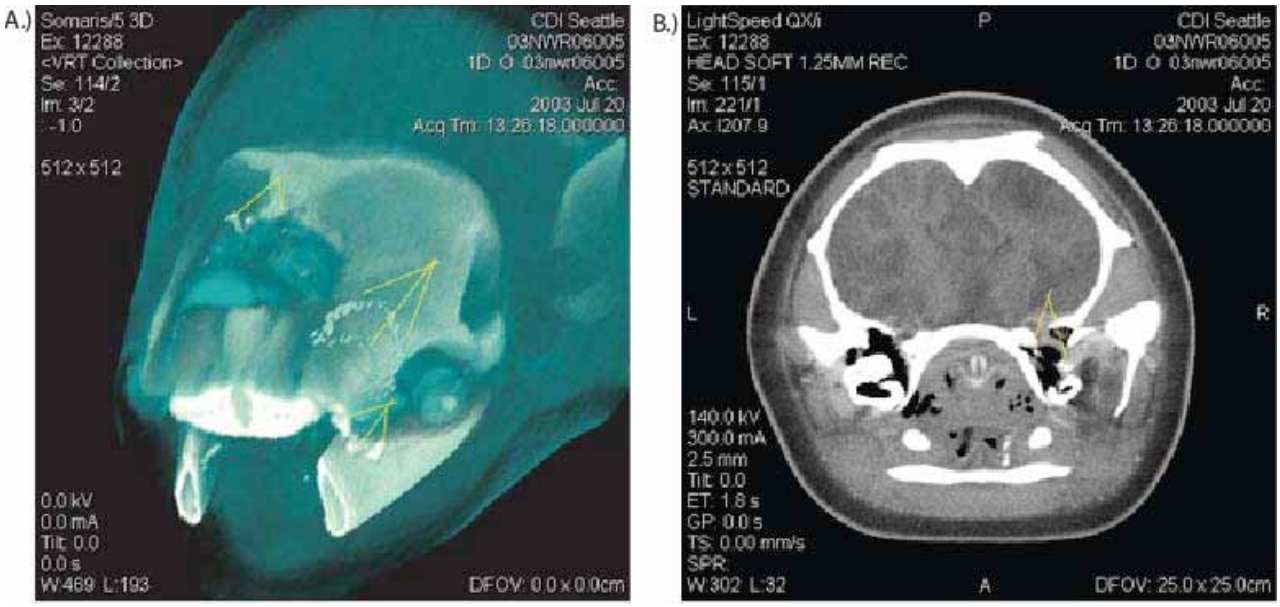

Figure 4.3. CT scans of a harbor porpoise with calcified parasites. Left: A 3-D reconstruction of the midregion of the head shows multiple extensive calcified parasites (white tracks, yellow arrows) that were located near the right eye and extended over the melon. Right: A 2-D transaxial image at the level of the ears shows a peribullar soft tissue mass with small calcified inclusions that represent a group of nematodes and calcified cysts,(yellow arrows). Additional cysts are evident as bright white inclusions in the tissues on the right of the esophagus. Parasites are a common finding in this species in the middle ear and retrobullar sinuses, but the extensive calcified tracks are an unusual finding. (Images copyright D. Ketten, 2005)

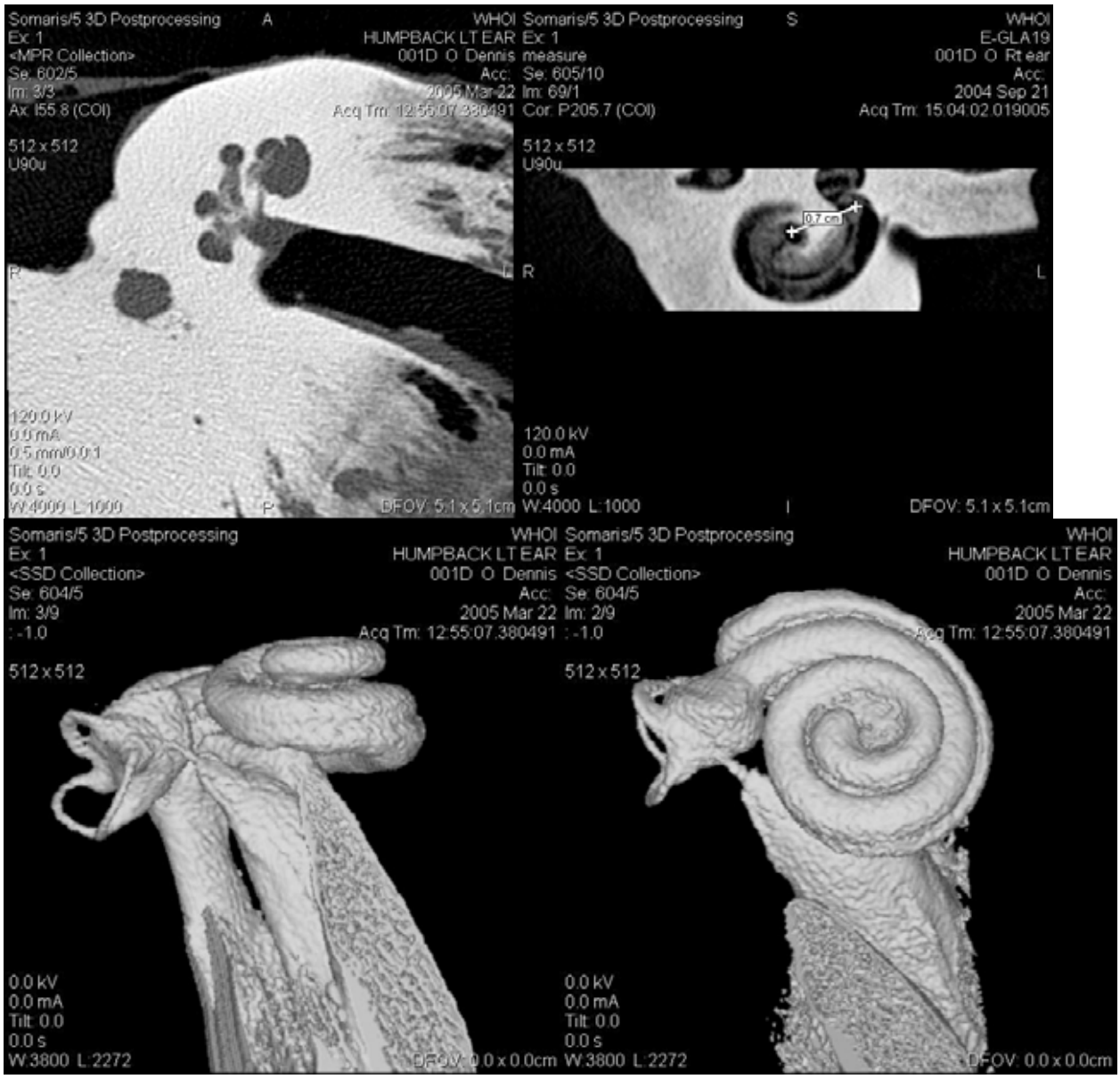

Figure 4.3. Mysticete Inner Ears. 2-D CT images (top) show mid-modiolar and coronal views of the inner ear of a right whale *(Eubalaena glacialis)* that illustrate the number of turns (left) as well as soft tissue neural tracts (right). 3-D images of a humpback whale ear *(Megaptera novaengliae)* in similar orientations show the dimensions and angular relationship of the vestibular canals and the cochlear canal. (Images copyright D. Ketten, 2004)

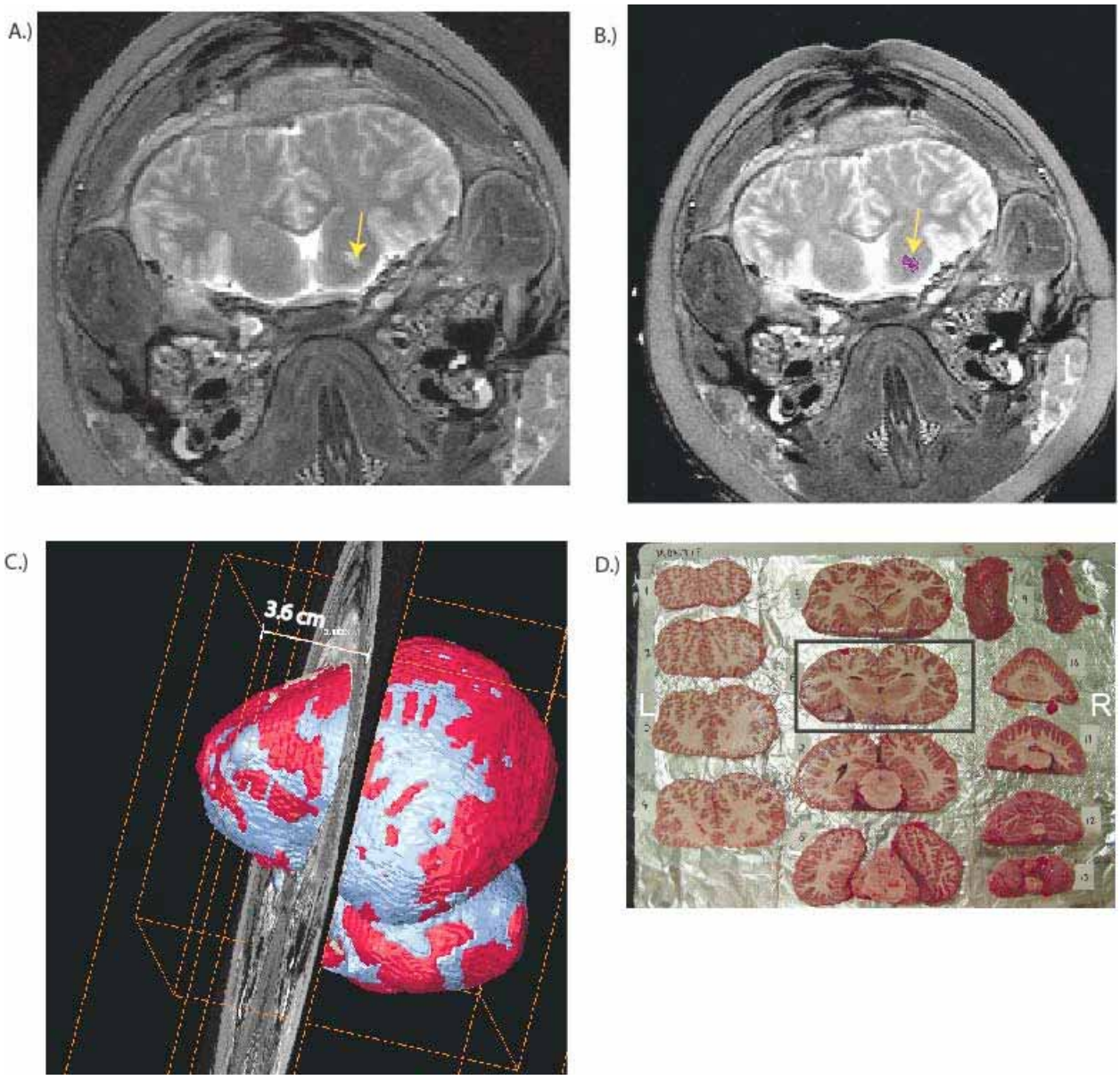

Figure 4.4. 3-D models of the brain in conjunction with virtual reconstructions of lesions as a guide to small lesions. A.) The brain lesion in this Atlantic white-sided dolphin (indicated by the yellow arrow) was identified by MR imaging. B.) A 3-D model of the brain was constructed to measure the distance of the lesion (purple) from the anterior boundary of the frontal lobe (3.6 cm). C.) Red = gray matter; blue = cerebrospinal fluid. This distance was measured on the gross brain to find the lesion. D.) The black rectangle indicates the brain section that contained the lesion. (Images copyright E. Montie, 2006)

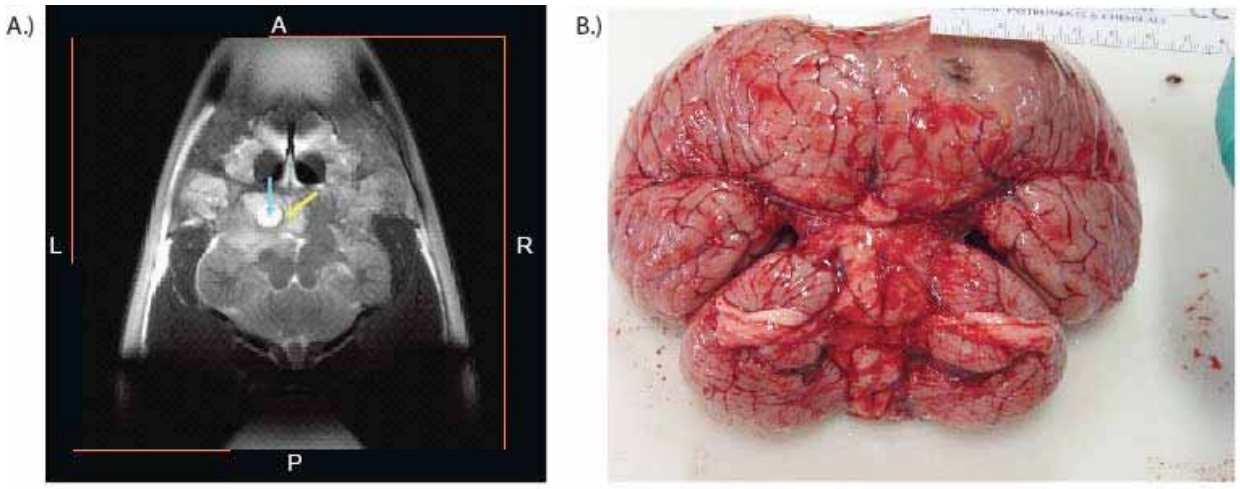

Figure 4.5. Brain lesion in CCSN04-177-Dd common dolphin. A.) The MR image indicated a 2 cm spherical abscess identified in the left frontal lobe. A halo of damaged tissue is noted by the yellow arrow. A fluid-filled necrotic core is indicated by the turquoise arrow. B) The lesion was revealed during the dissection. Brain removal revealed pus in the left frontal lobe. (Images copyright E. Montie, 2006).

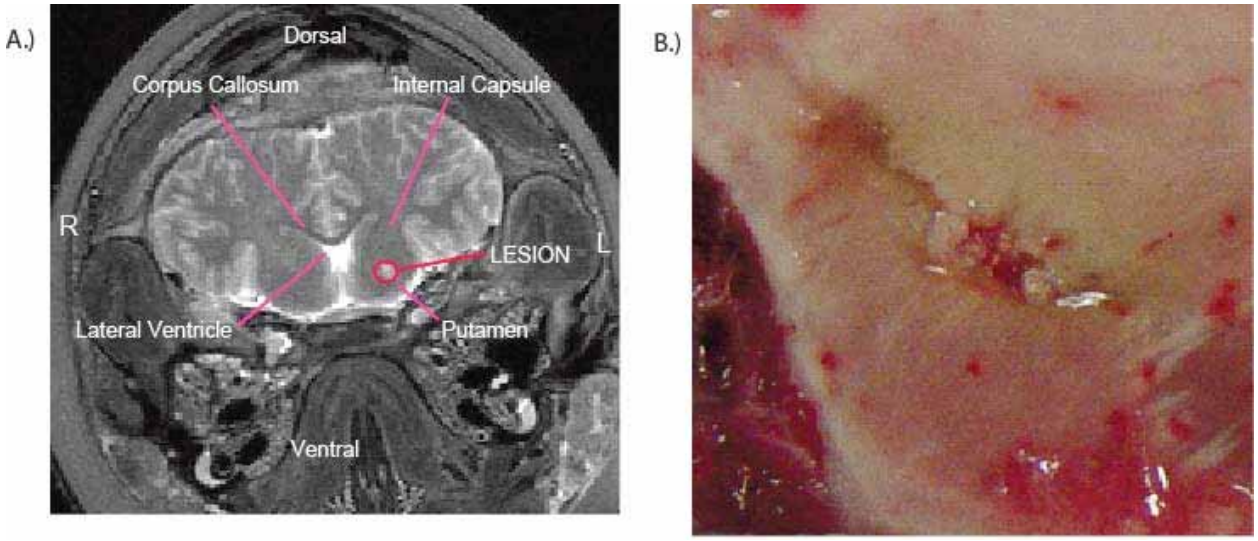

Figure 4.6. Brain lesion in CCSN04-191-Dd common dolphin. A.) The MR image indicated a small lesion in the region of the putamen and globus pallidus or, collectively termed, the lentiform nucleus. B.) The lesion was revealed during the dissection. (Images copyright E. Montie, 2006.)

Appendix 4.1
CT Scans and Archived Images of Cetaceans

WHOI COMPUTERIZED SCANNING AND IMAGING CENTER (WWW.WHOI.EDU/CSI)

Classification	Scientific Name	Common Name	Scan Sets
Fissipedia	*Enhydra lutris*	sea otter	8
Mysteceti	*Balaenoptera acutorostrata*	Minke whale	9
Mysteceti	*Balaenoptera musculus*	blue whale	2
Mysteceti	*Balaenoptera physalus*	fin whale	1
Mysteceti	*Eubalaena glacialis*	right whale	24
Mysticeti	*Eschrichtius robustus*	gray whale	4
Mysticeti	*Megaptera novaeanglia*	humpback whale	9
Odontoceti	*Cephalorhynchus hectori*	Hector's dolphin	1
Odontoceti	*Delphinus delphis*	common dolphin	34
Odontoceti	*Tursiops truncatus*	bottlenose dolphin	65
Odontoceti	*Phocoena phocoena*	harbor porpoise	75
Odontoceti	*Delphinapterus leucas*	beluga whale	4
Odontoceti	*Feresa attenuata*	pygmy killer whale	1
Odontoceti	*Grampus griseus*	Risso's dolphin	18
Odontoceti	*Globicephela macrorhyncus*	short-finned pilot whale	34
Odontoceti	*Globicephala malaena*	long-finned pilot whale	12
Odontoceti	*Kogia breviceps*	pygmy sperm whale	22
Odontoceti	*Kogia simus*	dwarf sperm whale	6
Odontoceti	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	39
Odontoceti	*Lagenorhynchus albirostris*	White-beaked dolphin	3
Odontoceti	*Mesoplodon bidens*	Sowerby's beaked whale	13
Odontoceti	*Mesoplodon densirostris*	Blainville's beaked whale	6
Odontoceti	*Mesoplodon europaeus*	Gervais' beaked whale	9
Odontoceti	*Mesoplodon mirus*	True's beaked whale	1
Odontoceti	*Mesoplodon sp.*	beaked whale	3
Odontoceti	*Monodon monoceros*	narwhal	4

Classification	Scientific Name	Common Name	Scan Sets
Odontoceti	*Physeter catadon*	sperm whale	15
Odontoceti	*Peponocephala electra*	melon-headed whale	1
Odontoceti	*Stenella attenuata*	pantropical spotted dolphin	4
Odontoceti	*Steno bredanensis*	rough-toothed dolphin	3
Odontoceti	*Stenella clymene*	Clymene dolphin	1
Odontoceti	*Stenella coeruleoalba*	striped dolphin	6
Odontoceti	*Stenella frontalis*	Atlantic spotted dolphin	1
Odontoceti	*Stenella longirostris*	spinner dolphin	1
Odontoceti	*Ziphius cavirostris*	Cuvier's beaked whale	9

Appendix 4.2
Cetacean Brain MRIs (for access contact E. Montie)

Field ID	Species	Common Name	Sex	Length
CCSN04-177-Dd	*Delphinus delphis*	common dolphin	m	209
CCSN04-195-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	m	192
CCSN04-217-Dd	*Delphinus delphis*	common dolphin	f	210
CCSN04-218-Dd	*Delphinus delphis*	common dolphin	m	139
CCSN04-219-Dd	*Delphinus delphis*	common dolphin	f	169
CCSN05-014-Dd	*Delphinus delphis*	common dolphin	f	205
CCSN05-014-Fetus-Dd	*Delphinus delphis*	common dolphin	m	41
CCSN05-037-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	f	206
CCSN05-038-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	f	208
CCSN05-039-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	f	211
CCSN05-039-Fetus-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	m	44
CCSN05-040-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	f	204
CCSN05-040-Fetus-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	m	54
CCSN05-084-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	m	156
CCSN05-231-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	f	137
CCSN05-232-La	*Lagenorhynchus acutus*	Atlantic white-sided dolphin	f	185.5
CCSN05-236-Dd	*Delphinus delphis*	common dolphin	m	179
CCSN05-237-Dd	*Delphinus delphis*	common dolphin	m	166