Odontocete Necropsy Procedure

(Sentiel Rommel, William McLellan, Alexander Costidis, Michael Moore)

This is a description of the procedures for the necropsy of odontocete carcasses. Several marine mammal programs have standard protocols [Alaska M.M Tissue Archive Project Revised Collection Protocol – Becker et al, Small Cetacean Dissection & Sampling – A field Guide – Jefferson et al – NOAA – NMFS – SWFSC 198 -Killer Whale Necropsy And Disease Testing Protocol by Raverty and Gaydos, Hensley et al 2005 heart protocol?], many of which are adequate to properly sample odontocetes.

These protocols, however, tend to focus on specific conditions or sampling needs. In recent years mass strandings associated with high intensity underwatersounds have presented new lesions associated with fat and gas emboli. This procedure incorporates new suggestions to document these lesions and provide guidelines for a complete examination of each case.

Hierarchy - person on beach decides (with advice from protocol and specific *authorities - to be decided when?*) A thorough examination may be constrained by many variables – time, equipment availability, condition or access to a carcass, to name a few. Although the entire procedure presented here may not be feasible in all cases, it is important (particularly in mass stranding investigations) to be consistent in following guidelines. Modifications to procedures should be noted.

Authority / Jurisdiction. As protected wildlife, odontocete 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.

These directions are to accompany several illustrations of the left lateral aspect of a carcass (Appendix 1 is a copy of the illustrations of *Tursiops* gross anatomy from the CRC Handbook (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).

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NECROPSY PROCEDURE [1]

There are three purposes of a necropsy: to collect natural and life history information, to scientifically determine the most probable cause(s) of death and 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 a mortality. One should document both immediate and any secondary conditions contributing to a 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, each carcass is processed in order to obtain general and detailed biological information. General information (such as – make a list – maybe a TEXT BOX to help highlight points) collected upon primary examination must include: morphometrics, total body weight (TBW), description and measurement of wounds and scars, photographs and sketches for individual identification, and epibiota. More detailed information may include: gross appearance of organs and tissues, description of fat stores and fullness of the gastrointestinal (GI) tract, documentation of natural, anthropogenic, and pathogenic 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

[1] This written protocol is based on procedures followed at the Florida Fish and Wildlife Conservation Commission

carcass and sampling logistics, and may provide useful insights into normal and abnormal conditions.

Preparing for a Necropsy

Tissue labels should be written with a bold 'permanent' marker (e.g., pencil or fine point *Sharpies*: http://www.sharpie.com/sanford/consumer/sharpie/index.jhtml?_requestid=121078) and histo pens for tissue cassettes . 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 should be made of waterproof non reactive material – for example Tyvek or *Write In The Rain*. Metal tags or tag parts should be non reactive metal – samples should be packaged so that tags are not in direct contact with sample.**

SAMPLING – [NOTE: put this section at the end – one sheet per sample type (so if it changes you would only have to change one page) and I would put a scale image of the size of the sample. So if someone was sampling for biotoxin, they could copy off one sheet and take it as a guide (laminate and reuse). I would also include a spreadsheet checklist for all samples (like what CCSN or the OHHI protocol – uses) to keep all of the samples on one page.]

Tissues and labels include (one tissue type per container (e.g., histology, microbiology, virology, blubber for archive toxicology, reproductive tract) unless otherwise specified:

(FWC) Marine Mammal Pathobiology Laboratory (MMPL) and those followed at the University of North Carolina

1. Test Modality/Sample
2. Purpose of Test
3. Samples Required
4. Storage Specifications

Archive Toxicology (approximately 5 x 10 x 2 cm samples) - from fresh carcasses or carcasses of special concern: blubber, kidney, liver (vacuum sealed[2], frozen—temp?), water samples from stranding location?

NIST protocol???

Harmful Algal Bloom (HAB) Toxicology for ELISA (thumb-size samples): liver, lung (cranial pole?), urine (if not available, then kidney), contents from stomachs or duodenal ampulla (whirl-pack, frozen), feces, blood.

Histology - (max thickness 0.5 cm); all tissue types, see histology check list (Appendix 6) samples should be thin enough for the formalin to penetrate and to properly fix the tissue, no larger 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 (neutral buffered formalin): all major organs, appropriate lesions, all major lymph nodes - the ratio of formalin volume to tissue volume should be at least 10 volumes of formalin to1 volume of tissue- for tissues that are not unique (e.g., lymph nodes, right and left lungs &

Wilmington

[2] Vacuum sealers, available from meat precessing suppliers, are useful (but not necessary) for archive storage, because they reduce freezer burn and minimize package volume. Sealable plastic bags are adequate, be sure the bags are well sealed to reduce freezer burn.

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

adrenals, etc.) and for which it is important to distinguish, identifiers such as laundry tags, spaghetti tags, or histology cassettes clipped on to an edge of the tissue should be used. Photos of these products for international audience?

Imunohistochemistry – (<6 days in NBF) D Rot – state basic principle – transfer to alcohol if processinf delayed?

Entire head -

Eyes - collect aqueous humor, collect eyes entire (sans fluid from one) [need collection procedure need fixing procedure – Bouins Solution? Inject eyes w/ formalin if Bouins disliked]

'Core' Temperatures - postmortem temperatures (e.g., epaxial 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 do and can help fix time of death relative to time of recovery. 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]

Epibiota - Barnacles, _____; other (copepods – *Penella sp*.)

Genetics -

Tissues for Ancillary Diagnostics: Microbiology, Virology, and Immunology

Test Procedures:

- **Bacterial/Fungal Culture and Sensitivity**
- **PCR**
- **Culture (bacteria and fungus)**

Immunology - microbiology and virology – Cultures (&/or -80^{o} freezer?) of the blowhole, anus, U/G opening, eye, and mouth are in descending order of importance – other organs? Liver, spleen, lymph nodes, kidneys, abdominal cavity when first opened

Blood culture

Cerebro-spinal (CSF) fluid – need a procedure CSF degrades quickly, so freezing some; putting some with formalin may help to preserve cells if can't analyze within a few hours; labs-alk phos, creatinine, TOTAL PROTEIN

Blood - acidosis, pH, RBC, creatinine, ... save serum, plasma, WBCs (check w/ Craig Harms)

Reproductive system

- males: cross section (0.5 cm thick) from the center portion of left testis & section of vas or epididymis

-females: 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 and label one horn

Skeleton - flense, dry, and store (frozen if practical) for final osteoprep

Teeth – for age & life history, extract store in _____

Stomach contents - collect contents from each stomach in separate labeled containers [(plastic bag, freeze at ..... ^{0}C) - and freeze for further analysis. There are three basic

analysis – identification of prey, identification of parasites and toxicology (biotoxin or anthropogenic). As each type can impact the others these analysis should be carefully coordinated.

Tympano-periotic bones ---------D Rot: This is one that I wonder about as well..if formalin ok? Do we need to figure out some sort of gravity perfusion? Should we be vacuum sealting these tissues (well, guess a cetacean deals with greater pressure than that.

? Note that beakers have a mastoid process and a slightly different periotic morphology – maybe put the skull morphology figs here or refere to them in an appendex here? Do we worry about large whale ears here too?

Special tissues: packaged and labeled (inside and out) for appropriate storage as per instructions for the requestor

Curation of samples collected

Microbiology and virology samples need to be mailed priority (if fresh – if on "transport media" I think regular shipping would be ok) to appropriate laboratories.

Maybe this could be broken out into categories:

Bacteriology
Sample handling testing
Shipping

Virology
Sample handling testing
Shipping

Make sure to note whether the sample or samples are to be "pulled" (i.e. sampled as one), test-aerobic, anaerobic, both, sensitivity required, region/tissue of origin, the date it was collected, and the carcass Field ID. Keep in mind that some bacteria and fungi do not handle extreme temperature and that room temperature microbiology swabs may be advisable. Contact your reference lab regarding acceptable sample types. Pack the sample with dry ice (most express

carriers do not like dry ice and dry ice can only be shipped – by air – if a "trained" shipper has packed it and filled out the HAZMAT form (big fine) – otherwise it can be shipped by ground transport) — (Also I don't think dry ice is a good idea for bacteriology – most are ok on transport media at room – temp) and some Styrofoam chips to fill up any extra space. Schedule the pick-up for late afternoon so the samples can stay refrigerated as long as possible before being shipped priority overnight. Notify the destination laboratory before shipping to assure the samples can be correctly handled on arrival.

Packaging large organs/parts: attach labels with both the sample (suitably protected if necessary) and on the outside of the container.

Frozen samples for archive storage -80^{o} other

Frozen in ultracold, transport in liq N_2? See NIST guidelines – ideally one should try to use a "dry shipper" charged with LN2 a dry shipper maintains LN2 temperature but has no free liquid. These dry shippers can be shipped priority mail as non hazardous material (they can, however, be heavy and packages greater than 75 pounds may have to be dropped at a fed/ex depot.) Will likely need N2 in the field for storage prior to ultracold and/or shipping.

Other curatorial guide lines - WAM?? Mead - CWP? Yamada? Anton?

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. NOTE: If at all possible record as much information as possible about the carcass and it's collection. This can make a big difference (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 is the most important part of determining circumstance of death.]

Wound and scar data sheet: (blank *Wound & Scar Data sheet* in Appendix 2)

Wounds: Wounds are relatively recent superficial lesions that may have some important part 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 (Figs. 1&2). If there are multiple wounds from a single event (e.g., a series of similar lesions), label each lesion in the wound series sequentially (histo and frozen samples should be matched as well).

Scars. Scars are superficial skin lesions that are healed or show significant resolution, and have recovered sufficiently to unlikely be 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. Re-pigmentation 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: Sketch the major scar patterns on the wound and scar data sheet. Note whether each lesion is a wound or a scar. If there are prominent scars that extend down the sides or are found on the ventrum, sketch them on an appropriate projection. If there are signs of human interaction (HI) then follow specific procedures to document these lesions. (W/C and HI procedures as appendices?)

External photographs:

A 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. If possible, photograph each image perpendicular to 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 3 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 & left), ventral full body photos and close ups of specific lesions are recommended. The whole body photos should be taken as perpendicular as possible to the long axis of the carcass (and of the scale) so that

measurements can be made from the photos (how about a standard photo illustrating good technique). 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 & weight (blank *Necropsy Carcass External Data Sheet* in Appendix 4):

If a hoist or front-end loader is available, the carcass can be lifted by straps and weighed (Fig.1 insert). 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, gender, age, superficial lesions, and condition) must be recorded (Fig. 3). If the carcass is intact, at least a total body length (TBL) must be measured 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, then 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. As observations are recorded they should be checked off on the *Necropsy Morphometrics Data Sheet* (Appendix 4).

NECROPSY NARRATIVE (blank *Odontocete Necropsy Narrative* data sheet in Appendix 5):

Each necropsy should be permanently recorded as a written narrative using a word

processor. 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.

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. 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 assure that all statements are accurate. This final read also serves as a check to make sure all steps in the procedure have been completed.

Carcass Condition (put in as an appendix *7 quote from Geraci & Lounsbury*):

The details recorded for each part of the necropsy procedure are dependent 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 can not be collected after the carcass has been disposed of. Additionally, some biochemical assays (i.e., ELISA test for HAB) can be completed reliably on even autolyzed tissues.

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 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 may often times 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 animal prior to death. The duration of the condition is also variable, but is typically measured in hours or, under cool conditions, perhaps 36-72 hours (we should consider if supersaturation of N_2 or depletion of O_2 may affect rigor). 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/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. 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 non-verbal record of all significant observations. Again, take wider view photographs before close-ups, for perspective. Whenever possible, also photograph structure/organ/lesion after removing and placing on a white background (often improves color in photos). Use of a color strip can also help with standardization of color perception and description. When documenting anthropogenic lesions, Polaroid photographs should be added to the electronic and/or film record; the Polaroid pictures help the reviewer when editing necropsy reports. Polaroid pictures can be written on with a permanent marker. This provides prosectors an additional medium on which to record observations or comments.

Necropsy Report Outline

I. HISTORY

II. GROSS DESCRIPTION

A. EXTERNAL

B. ABDOMINAL ORGANS AND GI TRACT
1. ABDOMINAL CAVITY
2. STOMACHS
3. INTESTINES
a. DUODENAL AMPULLA
b. PROXIMAL INTESTINE
c. MID INTESTINE
d. DISTAL INTESTINE
4. PANCREAS
5. SPLEEN
6. LIVER

C. UROGENITAL SYSTEM
1. FEMALE REPRODUCTIVE TRACT
2. MALE REPRODUCTIVE TRACT
3. URINARY BLADDER
4. KIDNEYS

D. VASCULAR SYSTEM
1. HEART
2. GREAT VESSELS
3. PERINATAL INDICATORS
4. OTHER – all of the new info – all of the questionalble paths that emboli can take or the places they may be engendered?

E. RESPIRATORY SYSTEM
1. DIAPHRAGM
2. LUNGS

F. LYMPHOID TISSUES
1. LYMPH NODES
a. SUPERFICIAL and/or ASSOCIATED with an EXTREMITY
b. THORACIC
c. ABDOMINAL
2. THORACIC DUCT & lymph channels of mesenteries
3. Other lymph channels
4. THYMUS

G. HEAD AND NECK
H. SKELETON
I. OTHER – how about a section on FATTY TISSUES? Or FACIA & FATS??

III. MORPHOLOGICAL DIAGNOSIS
A. SIGNIFICANT FINDINGS

IV. MOST PROBABLE CAUSE OF DEATH
MARINE MAMMAL NECROPSY REPORT FORM (see Appendix 3 for blank form).

V. FORMS, DATA SHEETS.

FIELD I.D. (unique identifier) eg,. **MSW0312** SPECIES *Tursiops truncatus*

RECOVERY DATE (*date animal was recovered, rescued, or died*) NECROPSY DATE

Gender M / F TL *(total length*) cm WT *(weight)* kg CONDITION (*decomposition)*

I. HISTORY *(sighting, rescue, salvage, recovery information).*

History of the animal since its rescue or between the time of the initial sighting, to the recovery of the carcass and necropsy. The history of the individual may be important in interpreting the results of the necropsy. For example, knowing which side of the carcass was recumbent can help interpret gross observations.

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 along the north fishing 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.

Procedure: 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, transportation, refrigeration, and in the case of mass strandings the temporal and spatial occurrence of each carcass and of military activities and seismic testing.

II. GROSS DESCRIPTION

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, intact) emaciation (peanut head, rib outlines, body folds)).*

This section describes the external appearance of the carcass. In addition to verbal and quantitative descriptions of wounds and scars, a verbal description of the external appearance of the carcass must be dictated. If human interaction (HI) is suspected (e.g., military or seismic

surveys nearby) or observed (e.g., entanglement in fishing gear) then a HI report should be completed (Appendix 2).

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.

Procedure: Be sure to note which side the carcass has been laying on, as 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), peeling due to exposure. Note the presence and types (species) of barnacles or other epibiota, record the dimensions of the largest individual

of each type of epibiota. Describe fitness/emaciation general appearance. Collect samples if appropriate. Examine the body openings and describe any lesions or foreign materials (e.g., fishing gear) that are present.

*Initial incisions (*Fig. 4). 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 explosively extruded from the carcass. With the carcass right laterally recumbent (if possible), make cuts through the blubber and superficial muscles as illustrated in Figure 4. Make cuts parallel to the long axis of the body along the dorsal and ventral midline, and perpendicular to long axis at nuchal and umbilical regions (or caudal insertion of dorsal fin).

Keep any HI evidence such as fishing gear or lines; if it looks like it might be a criminal case notify law enforcement and make every effort to maintain chain of custody/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) then 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 (Fig. 4). Examine and measure nuchal fat thickness. Be sure that the skin and blubber are not stretched from bloating when measured; 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 Figs. 4 & 5).

If the carcass is a 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 U/G opening.

Collect a sample of blubber at the level of the umbilicus, on the dorsal or dorso-lateral aspect of the carcass. Once the blubber is removed from the dorsal body, epaxial muscle samples can be collected (collect histology and archive toxicology samples from.....). If practical and gas bubble disease is suspected, collect a cube of muscle ~ 10cm on a side and place it in a sealed plastic bag. Exclude as much air as possible and ensonify with a ultrasound unit set at – kHz to check for supersaturation. D-Rot: My comment here is that this really needs to be explained regarding the principles of why and how this works. Who will be doing the US? What do you do for quality control? What muscle is best? Why muscle? (I'm asking because I can tell you I don't' know!!!!!!!...but I want to!)

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 lateral, ventral and medial aspects for vascular lesions before continuing. Look for evidence of emboli and describe (include photographs) if present.

The intestines and gonads may be exposed at this point. Without cutting anything, examine the serosal surface of all organs visible at this time and describe the occurrence of any lesions (e.g., blood clots, fibrin, adhesions, lacerations, parasites, and gassiness). The stomachs and liver may also be visible at this time. The condition of the liver (firmness, sharpness of

margins, 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.

Tissues to be collected. Collect samples of epibiota (in ETOH) if appropriate, superficial lesions (in 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 (an idea formulated at Baltimore, subsequently argued against – check on validity). Blubber and muscle samples for archive toxicology; mammaries if appropriate. Collect a muscle sample for ensonification if appropriate.

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 may be sites of sound-related lesions. Be sure to carefully examine the thoracic and epidural retia before the cranial ribs are removed.

1. ABDOMINAL CAVITY *(fluid description (i.e. color, texture, volume*[4]*) -foreign objects (i.e.,*

[4] To simplify estimates of volume and length, you can measure the sizes of parts of your hand and arm. By submerging each part 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.

blood clots, GI tract contents) -adhesions, mesenteric and perinodal fats)

Normally, the abdominal cavity contains a small amount of clear serous fluid. 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 fluid for microbiology. 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 (photo? Put photo in which Fig?).

Sample narratives:

e.g., The abdominal cavity contained approximately 0.5 liters of clear ascites. NGVL.

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

Procedure: Take care when cutting the 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 before making any additional cuts. (Antonio and Paul can supply photos of this). Note if there is milky fluid in the lymph channels of the mesenteries.

Remove the GI tract (Figs. 5 & 6), starting with the intestines, by cutting their mesenteries at the root, a few centimeters from the intestines. Tie off the ends of the GI tract (at the esophagus and rectum) with string. Remove and examine (Page: 24
We may want to not open the stomach so that an expert can do the stomach contents analysis. Perhaps only take a small sample of the stomach wall (in cases where I want stomach wall but have to save the stomach for analysis, I take a piece and then use string to suture it back up.) the stomachs and duodenal ampulla with the intestines attached. Examine the GI tract and describe its appearance. The pancreas and spleen can be removed before or after removal of the GI tract. Remove and examine the mesenteric lymph nodes and sample for histology and virology (see section **F** below).

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. The entire adrenal glands should be collected and bread-loafed for histology (a small amount from one adrenal can be collected for virology, if appropriate); save to evaluate cortico-medullary ratio (? Maybe Lance Clark's '05, '06 work on the adrenal?).

Pressure from adjacent loops of intestines may leave impressions characterized by distinct color patterns.Describe the patterns but be sure these are not interpreted as lesions. Always note the presence and attempt to quantify contents and parasites or other lesions, and take Polaroid and high resolution (electronic preferred — or 35 mm) photographs.

Note presence, number and location of parasites (*monorhgygma*) on/in the body wall and blubber of the U/G region.

Tissues to be collected: When working with fresh carcasses, be sure to obtain weights of all organs. Measure maximum linear dimensions of organs (particularly gonads & adrenals) if they cannot be weighed. Make sure that excess tissues (i.e. superficial fats, fascia, mesenteries) or internal fluids (i.e. blood, blood clots) are removed prior to weighing, in order to obtain representative weights of the organs themselves *(see Crile and Quiring)*. (Figs. 6 & 7)

When collecting thin-walled GI tract histology samples, be careful that the thin layers are not disturbed; collect an entire 'ring' about __ cm long from each section of the intestine to be sampled. Samples from stomach walls should be _____ (see Fig 6). Do not rub or scrape the mucosa to clean it. Rinse the sample with gently flowing water and then firmly place the sample against the paper.

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/dead)

Normally, the stomachs are empty in most single strandings; 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 / 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 were 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 be determined). Stomachs two and three were empty and the mucosae were unremarkable.

Procedure (unless sent to GI tract contents specialist — in which case keep intact): Open each of the stomach chambers. 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. 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. Take Polaroid and high resolution

electronic (preferred or 35 mm) photographs of any significant lesion. Collect samples for histology, HAB, and stomach analysis.

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

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 7. Subdivide the intestines into three (equal) parts/sections and then collect histology samples from the center of each section. These samples can be collected before the entire intestine in examined or as that general region of the tract is reached.

***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)*

Normally, the duodenal ampulla is loosely filled with bile-stained, watery digesta and mucus, or mucus.

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.

Procedure: Cut open the duodenal ampulla and examine its contents. Be sure to note the quantity, color, and wetness 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

Tissues to be collected: wall of the duodenal ampulla and contents if present; caution with the mucosa.

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/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. The mucosa color should be.....

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.

Procedure: Slit and examine the proximal intestine. 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.

Tissues to be collected: If the carcass is fresh, be sure to collect tissue samples for histology from the wall of the middle section of proximal intestine. Tissue samples should be placed on a stiff paper tag (see above). Additional samples can be collected from specific lesions of interest.

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/dead)*

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. The mucosa is light tan in color and smooth (with longitudinal folds) in appearance.

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.

Procedure: Slit open and examine the entire length of the mid intestine. 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.

Tissues to be collected: If the carcass is fresh, collect a histology sample from the middle part of this region.

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/dead)-*

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. The mucosa is light tan in color and smooth (with longitudinal folds) in appearance.

Sample narratives:

e.g., The distal intestine was empty of contents. Patches of bile stained mucosa were evident and some bile stained, slightly moist feces were also present. No parasites were observed.

e.g., The distal intestine contained wet, poorly-formed, brownish feces. A fish hook was embedded in the proximal third of the colon. The trailing line was embedded in the mucosa, causing a chronic longitudinal contraction of the colon.

Procedure: Slit and examine this part of the intestine. 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.

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

part of this region. Place the mucosal surface of each sample on a paper card to allow it to fix.

4) PANCREAS *-serosal surface description (i.e. color) -texture (fibrosis, extent of decomposition)*

The pancreas is located in the lesser curvature of the U-shaped third stomach (in species, like Tursiops, that have 3 stomachs) proximal to the duodenal ampulla. Take care when separating the pancreas from the adjacent organs and note any indications of fibrosis or parasites in the region. Normally, the pancreas has a light pinkish grey color, it is firm but lobulated. Its enzymes make it autolyze quickly.

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. Pancreas weight was __ g.

Procedure: 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.

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

5) SPLEEN **-***serosal and cut surface description (i.e. color, texture) -lesions -number of accessory spleens*

The spleen is a subspheroid located on the right side of the first stomach. Normally the spleen has a slightly mottled dark plum serosal surface and has a firm dark reddish-plum parenchyma. If accessory spleens are present in the region, note their number and sizes.

Sample narrative:

e.g., The spleen was pale on cut and serosal surfaces. The spleen was __ x __ x __cm in diameter and weighed __ g.

e.g., The spleen was dark plum on serosal and cut surfaces and was enlarged. The spleen weighed __ g and measured __ x __ x __ cm.

Procedure: Note the presence of accessory spleens. Examine the serosal surface 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.

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

6) LIVER *-serosal and cut surface description (i.e. color, texture, margin roundness, bloody cut surfaces)*

-lesions

Prior to removing the liver, examine portal circulation for evidence of gas. 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. The parenchyma should be reddish brown, firm, and moist. There are large venous sinuses in the liver, palpate their margins and carefully look for evidence of gas bubble trauma (we will have **photos** (add to fig 6? - of this from Paul & Antonio?).

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. The liver

weighed __ kg.

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. The liver weighed __ kg.

Procedure: 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. Examine sinuses and hepatic portal system. Bread-loaf the liver and examine the parenchyma on all cut surfaces. Record the color, texture, and bloodiness/wetness of the cut surfaces. Examine liver sinuses and portal veins.

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 should they be proximal and distal to the portal circulation to reflect distribution of emboli?). Collect a card-sized sample for archive toxicology. If the carcass is from a HAB region, a finger-sized sample of liver should be collected for ELISA regardless of decompositional state. Collect for virology?

Is this sequence ok? If so move figs around – if repro is typically done later then move this section###

C. UROGENITAL SYSTEM:

REPRODUCTIVE *-mature/Immature -**Females:** multiparous/parous/nulliparous, description of ovaries/follicles -**Males:** dimensions of testes, weight of testes and epididymides, seminal*

fluid presence, sperm presence

e.g., Right testes had dimensions of 27.5 x 8 x 2.5 cm and weighed ___ g; left testes had dimension of 29 x 8.5 x 2 cm and weighed __g. The testes were flaccid and autolyzed. There was no milky fluid.

e.g., Left mammary was 32 x 10 x 3cm. The carcass was pregnant and the fetus was in the left uterine horn. The male fetus's total length was 98cm.

Procedure:

1. FEMALE REPRODUCTIVE TRACT: Examine the serosal surfaces for any lesions. Take particular care with the nearby vascular plexuses for signs of emboli (Figs 6 & 8).

Uterine exam- Examine the broad ligaments for edema, parasites, vascularization, opacity, and thickness. Remove the entire reproductive tract and cut open the cervix and uterine horns. Examine the mucosa for evidence of placentation, areas 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 (Fig.9_).

If the carcass is pregnant, the uterine horn in which the placenta is located is defined as the pregnant horn. Fetus gender and length should be recorded, and the fetus preserved for additional work up. If possible, collect and freeze a sample of the amniotic fluid for bioassay (seal and freeze).

Ovarian exam- Look for surface irregularities such as corpora lutea of pregnancy (colors___) and ovarian scars from past pregnancies. These qualities are used to determine reproductive maturity, history, and status. Collect both ovaries, bread-loaf if large, and store in 10% NBF. In an unambiguous and permanent way, uniquely identify one of the ovaries to

distinguish it from the contralateral ovary (a labeled tag is preferred, but a string tied to the right ovary or distal tip of the uterine horn will work).

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 surface before weighing and measuring. 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.

2. MALE REPRODUCTIVE SYSTEM. Examine the serosal surfaces of the testes and epididymides for general appearance (color, size, shape, firmness, fullness, flaccidness) and lesions (Fig. 10). Take particular care with the vascular plexuses associated with 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 mucosal 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.

Tissues to be collected: Collect a smear from one of the testes or epididymides on a glass slide and examine under a microscope (40X) for the presence of sperm. If the carcass is fresh, collect a tissue sample from the testes and from the proximal and the distal epididymides for histology.

3. URINARY BLADDER *-mucosa description (i.e. congested) -contracted/dilated -empty/full (i.e. volume, color, transparency) -lesions*

Sample narrative:

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

Procedure: Make a small incision at the cranial apex of the bladder. Note the total amount, color, and transparency of the urine before draining the bladder. Examine the mucosal and serosal surfaces for any lesions, and note whether or not the bladder is contracted. Examine the ureters for patency, note any lesions.

Tissues to be collected: If the carcass is fresh, collect a sample of the bladder wall 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 toxicology; to store, either tape the syringe or transfer fluid to a small sealable container. If no urine is available, collect a finger-sized (~10 ml.) piece of kidney for ELISA.

4. KIDNEYS *-serosal/cut surface description (i.e. color, wetness/bloodiness, presence of fat) -corticomedullary boundary description (i.e. cortex/medulla color) -lesions (i.e. infarcts, gas bubbles)*

Check the region surrounding the kidneys for lesions such as hemorrhage, infarcts, and emboli. The serosal surfaces of each reniculus are typically plum in color, though levidity may make some surfaces darker than others. On cut surfaces the cortices are lighter/darker than the medullae and there are distinct cortico-medullary boundaries. Parasites are common in some species of odontocetes (list_____).

Sample narratives:

e.g., The kidneys were unremarkable. The ureters had NVL.

e.g., The kidneys were pale to dark red on serosal and cut surfaces. The ureters were unremarkable. No parasites were observed.

Procedure: Examine the intact kidneys and then remove them. Closely examine all the surfaces and remark on the color, firmness, and texture, note the presence of any blood clots, kidney fractures, 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. Open and follow the ureters, look for stenoses or blockages.

Tissues to be collected: If the carcass is fresh, collect a tissue sample for histology from each kidney, as well as an additional sample for archive toxicology. Note and collect parasites, if present.

D. VASCULAR SYSTEM (see figs. ____):

1. HEART *-valves and chambers (i.e. check color, transparency, and texture of myocardium and valves), look for contraction band necrosis.*

Normally, the heart should be uniform in color and the valves should be smooth in texture and translucent.

Sample narrative:

e.g., Valves and chambers were unremarkable. On cut section, there was banding on the myocardium.

Procedure: Remove the heart from the pericardial sac. Note the quantity and color of the pericardial fluid. Make a cross sectional cut at the apex of the ventricles and look for discoloration and/or banding 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 atrioventricular (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.

Tissues to be collected: For histology, collect a sample of each atrium and ventricle and any areas with lesions.

2. "GREAT" VESSELS

Closely examine the serosal and luminal aspects of the aorta, vena cava, and pulmonary trunk. Look for adhesions and other lesions. Examine the thoracic retia for signs of emboli (photographs).

Tissues to be collected: For histology, collect a 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.

3. PERINATAL INDICATORS (new figure in progress) ?? should this section be moved to an appendix???

Procedure: 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 patency of the umbilical arteries, umbilical vein, the urachus (a neonate odontocete may have a patent urachus), the ductus arteriosus (if patent, then also its flat diameter and the flat diameters of both pulmonary arteries – note if flat or round

diameters), and the foramen ovale (note overall closure — as a % — if multiple openings in septum are observed). 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).

Tissues to be collected: For histology, collect a sample of the umbilicus, the umbilical arteries and 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.

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

1. DIAPHRAGM

Normally, the diaphragm is intact and tautly (if the pleural cavity has not been compromised) stretched between the midline and the lateral aspects of the abdomen, with a dorsal bulge (convexity?). 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 and light tan or pink.

2. TRACHEA & 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.

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

Procedure:

3. LUNGS

The lungs should be spongy and well inflated. The serosal surfaces of the lungs should be uniformly light pink. On cut surfaces, the lungs should be pink to light red and moist. The primary and secondary airways should be clear and have a thin moist coating. Lung worms may occur depending on species and feeding history, note parasites or tissue reactions to them.

Sample narratives:

e.g., The lungs had scattered small focal nodules. Both lungs were dark red on serosal surfaces and dark red to purple on cut surfaces. The airways were unremarkable. The left lung weighed __ kg. The right lung weighed __ kg.

e.g., The left pleural cavity was distended with gas. The diaphragm was intact. There was a moderate trematode infestation observed 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. 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 x 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.

Procedure: Prior to entering the pleural cavity note any 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. 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, as well as 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, congestion, and any lesions observed. Photodocument any irregularities that are observed.

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 a tissue sample (Page: 41
We should be collecting more than just one sample. One on the margin, one in the middle, and one along the main airways. As I recall, fat emboli may localize in the middle of the lung where the vessels are of the right size for trapping normally sized emboli. Margins they're too small, near the pulmonary arteries they're too big.) for histology from each lung, lateral to the bronchus and approximately one hand span from the cranial pole of the lung. Include a piece of bronchus in at least one of the lung sections.

F. LYMPHOID TISSUES

Lymph nodes can be quite variable in appearance; however a healthy, inactive lymph node should be a pale yellowish-brown or off-white in color, should be located within a perinodal (Rather redundant since we say "within" right before it. May be good for familiarizing people with the "lingo" though.) 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 edematous/serous atrophied 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 external appearance, and the serosa may have irregularities. Prior to fixation, section lymph nodes and describe cut surfaces. Describe the color, presence of serous fluid, and any structural irregularities. Note that in some species, some lymph nodes (i.e. axillary) are nearly impossible to find unless active and enlarged.

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 surface of one mesenteric lymph node was nodular in texture, and wept serous fluid when cut.

1. LYMPH NODES

Procedure: The narrative for this section should be dictated as one progresses through the carcass and each lymph node is encountered. Be sure to note on the general appearance of the lymph nodes, as well as any lesions or irregularities.

a) Superficial and/or associated with extremity (Fig 6A – possibly combined w/ Fig 5 superficial circ, parasites and LNN??): 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.

b) **Thoracic** (Fig 6A): Examine all pertinent thoracic lymph nodes after exposing the pleural cavities and during the lung examination.

c.) **Abdominal** (Fig 6B): Examine all pertinent abdominal lymph nodes after removing the gastrointestinal tract and during the abdominal organ examination.

Special attention should be given to the major lymph nodes draining important physiological systems such as the respiratory and digestive system. Pulmonary and mesenteric lymph nodes are often times noticeably irregular if there are respiratory or digestive complications respectively.

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 both sides. Lymph nodes may also be frozen (preferably at -70^{o}C) for immunological studies.

2. THORACIC DUCT & MESENTERIC LYMPH CHANNELS

The thoracic duct is located just dorsal to the distal/caudal aorta. External appearance of the thoracic duct is variable, however examination can reveal fat and/or gas emboli.

Sample narrative:

e.g. The thoracic duct was unremarkable externally. When opened, the thoracic duct revealed…

Procedure: Make a transverse cut at the distal thoracic duct (? Is there a significant enlargement similar to the receptaculum chyli also ampulla chyli, chyle cistern ** illustrate or photograph this structure**) and open the duct cranially as far as possible. Examine the lumen for signs of emboli. (Antonio and Paul will probably have to write this section, ...

Tissues to be collected: Collect a cross-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.

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.

e.g. Section written by Antonio and Paul

Tissues to be collected: Collect a cross-section of the channels 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). Collect any irregularities or lesions observed.

4. THYMUS

In fresh carcasses, the thymus should be pale pink. The thymus involutes with age and therefore in older animals can be significantly reduced in size and/or very fatty. The thymus should (Fig 6 – do we need a ventral view?) be examined for any irregularities. If possible and if in good enough condition, be sure to dissect out the entire thymus and weigh it.

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. There was a …

Tissue to be collected: Collect a piece of thymus for histology. A piece of thymus can also be frozen (preferably at –70^{o}C) for immunological studies and for virology.

G. HEAD & NECK -l*esions (i.e. bruising, abscesses, edema, clots) - description of lesions present - earbone collected,* tooth *collected, nuchal fat collected*

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 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.

Procedure: 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.

1. ORAL CAVITY & THROAT

The tongue, lips, gums, and oropharynx should be examined for orogential ulcerations. The goosebeak/larynx should be examined for signs of HI and other lesions. The tonsils should be sampled. Teeth should be examined and the extent of fractures and/or truncation should be described.

e.g. There were small (~0.5cm), focal, bilateral ulcerations visible along the gum line. There were large (1.5-2.5cm diameter), diffuse and coalescing, nodular proliferations on rostral half of the lip of the right mandible. Most of the teeth were severely truncated, except for 5 teeth with oblique fractures.

Procedure: 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. Describe the color and consistency of the thyroid, and note any fluid filled follicles. Remove the dorsal blubber caudal to the blowhole and examine and collect the E.A.M. lnn. **(**Page: 46
Should there be a separate head/neck lymph node section? If so, should each section (thoracic, abdominal, etc. OR respiratory, excretory, etc.) have a separate lymph node section?
)

Tissues to be collected: Collect a piece of tongue and any oral lesions present for histology. Additionally, collect a cross section of the larynx/goosebeak, and sections of the oropharynx, thyroid and tonsils. EYES vitreous/aqueous, etc?

2. SINUSES & VASCULARIZED SPACES

Special attention should be given to the mandibular fat pads, the pterygoid sinuses, and

the fibrovenous plexuses between them (Figs 4, 5, & 6 – move FVP vasculature in 6 so it can be seen or put details in an appendix w/ photos and reference it here). This region may be susceptible to acoustic and/or barotrauma and could be a source of fat emboli (extra section 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.

3. BRAIN & 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, the brain should be extracted. Once the brain is removed, examine the nasopharyngeal mucosa for signs of congestion. (Page: 47
Is this from the manatee manual? Would be hard to examine nasopharynx in these guys wouldn't it?)

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.

Tissues to be collected: If the carcass is fresh, collect a tissue sample of pituitary, cerebrum, cerebellum, and nasopharyngeal mucosa for histology. Prior to sampling, the brain can be placed in NBF to increase firmness, and aid in sectioning. Collect any active lymph nodes or other affected tissues according to your needs. Collect a nuchal fat samples (frozen? and histology?).

4. EARS

Not sure what you guys want to do about the ear section. I know there's debate regarding whether to remove or leave in and scan, or not bother much with them. Eustachian tubes and

cranial hiatus should however be examined when possible, and peribullar sinuses should be examined for clots.

H. SKELETON *-lesions (i.e. any fractures, luxations, lacerations caused by broken/luxated bones)-remodeling -joint problems (i.e. dry/lumpy nucleus pulposus, osteoarthopathy)*

Sample narrative:

e.g., Left ribs #s 9, 10, and 11 were broken proximally and beginning to heal. Left rib #11 had compound, comminuted fractures. 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. The vertebral epiphysis of T-__ was closed.

Procedure: 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 (D-Rot: for what? Will we see lesions in acute DCS?). After exposing the ribs when flensing, the ribs can be cut with tree/hedge clippers or disarticulated at the costo-vertebral joints. At least one costovertebral joint set (double headed rib – histo #_, Fig_11) should be collected for histology (the caudal vertebra of this pair could be examined for skeletal maturity – histo #_, Fig_). Additionally a chevron-vertebral joint should also be collected (– histo #_, Fig_). After removing the lungs be sure to run your hand across all the ribs while moving the rib tips, in order 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. If fractures are observed, be sure to note whether there is evidence of healing. Look for mandibular fractures (fresh or healing),

particularly in adult males and record presence and location. Photodocument any evidence of trauma or remodeling. Should we have a skeleton lesion sheet – yes, in progress

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

I. OTHER *-lesions (i.e. any comments that don't fit in above categories)-Organ weights/dimensions (i.e. of organs not in above list)*

e.g., The adrenals were very firm. The left adrenal measured _x_x_ cm and weighed __ g. The right adrenal measured _x_x_ cm and weighed __ g. Periadrenal fats were serous atrophied but abundant. Maybe use Clark's stuff to add something here on adrenals? (Page: 49
Adrenals will likely be very important in cetaceans. May deserve their own section for sampling, measurement, and corticomedullary ratio. If so, there's already a note on examination/extraction in the abdominal section.)

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

Tissues to be collected: If the carcass is fresh and the lesions are indicative of cause of death or are of special interest then collect a sample (see tissue checklist, Appendix 4).

III. MORPHOLOGICAL DIAGNOSIS

D-Rot: Personally, I am big fan of systemic-based diagnoses; that is Respiratory System-list findings, Digestive System-list findings, etc.

That's not to say it's the best way, but I know it helps me to put the lesions and case in context.

A. SIGNIFICANT FINDINGS -important lesions/findings (i.e. enteritis, trauma, emboli)

Sample narrative:

e.g., Lesion in colon with associated 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; Well-formed blood clots.

IV. MOST PROBABLE CAUSE OF DEATH *-proximal cause of death*

Sample narratives:

e.g., natural; other (red tide)

e.g., watercraft, both

NECROPSY CONDUCTED BY *-examiners / participants / observers present during necropsy*

Bibliography:

Bossart, G. D., T. H. Reidarson, L. A. Dierauf, and D. A. Duffield. 2001. Clinical Pathology. Pp. 383-436 in: L.A. Dierauf and F.M.D. Gulland, Eds., CRC Handbook of Marine Mammal Medicine, 2nd Edition, CRC Press, Boca Raton, FL.

Hensley, G., Gregory Bossart, G., Ewing, R., et al. 2005 *Kogia* Heart Dissection Manual. Harbor Branch Oceanographic Institution, Inc. Technical Report No. 90

Clark, L.S. , Pfeiffer, D.C, and Cowan, D.F. 2005. Morphology and Histology of the Atlantic Bottlenose Dolphin (Tursiops truncatus) Adrenal Gland with Emphasis on the Medulla. Anat.. Histol. Embryol. 34, 132–140

Clark, L.S., Cowan, D.F., and Pfeiffer, D.C. 2006. Morphological Changes in the Atlantic Bottlenose Dolphin (*Tursiops truncatus*) Adrenal Gland Associated with Chronic Stress

Crile,G.; Quiring,D.P. 1940. A Record of the Body Weight and Certain Organ and Gland Weights of 3,690 Animals. Ohio Journal of Science 5:219-259 – *for standards on trimming organs for weight comparisons*

Daily, M. D. and R. L. Brownell, Jr. 1972. A checklist of marine mammal parasites. pp 528-589 in: Ridgway, S.A. (ed.) Mammals of the Sea. Charles. C. Thomas, Springfield, IL.

Evans, D. L. and England, G. R. (eds.), 2001. Joint Interim Report Bahamas Marine Mammal

Stranding Event. US Department of Commerce (NOAA)/US Navy.

Fernández A., M. Arbelo, R. Deaville, I. A. P. Patterson, P. Castro, J. R. Baker, E. Deg, Ollada, H. M. Ross, P. Herrááez, A. M. Pocknell, E. Rodrííguez, F. E. Howie, A. Espinosa, R. J. Reid, J. R. Jaber, V. Martin, A. A. Cunningham & P. D. Jepson. 2004. Pathology: Whales, sonar and decompression sickness (reply). Nature 428:

Frantzis A. 1998. Does acoustic testing strand whales? Nature 392: 29.

Geraci, J. R and V. J. Lounsbury. 1993 new version!. Marine Mammals Ashore; A Filed Guide for Strandings. U.S. National Oceanic and Atmospheric Administration.

Houser, D. S., Howard, R., and Ridgway, S. 2001. Can diving-induced tissue nitrogen supersaturation increase the chance of acoustically driven bubble growth in marine mammals? J.l Theor. Biol. 213:183-195.

Jepson, P. D., Arbelo, M., Deaville, R., *et. al.* 2003. Gas-bubble lesions in stranded cetaceans. Nature 425:575-576.

Reynolds, J.E., III, S.A. Rommel, and M.E. Bolen. 2002. Post-cranial anatomy: orientation and organization of the soft tissues. Pp. 21-30 in: W.F. Perrin, B. Wursig, and H. Thewissen, Eds., Encyclopedia of Marine Mammals, Academic Press, San Diego, CA.

Rommel, S., E. Haubold, A. Costidis, G. Bossart, and R. Meisner. 2002. Comparative Distribution of Lymph Nodes in Marine Mammals. Poster presented at the Florida Marine Mammal Health Conference. University of Florida Hotel and Conference Center, Gainesville, Florida. April, 2002.

Rommel, S.A., and L.J. Lowenstein. 2001. Gross and microscopic anatomy of marine mammals. Pp. 129-163 in: L.A. Dierauf and F.M.D. Gulland, Eds., CRC Handbook of Marine Mammal Medicine, 2nd Edition, CRC Press, Boca Raton, FL.

Rommel, S.A., D.A. Pabst, W.A. McLellan. 2001. Functional morphology of venous structures associated with the male and female reproductive systems in Florida Manatees (Trichechus manatus latirostris. Anatomical Record 264:339-347.

Rommel, S., D. Pabst, and W. McLellan. 2002. Skull morphology of marine mammals. Pp. 1103-1117 in: W.F. Perrin, B. Wursig, and H. Thewissen, Eds., Encyclopedia of Marine Mammals, Academic Press, San Diego, CA.

Rommel, S.A. and J.E. Reynolds, III. 2002. Skeletal Anatomy. Pp. 1089-1103 in: W.F. Perrin, B. Wursig, and H. Thewissen, Eds., Encyclopedia of Marine Mammals, Academic Press, San Diego, CA.

Schaller, O. (ed.) 1992. Illustrated Veterinary Anatomical Nomenclature. Ferdinand Enke Verlag, Stuttgart.

Delyamure, S. L. 1955. Helminthofauna of Marine Mammals (Ecology and Phylogeny). Izdatel'stvo Akademii Nauk SSSR, Moskva. Translated from the Russian, Israel Program for Scientific Translations, Jerusalem, 1968.

PDFs of Alaska M.M Tissue Archive Project Revised Collection Protocol – Becker et al, Small Cetacean Dissection & Sampling – A field Guide – Jefferson, Myrich, Chivers – NOAA – TM – NMFS – SWFSC 198 -KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL by Raverty and Gaydos],

Acknowledgments: We thank

drawings in text:

Wound and scar sketch/HI; Lifting methods

Wound and scar sketch/Watercraft Interactions

External morphometrics

Initial incisions; Flensing

Superficial vasculature, musculature, mammaries, & Phyllobothrium sample procedure

GI tract sample sites

Anatomical Guide to LNN, viscera and pericardial veins

Anatomical Guide to large veins

Female Reproduction

Male Reproduction

Special Prceedure for collection of Skeleton lesions part 1 joints

Special Prceedure for collection of Skeleton lesions part 2 broken bones

Special Prceedure for collection of Skeleton lesions part 3 aging

Special Proceedure for Perinatal Evaluation

photos in text:

tags, good example of an external photo

good example of in internal photo (emboli?)

Appendices:

Reference gross anatomy from CRC handbook

Wound and Scar data sheet

Scale/ruler

External data – morphometrics data sheet

Blank Necropsy form

Blank histology check list

Carcass condition

Blank perinatal protocol?

Comparative LNN handout? w/ enlarged Tt LNN ??

Blank Human Interaction form

Authority / Jurisdiction

International Contact information

Some updatable table/list of suppliers sources shippers requestors?

Separate photo data sheet check list? or make columns on histo sheet?

Greg Early suggested that we develop an interactive electronic version – similar to one he uses or the one Pat Rotstein has developed??

WAMs comments 04: Some overview comments at first read:

Start right off with caveat that any potential barotrauma or gas emboli stranding event has the potential of turning into a mass stranding/epizootic event. As these events are a paradigm shift for stranding response, they might tie together events that were disparate before now, i.e. single species events now being investigated together or multiple species being tied together?? It is a whole new field investigation out there now…

How long to store recently collected tissues in 10% NBF before adequate fixation and therefore can be shipped safely? Is one day fixation enough?

Add a cross section of the uterine horn along w/ both ovaries and a section of mammary

Dry ice is fast becoming a non-starter w/ Fed Ex. I personally try to not ship w/ dry ice, then you can check the box “no hazardous goods” which seems to make it simpler.

Temperature should be taken in deep epaxial muscle, mid lumbar or half way between dorsal fin and blowhole. Drive deep temperature probe through blubber 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).

Probably need some dissection protocol for removing the head and suggestions for how to wrap and store either frozen or shipped immediately? This brings up the question as to who is getting all of this stuff and arrangements/collaborations.

Under histo checklist should there be specific prompts to collect sections across any potential air/tissue interface, *i.e.* bronchi, lots of lung sampling, major liver sinuses, stomach, intestine, colon- where ever there could be a buildup of gas??

Also need bone and joint sampling ala Michael Moore. Collect humeral joint, intervertebral joints, rib articulations??

Polaroids cameras are great, but digital is a must as well now. Images can be emailed immediately around the world (which is a HUGE benefit to folks getting a possible emboli case) and are user friendly inserting images into necropsy report and presentations down the road. A single flash disc now can hold hundreds of images which saves changing film during necropsy- a person can just about shoot all day, and saves on film costs over the life of the camera- basically pays for itself.

Do you want to include a blank necropsy form to fill out?

How would you like me to proceed with small changes in text- track with color edits??

WAM

After Baltimore and during Steno UME:

APPENDIX: Suggested Necropsy and Histopathology Procedure S. Rommel, A. Costidis, and J. St.Leger

Standardized gross examinations accompanied by detailed necropsy reports should be part of the procedure when DCS is suspected - some of this detail is too extensive (and expensive) to perform on routine strandings. In order to see the big picture, the entire animal must be examined and described, not just regions classically associated with acoustic trauma. Additionally, new histopathology techniques must be added so that we can pinpoint certain acoustically-induced lesions such as the fat and gas emboli that have been described by Jepson et al. and Fernandez et al. 2003

We recently had a mass stranding of Tursiops and used these carcasses to develop a proceedure for collecting some additional histology samples and to develop a protocol that will address some of the missing pieces in terms of necropsy procedure to document DCS. Jepson's and Fernandez's published observations suggest gas and fat emboli (FE) are very important in DCS cases, unfortunately FE aren't diagnosed with standard techniques (so, in addition to adjusting technique so fats are made visible, we are suggesting collection of samples (#62-63) that will document vascularized density interfaces (other than bone marrow) that might introduce FE into the circulatory system. Bone marrow and some synovial joints may be sensitive to DCS. Additionally, signs of osteonecrosis (bone death) are not typically looked for yet may be important for diagnosing DCS; I asked Michael Moore and Greg Early (see their paper at the recent Biennial on osteonecrosis in sperm whales) to suggest some sample sites that might give us indications of these lesions, hence the samples #65-68. The braincase has been imaged electronically but not histologically, I suggest we consider taking a histo sample of the braincase with dura at the level of the cranial hiatus (#64). Because they include bone, samples 62-68 all require a saw and special processing (decalcification for the bone, special dyes and processing for the fats).

From Greg Early: (possibly use this as an appendix or use it as a template?)

Collection Protocols and Priorities (A guide for what to do with the nasty bits)

Adapted from "The Southeastern United States Tissue and Skeletal Collection Protocol" Prunier & Mase, NMFS Miami Laboratory

(revised April 2003)

The importance of data and specimens collected from stranded animals has been long recognized. Over one hundred years ago Frederick True (as in True's Beaked whale) established one of the first programs to respond to stranded marine mammals along the East coast of the US for the Smithsonian Institution. Today, stranding networks are a part of NOAA Fisheries Marine Mammal Health and Stranding Response Program. The MMHSRP goals are: 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, and to coordinate effective responses to unusual mortality events. This information can play an important part in management and policy decisions.

Dr. Joseph Geraci in his "Field Guide for Strandings" lists an number of factors that have an effect on the quality of information that can be obtained from stranded animals, including:

Condition and location of the specimen;
Size, skills, organization, interests and moral of the team responding to the stranding;
Adherence to clear, detailed protocols;
Availability of equipment and supplies;
Number of animal to be examined;
Amount of time available;
Care maintained in packaging and labeling samples;
Care in storing and shipping samples.

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

SAMPLE TYPES and DISPOSITION (revised April 2003)

Life History
Photographs
Voucher (archive)
Morphometrics

Parasites
Histology

Chemistry/Toxicology
Biotoxicology
Bacteriology
Virology

Serology

Genetics
Tissue Bank
Serum Bank

SPECIAL CASES – Generally with "special" cases documenting circumstance of stranding is equal or higher priority than establishing cause of death.

Suspected Human Interaction Protocol
Large Whale Protocol
Suspected Ship Strike Protocol

UNUSUAL MORTALITY

Contact NMFS for unusual signs, symptoms, or distribution of strandings. May require additional testing, special procedures or precautions.

NOTIFIABLE DISEASE – Contact NMFS if the presence of these diseases is suspected. **May require special procedures or precautions**.

Distemper
Brucella
Influenza
Rabies

"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.

Code 5 Collection Protocol

Code 5: Mummified carcass, organs not usually present.

Document/Record/Report (all)

1. **Assign Field Number**

2. **Level A Data**
   Use the National Stranding Form – send or fax to Dana/Amy.

3. **Morphometrics**
   Total length.

   Sex (?)

   Tooth/socket count

Sample Collection (ID verification/archive) (#1 for common-all for rare)

1. **Photographs**
   Suggested Photographs
   External
   Whole animal

2. **Life History Samples**
   Whole head or skull, or at least jaw bone with teeth.
   At least 2 unseparated vertebrae (from cetaceans) ***Entire skeleton if rare species.***
   (What's "rare"? Anything you see less than once per year

3. **Genetic Samples**
   "tissue" or bone (frozen or in salt sat. DMSO)

Code 4 Collection Protocol

Code 4: Advanced decomposition, bone partly exposed, skin peeling. Major bloating. Organs present but fragile and uniform color and texture. Body cavity opened (note once body cavity –thorax\abdomen opened organs should generally not be used for contaminant or archive)

Document/Record/Report

1. **Assign Field Number**

2. **Level A Data**
   Use the National Stranding Form – send or fax to Dana/Amy

3. **Morphometrics**
   Obtain as many measurements as possible. Use the Cetacean\Pinniped Data Record
   Tooth count

Sample Collection (ID verification/archive)

1. **Photographs**

Suggested Photographs (*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

2. **Life History Samples**

Stomach and contents, with ends tied off at the esophagus and small intestine.
Frozen in plastic
Whole head, lower mandible or 6 teeth taken from the center of the lower mandible. At least 2 unseparated vertebrae (from cetaceans) ***Entire skeleton if rare species.***

3. **Genetic Samples**

"tissue" or bone Skin sample, 1" x 1" minimum. Preserved in salt-sat.DMSO, if possible. If not, frozen.

Code 3 Collection Protocol

Code 3: from autolysed to decomposed…organs intact but losing individual color and texture.
Carcass intact.
Early – minor bloating, skin peeling to moderate decomposition.
Late - major bloating, penis may be extruded in males.

Document/Record/Report

1. **Assign Field Number**
2. **Level A Data**

Use the National Stranding Form – send or fax to Dana/Amy.

3. **Morphometrics**

Obtain as many measurements as possible. Use Cetacean\Pinniped Data Record.

Tooth count

Sample Collection (ID verification/archive)

1. **Photographs**

Suggested Photographs (*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

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 6 teeth taken from the center of the lower mandible. At least 2 unseparated vertebrae (from cetaceans) ***Entire skeleton if rare species.***

Reproductive Systems

Gonad measurements/ slice of testis preserved in 10% neutral buffered formalin.
Whole ovaries (preserved in 10% NBF).

3. **Genetic Samples**

"tissue" or bone
Skin sample, 1" x 1" minimum. Preserved in DMSO, if possible. If not, frozen.

Sample Collection (health/cause of death)

1. **Parasites** Preserved in ETOH or 70% isopropyl (or NBformalin (or substitute if neither is available.)
2. **Histopathology - Early code 3's** 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 attached AFIP Histopathology checklist. (AFIP for "special cases" call Dana)
3. **Virus isolation - frozen in plastic -** golf ball to softball sized sample if virus is suspected
   a. Lung (X4)
   b. Lung associated lymph node (X2)
   c. Spleen(X2)
   d. Thymus(X2)
   e. brain(X2)

Preserved in 10% Neutral Buffered Formalin

4. **Toxicology (general\chemistry)**
   a. **Organics**-50 – 100g samples (baseball size), frozen in foil (shiny side down), then in plastic. Liver, Kidney, Blubber, Muscle
   b. **Metals –** same size, same tissue frozen in plastic – or hair(without skin 2g)

Code 2 Collection Protocol

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

Document/Record/Report

1. **Assign Field Number**
2. **Level A Data**
   Use the National Stranding Form – send or fax to Dana/Amy.
3. **Morphometrics**

Obtain as many measurements as possible. Use Cetacean\Pinniped Data Record.

Tooth count

Sample Collection (ID verification/archive)

1. **Photographs**

Suggested Photographs (*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

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 6 teeth taken from the center of the lower mandible. At least 2 unseparated vertebrae (from cetaceans) ***Entire skeleton if rare species.***

Reproductive Systems

Gonad measurements/ slice of testis preserved in 10% neutral buffered formalin.
Whole ovaries (preserved in 10% NBF).

3. Genetic Samples
"tissue" or bone
Skin sample, 1" x 1" minimum. Preserved in DMSO, if possible. If not, frozen.

Sample Collection (health/cause of death)

1. **Parasites** Preserved in ETOH or 70% isopropyl (or NBformalin (or substitute if neither is available.)
2. **Histopathology - Early code 3's** 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 attached AFIP Histopathology checklist. (AFIP for "special cases" call Dana)
3. **Virus isolation - frozen in plastic** - golf ball to softball sized sample if virus is suspected
    a. Lung (X4)
    b. Lung associated lymph node (X2)
    c. Spleen(X2)
    d. Thymus(X2)
    e. brain(X2)

Preserved in 10% Neutral Buffered Formalin

4. **Toxicology (biotoxicology) (call reference lab)**
5. **Toxicology (general\chemistry)**
    a. **Organics**-50 – 100g samples (baseball size), frozen in foil (shiny side down), then in plastic. Liver, Kidney, Blubber, Muscle, Brain
    b. **Metals –** same size, same tissue frozen in plastic – or hair(without skin 2g)
6. **Bacteriology –** collect samples into sterile containers or transport media transport to lab (ck with lab for specific procedures for different organisms)(<24 hrs at room temperature >24 hrs refrigerate)
    a. External openings (mouth, nose, genital,)
    b. Internal – lesions, exudates discharge**)**
7. **Tissue Bank –** contact NIST for:
    a. cetaceans with known time of death (24hrs or less)
    b. mass strandings

c.

Code 1 Collection Protocol

Code 1: Live stranding; code 1 animals are reassigned to code 2 at death

Please contact NMFS Stranding Pager if a live cetacean stranding

Document/Record/Report

1. **Assign Field Number**
2. **Level A Data**
   Use the National Stranding Form – send or fax to Dana/Amy.
3. **Morphometrics**

Obtain as many measurements as feasible considering human and animal safety. Total length is the priority Use Cetacean\Pinniped Data Record.

Sample Collection (ID verification/archive)

1. **Suggested Photographs**
   Whole animal, left and right sides
   Dorsal fin, left and right sides
   Close up of the head
   Lesions, Abrasions, Net marks
   Flukes

2. **Genetic Samples**
   a. Sloughed skin,
   b. Buffy coat
3. **Clinical Samples** (hematology and chemistry)
4. **Serology serum collected for detection of antibodies for:**
   a. Morbillivirus,
   b. Brucella
   c. Herpes
5. **Serum Bank – save aprox 1cc serum frozen**
   a. Buffy coat (frozen White Blood Cells washed from green or blue top tubes – not EDTA)

Histology Sampling Procedures

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/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/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.

Whole hearts should be sectioned (bread-loafed) according to the Kogia heart dissection protocol

(Bossart et al.). After sampling for virology and bacteriology, whole brains should be placed in 10% NBF for a day 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. Center samples should include primary and/or secondary airways, for examination of mucosal surfaces.

Tissues collected for histology may also be tested using immunohistochemical (IHC) techniques. Some of these techniques require that tissues be kept in NBF no longer than 6 days (Dave said he'd look into this). In some instances, after allowing for an adequate fixation period, tissues can be placed in ETOH in order to preserve their utility in IHC.

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 or placed directly into the appropriate solvent (chloroform?), as described in the sampling protocol for lipid constituent analysis (Koopman et al.?). Histopathology samples of adipose tissues should be placed in 10%NBF, Penfix, or ???? (Dave or Antonio should comment on this) solution. If gross lesions not present, what should sampling location of fats be? Near ears, widest part of panbone, near sinuses?

Tissues collected (and specially preserved?) for examination of fat embolization:

CNS (brain and spinal chord), Lungs, Kidneys, Liver (portal and caval portion?), Spleen, Mesenteric lnn, Pulmonary lnn, Heart (if emboli bypass or pass through lungs to arterial circulation, they should appear in coronary arteries…?).

D-rot comments on acoustic fat sampling:

Response to fat trauma:

-**Hemorrhage** (variable depending on vascularizations)

May be able to get temporal determination based on presence of hemosiderophages (erythrophagocytizing macrophages with degraded erythrocytes and/or their components).

-**Inflammation**

Fat will typically saponify when necrotic due to release of intracellular calcium (dystrophic mineralization). May result in chalky, white foci. It can sometimes be a challenge to tell the difference between autolysis and inflammation. Autolysis may cause similar lesions, so presence of inflammatory cells (macrophages/neutrophils), granulation beds, etc. could help clarify/verify.

-**Fibrosis**

Given time could occur, and with enough time even collagen deposition could follow. Fibrosis would indicate chronicity, but once fully collagenized, would be hard to age. Also, mesenteric fat necrosis in livestock doesn't always result in appreciable fibrosis. Alex: Acoustic fats may have more fibrous/structural components, and thus may be more prone to fibrosis.

-**Embolization**

May not have evidence at site/source.

Histology for hearing impairment:

Histo could potentially help (nerve damage, hair cell loss, etc.), but cetacean ears are tough to process. Bony changes to periotic and tympanic bones may be a hint/suggestion of impairment, but would have to chronic.

Thursday, August 10, 2006 New possibilities – yesterday, at Mandy Hill's thesis defense we discussed the possibility of acoustic fats ageing as a possible source of hearing loss and the suggestion cam up about collecting fats at necropsy that might be of value - possibly melon and lower jaw fats - - perhaps a protocol to collect and analyze these fats (Heather Koopman & Dave Mann labs?) – need to assess value and importance ves time to collect, materials required for handling, shipping, storage, testing

D-Rot Responses to fat trauma can include:

1)hemorrhage (and depending on vascularity, this may vary as to amount). Over time, would have hemosiderophages (erythrophagocytizing macrophages with degraded red blood cells).

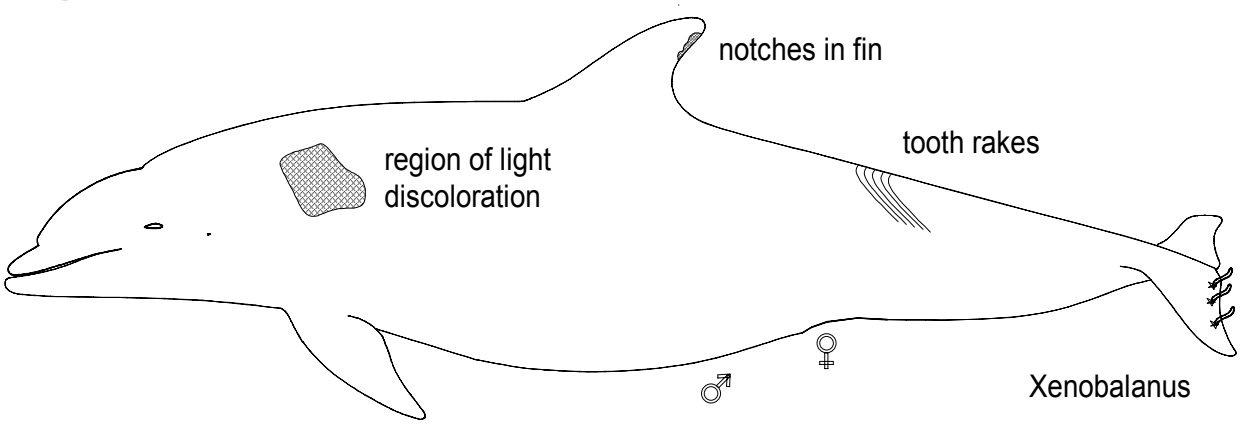
2)Embolization of fat--so may not have evidence at the site.

3)Inflammation--fat typically will saponify when necrotic due to the release of intracellular calcium (dystrophic mineralization). So, may appreciate chalky, white foci. HOWEVER, also get a degree of this with autolysis, so having inflammatory cells present would help.

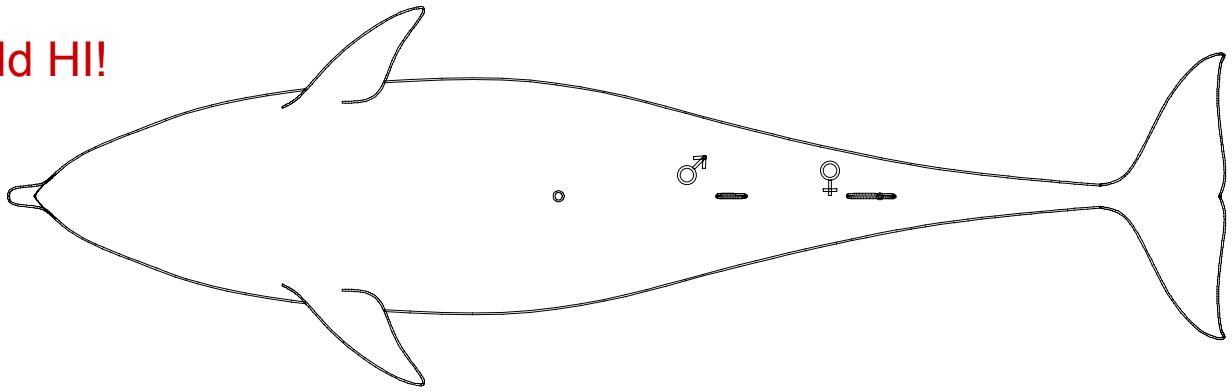
4)Fibrosis. Given time, this could occur, but I think of mesenteric fat necrosis in cattle and goats, and we don't always get an appreciable fibrotic response.

And I also would wonder..what's the response if the fat is actually in a liquid/liquidy state--emboli than, more likely than other changes?????

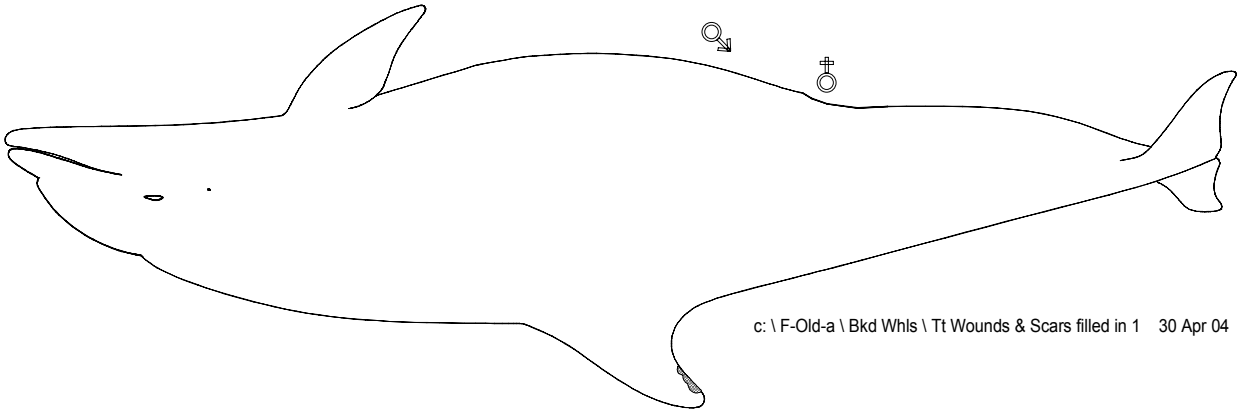
Fig 1



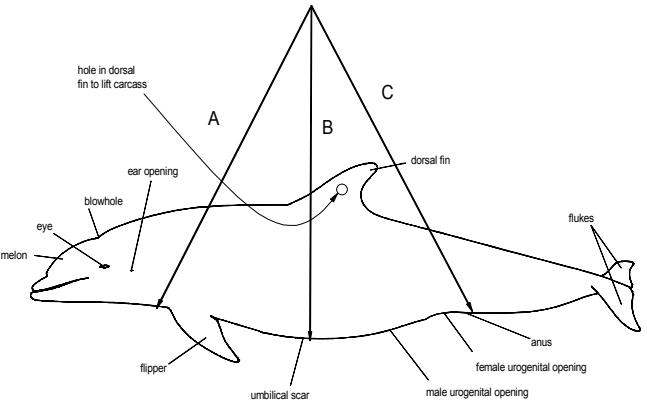
Add HI!



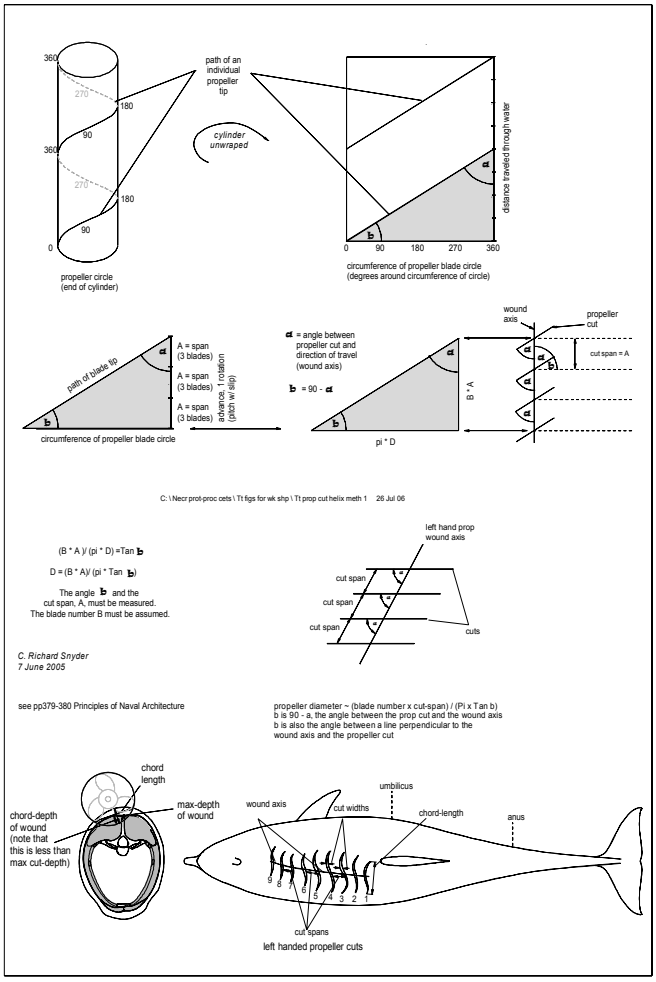
Filled in wound, scar, & HI report form

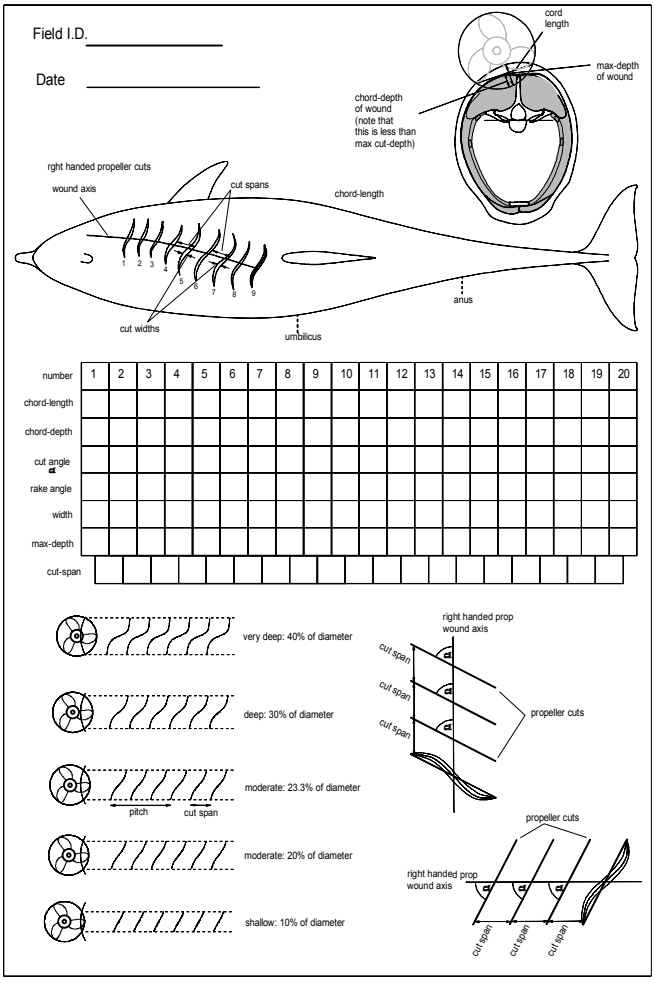


c: \ F-Old-a \ Bkd Whls \ Tt Wounds & Scars filled in 1 30 Apr 04



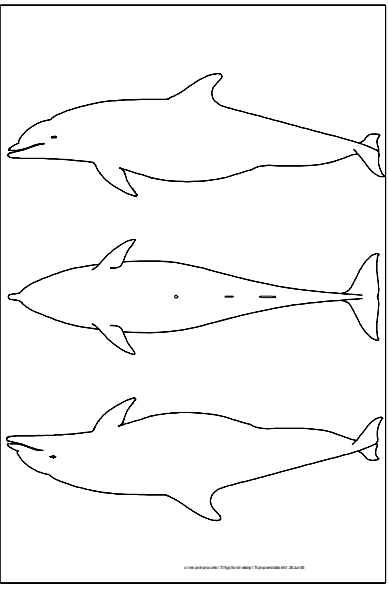
A small to medium-large odontocete that has a dorsal fin can be lifted via a hole in the center of the fin (assure the hole is round to minimize tearing). If lifting height is not a problem then the carcass can be lifted by the flukes. Strap locations are for carcasses without dorsal fins or for fins too far back from which to lift the carcass.

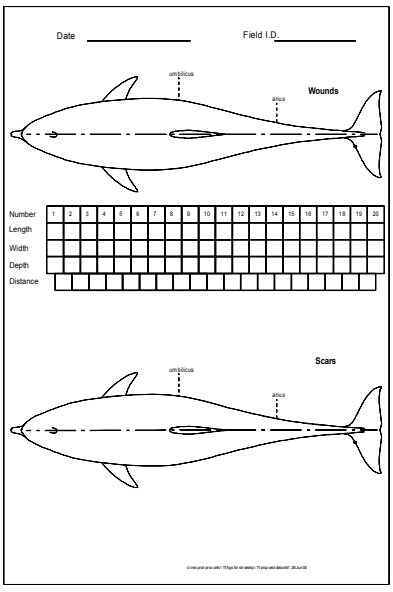


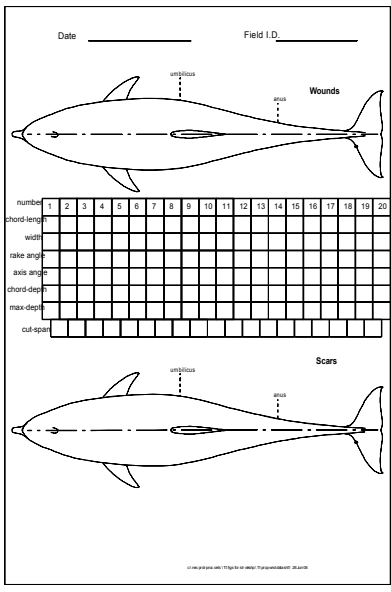


C:\ Necr prot cets \ Tt figs for strand \ Tt prop cut helix meth 1 3 Aug 06

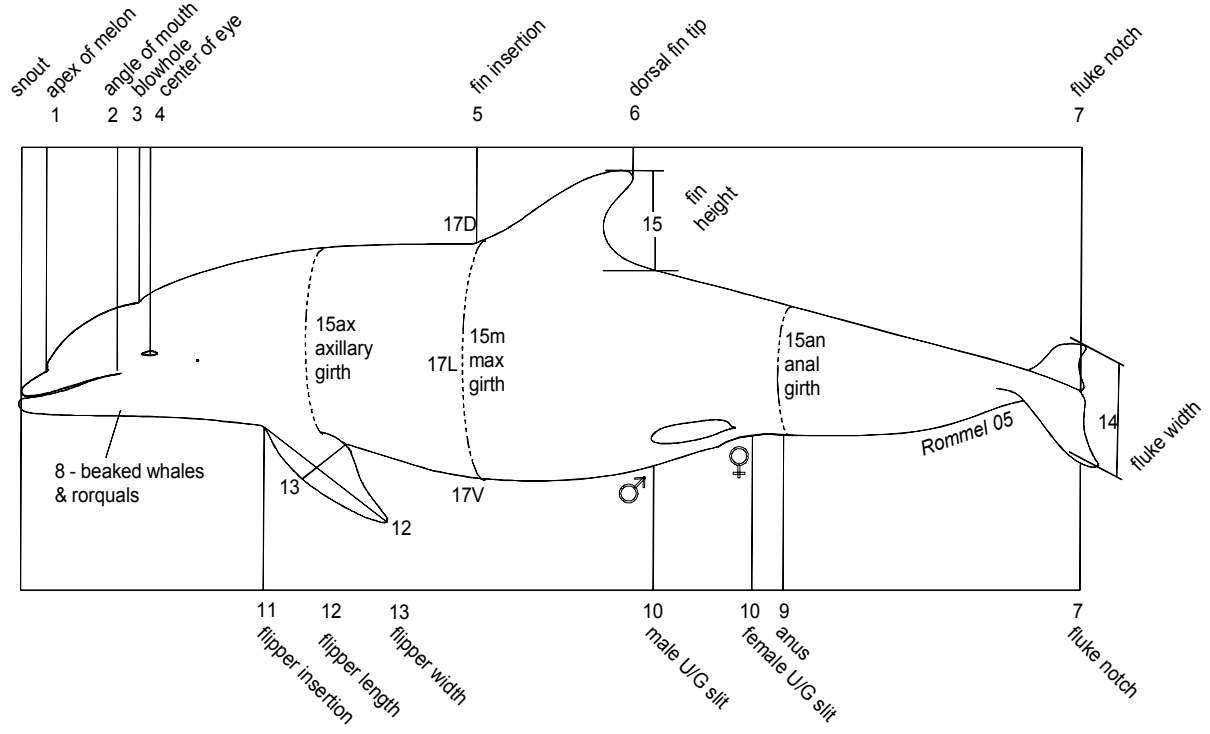
Right WNI protocol? Tt sheet? W/ Human Interaction???







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



Species _____Gender__M / F / Undet. ____Length _____Weight_____

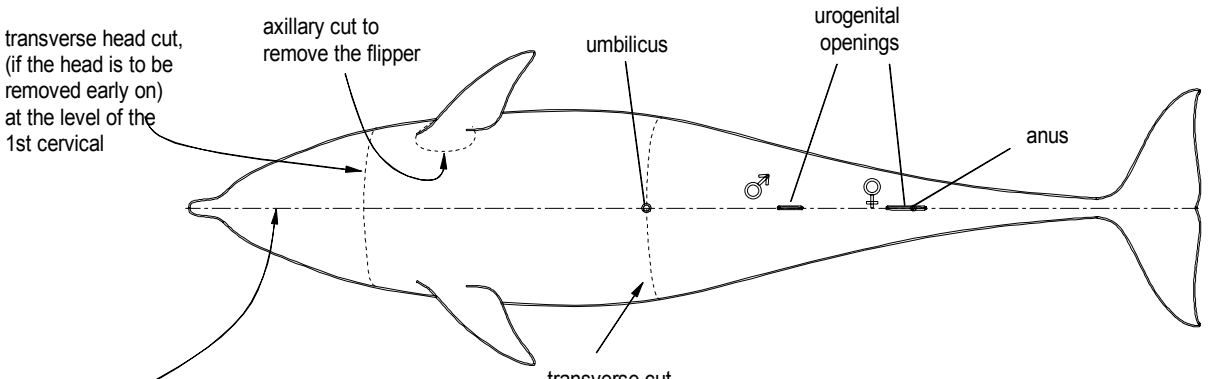
Condition: alive, freshly dead, mod. decomposed, extremely decomp., other _____

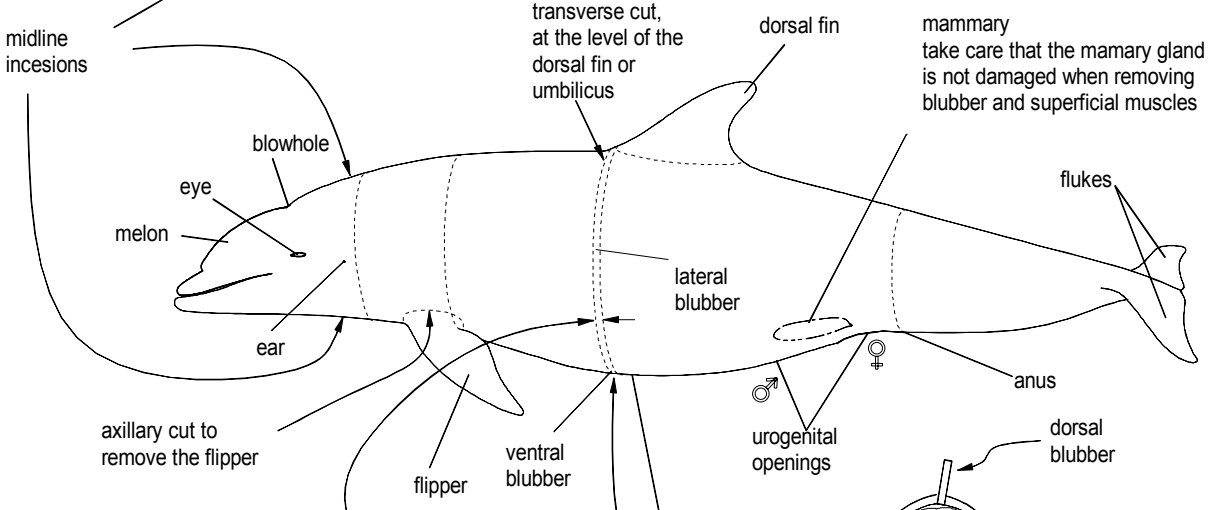
Locality: _____Observer:_____ Date: _____

Baleen/Tooth Counts (erupted or total) UL _____ LL _____ UR_____LR_____

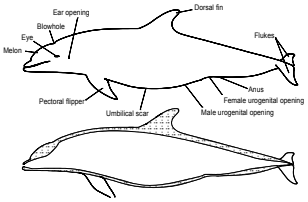
1. Snout to melon _____
2. Snout to angle of mouth _____
3. Snout to blow hole _____
4. Snout to center of eye _____
5. Snout to ant. insertion of fin _____
6. Snout to fin tip _____
7. Snout to fluke notch _____
8. Snout to caudal end of ventral grooves _____
9. Snout to center of anus _____
10. Snout to center of genital aperture _____
11. Snout to ant. insertion of flipper _____
12. Flipper length _____
13. Flipper width _____
14. Fluke width _____
15. Fin height _____
16. Girth: Axillary _____ Max (location) _____ Anal _____
17. Blubber thickness (excluding epidermis) Dors_____ Lat_____ Vent_____ Nuchal fat _____

Flensing – add 20 x 20 section near anus for Phyllobothrium fig _4__ 20 cm X 20 cm block of blubber (ventrolateral corner @ anus) to quantify *Phyllobothrium* infection
(to avoid distortion due to shrinkage, make the cranialmost and dorso-lateral cuts a few cm beyond the 20) - the result should a 20 digit table.

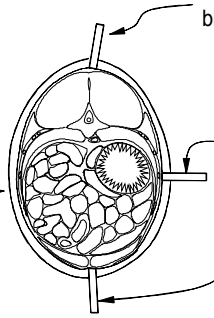




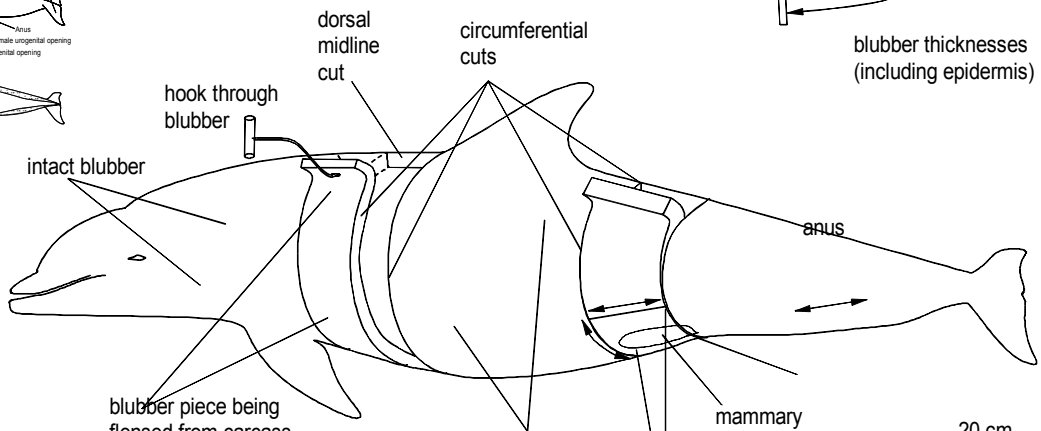
Fat depots – kinds of fats – sources of fat emboli – Marina's procedure for FE in lungs?

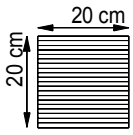


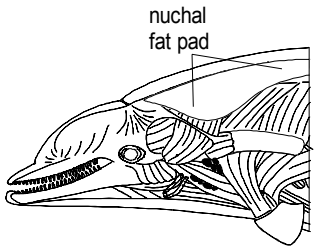
if the carcass is distended with gas, minimize skin distortion by making parallel circumferential cuts and measure blubber thicknesses on the minimally distorted pieces



blubber thicknesses (including epidermis)

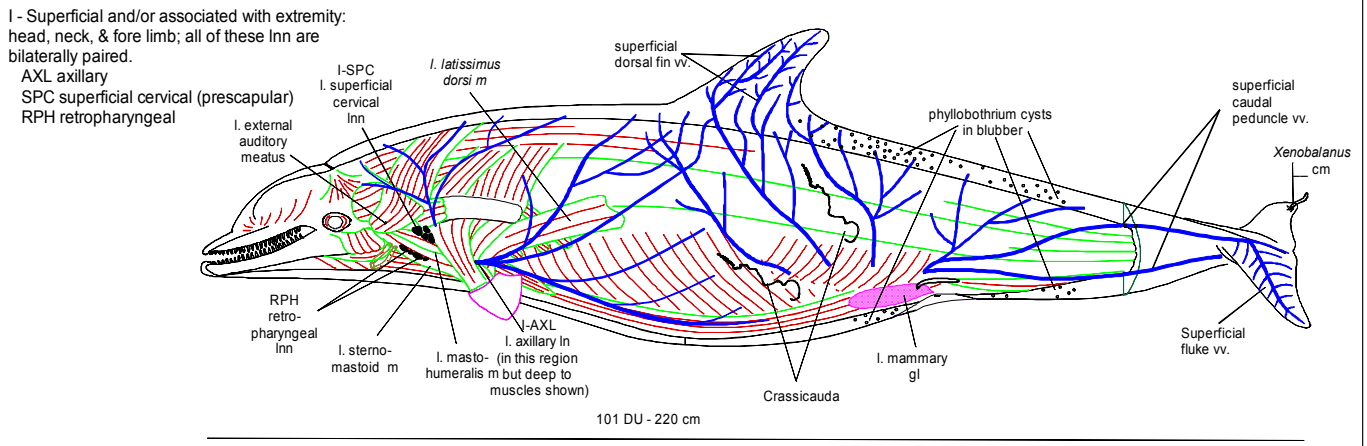


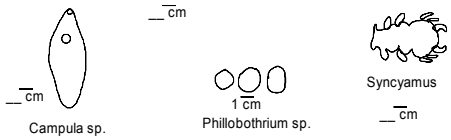


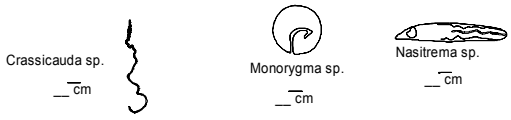


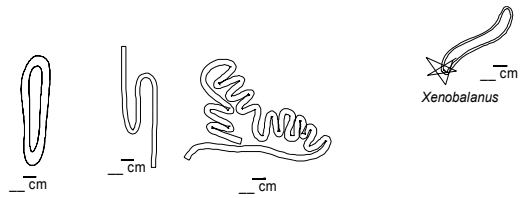
20 cm X 20 cm block of blubber
(ventrolateral corner @ anus)
to quantify phyllobothrium infection
(to avoid distortion due to shrinkage,
make the cranialmost and dorso-lateral
cuts a few cm beyond the 20) - the result
should a 20 digit table.

Initial ir

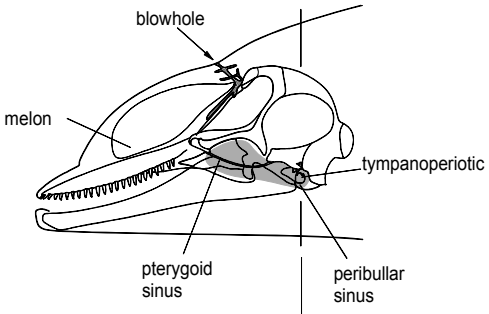








grossly visible signs of parasites
also see Geraci & Lounsbury
Daily
Brownell?



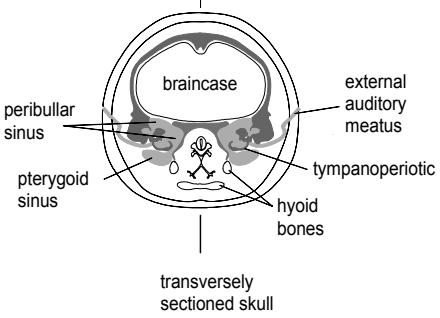
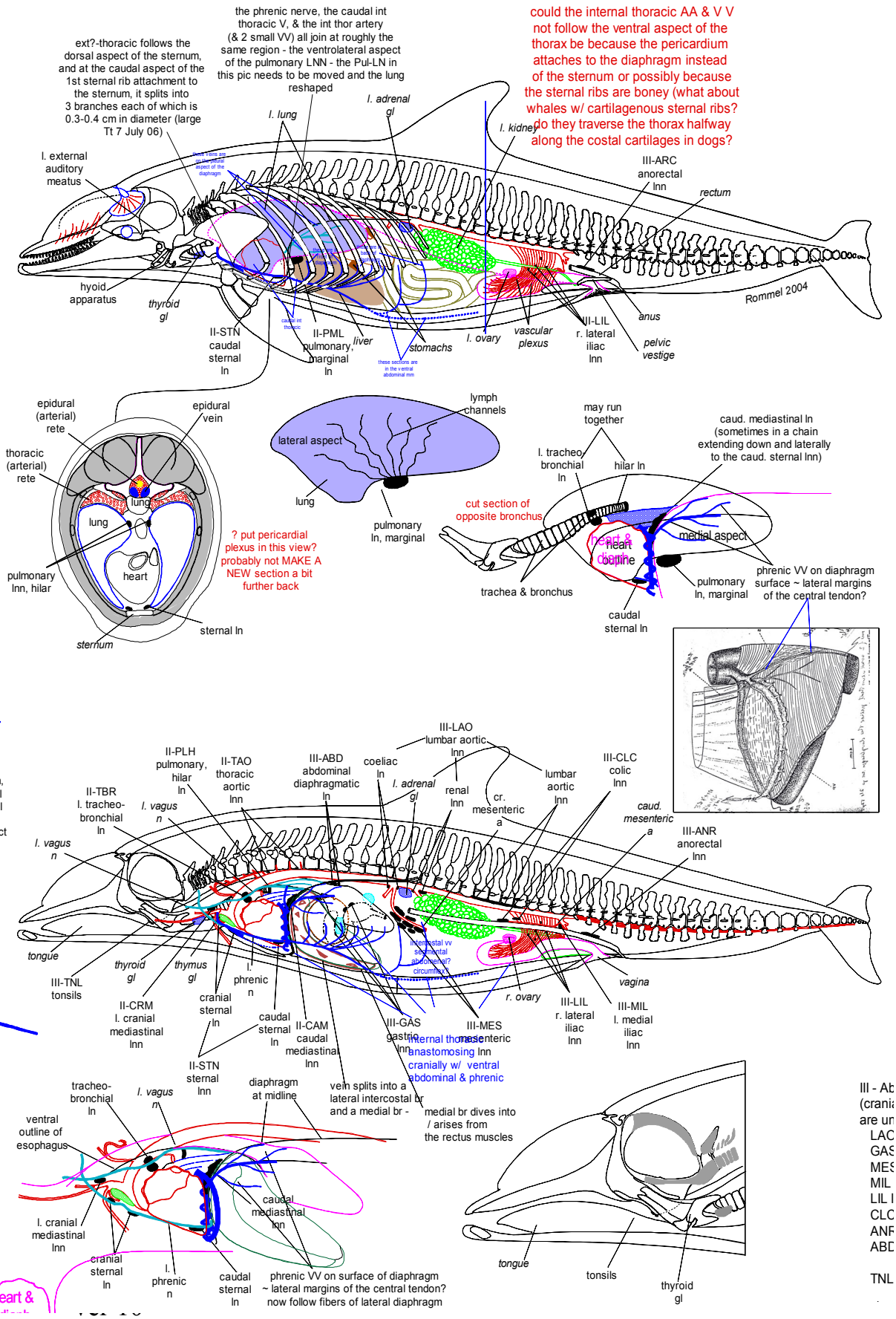


Fig _ sup mm VV lymph & parasites



17 Aug 06

Anatomical guide for lymph node identification fig _6__

Should we put monorhyma cysts here?

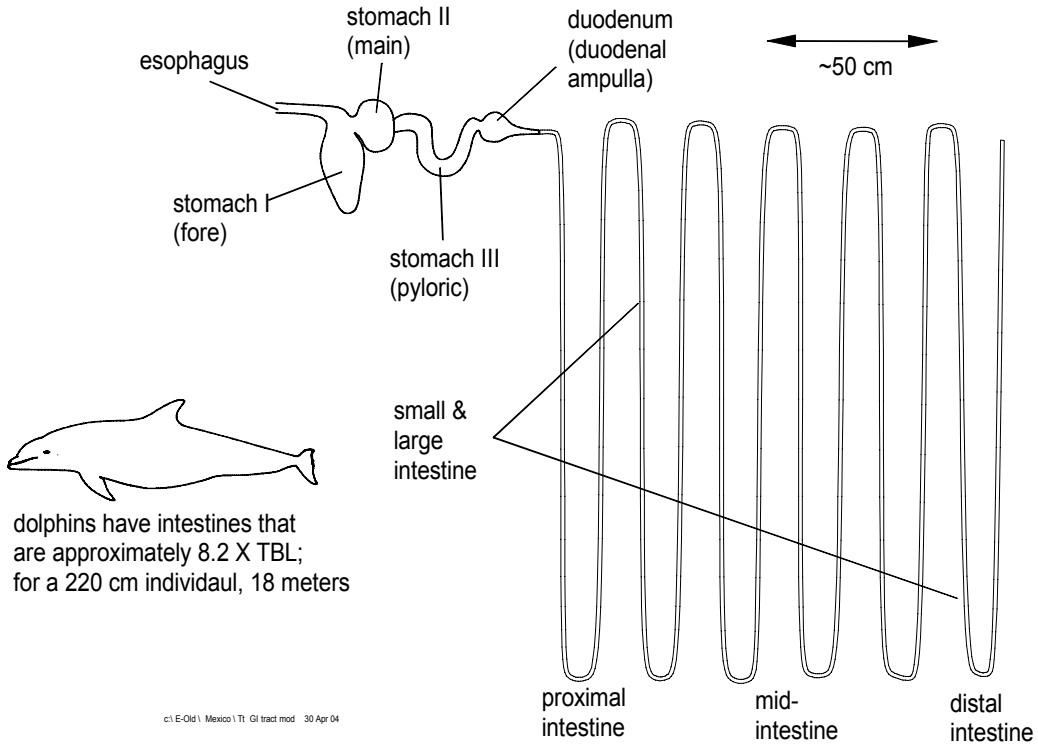
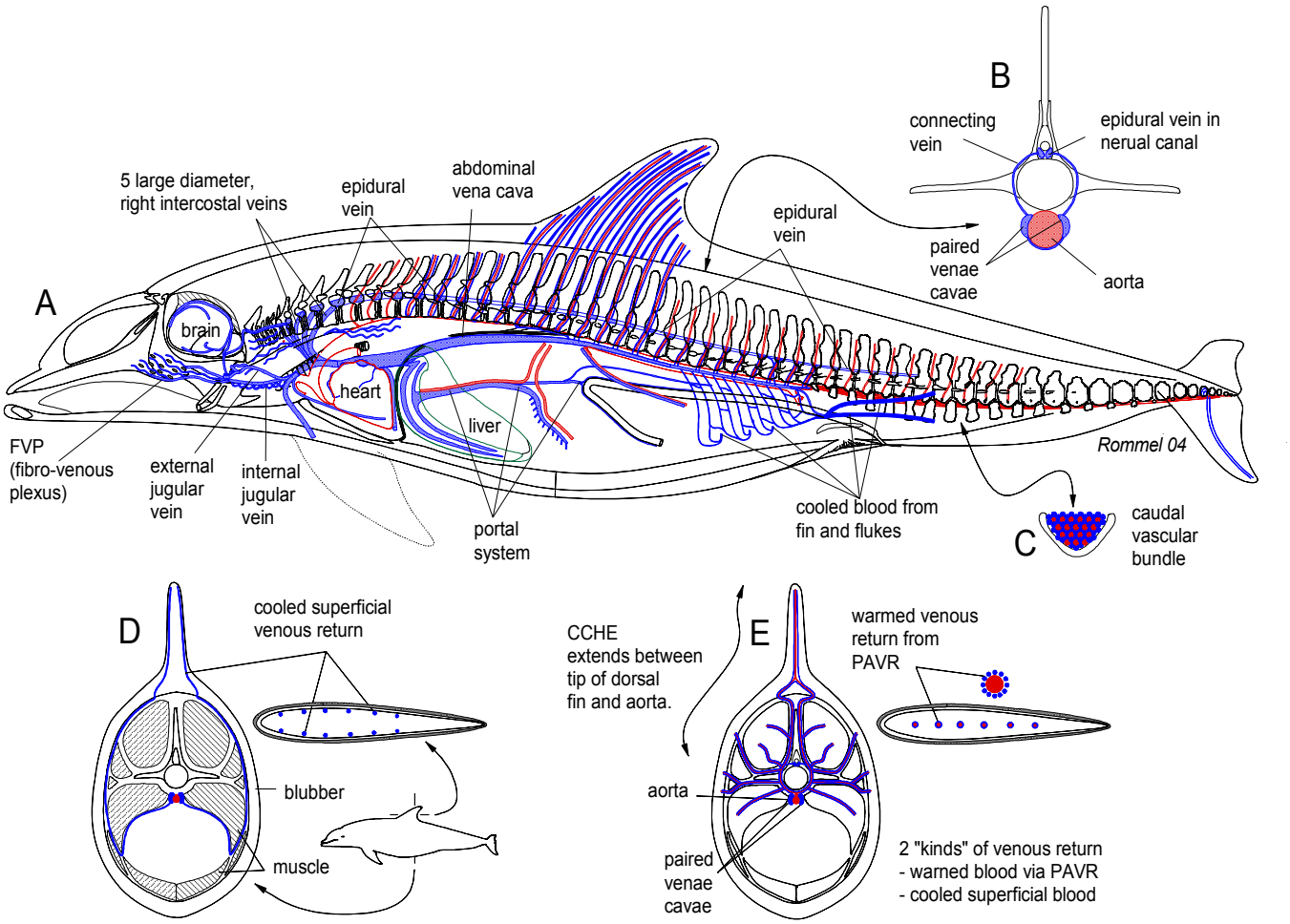


Fig 5 GI Tract sample sites – there have been some (silly) requests from those ignorant of the gross anatomy – we need some guide lines to keep (almost) everyone happy as to what is sampled and where

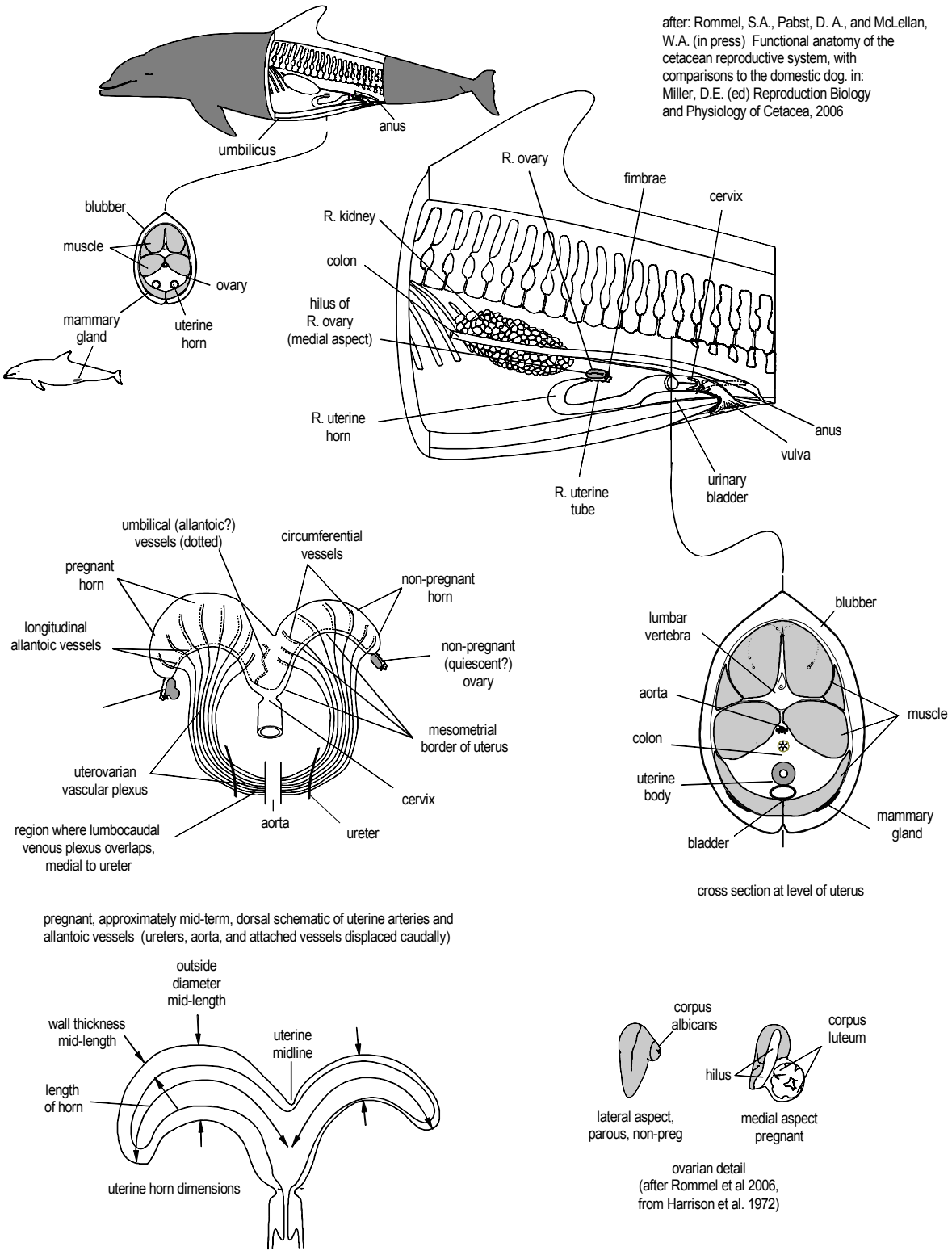
5

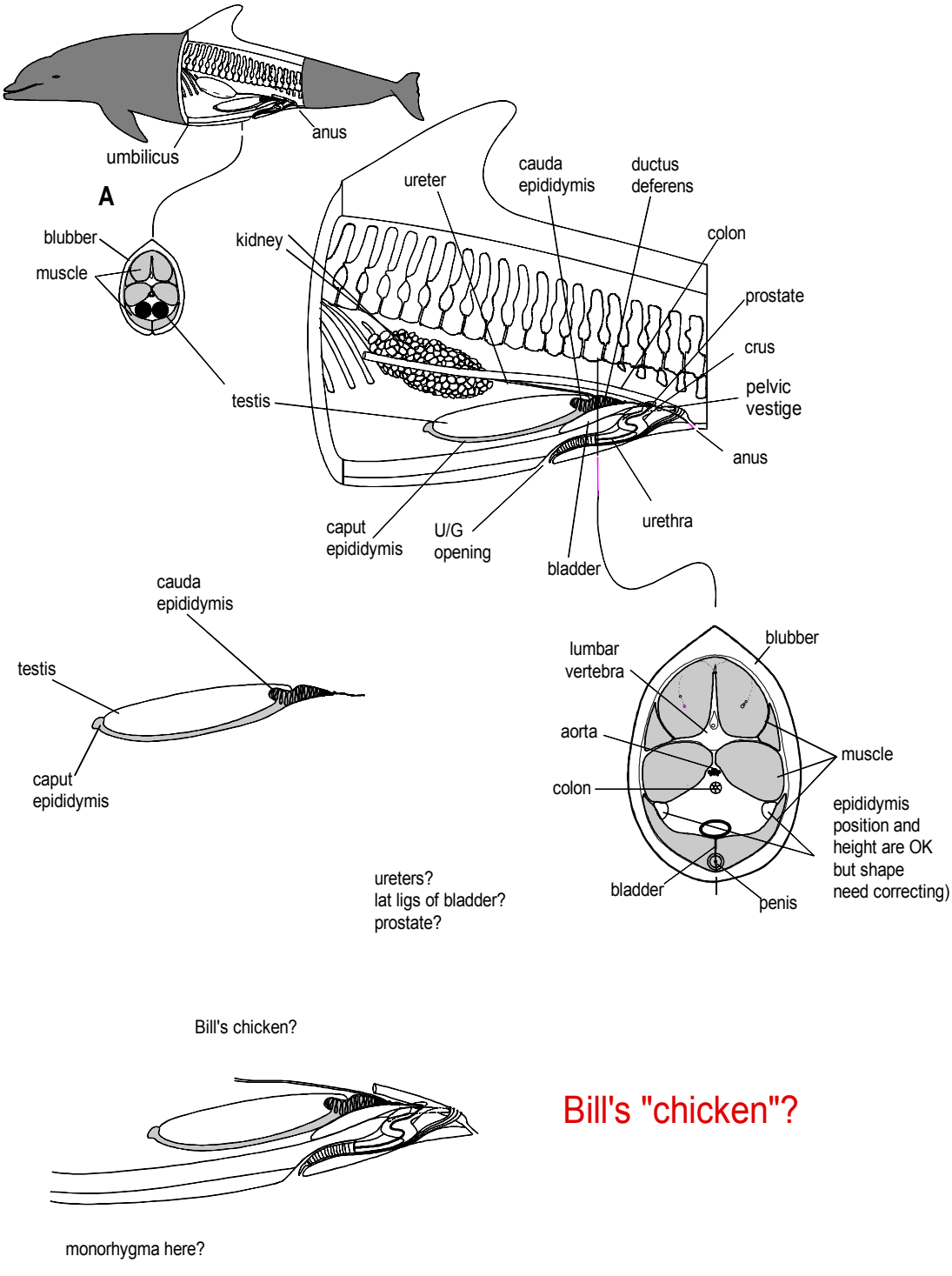
Fig 5 Photographs (from A Fernandez) of gas emboli in the mesenteric veins.



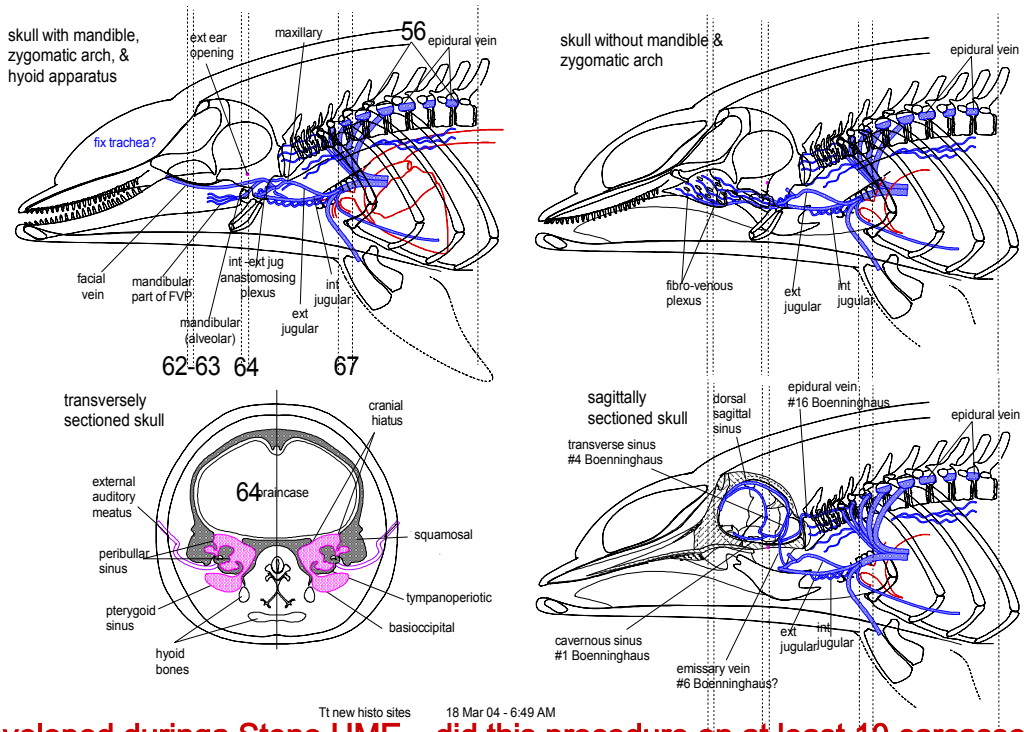
Anatomical guide for large vein identification fig _7__

Maybe put superficial veins (now w/ parasites) in this figure?


after: Rommel, S.A., Pabst, D. A., and McLellan, W.A. (in press) Functional anatomy of the cetacean reproductive system, with comparisons to the domestic dog. in: Miller, D.E. (ed) Reproduction Biology and Physiology of Cetacea, 2006
anus
umbilicus
blubber
muscle
ovary
mammary gland
uterine horn
R. ovary
fimbrae
cervix
R. kidney
colon
hilus of R. ovary (medial aspect)
R. uterine horn
anus
vulva
urinary bladder
R. uterine tube
umbilical (allantoic?) vessels (dotted)
circumferential vessels
pregnant horn
non-pregnant horn
longitudinal allantoic vessels
non-pregnant (quiescent?) ovary
mesometrial border of uterus
uterovarian vascular plexus
cervix
aorta
ureter
region where lumbocaudal venous plexus overlaps, medial to ureter
blubber
lumbar vertebra
aorta
muscle
colon
uterine body
mammary gland
bladder
cross section at level of uterus
pregnant, approximately mid-term, dorsal schematic of uterine arteries and allantoic vessels (ureters, aorta, and attached vessels displaced caudally)
outside diameter mid-length
wall thickness mid-length
uterine midline
length of horn
uterine horn dimensions
corpus albicans
corpus luteum
hilus
lateral aspect, parous, non-preg
medial aspect pregnant
ovarian detail
(after Rommel et al 2006, from Harrison et al. 1972)

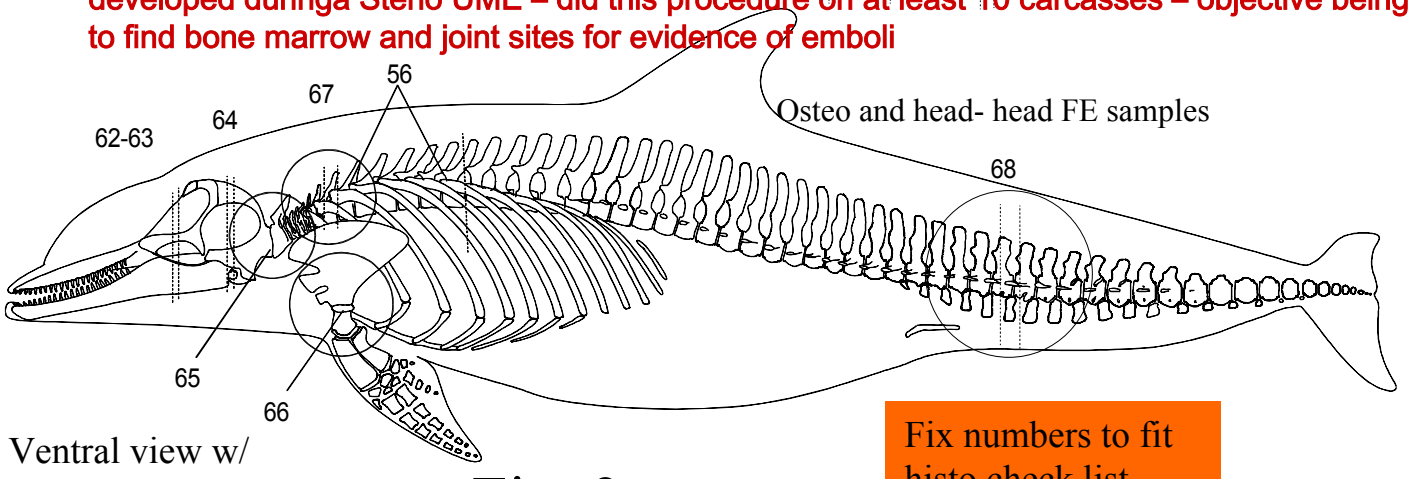

anus
umbilicus
A
blubber
muscle
testis
kidney
ureter
cauda epididymis
ductus deferens
colon
prostate
crus
pelvic vestige
anus
urethra
caput epididymis
U/G opening
bladder
cauda epididymis
testis
caput epididymis
ureters?
lat ligs of bladder?
prostate?
lumbar vertebra
blubber
aorta
muscle
colon
epididymis position and height are OK but shape need correcting)
bladder
penis
Bill's chicken?
monorhygma here?
Bill's "chicken"?

Emailed M Moore wed 2/4/04 834 PM about sample sies in Michael's email folder also check on emails w/ Greg Early about that time – Judy St leger?



Tt new histo sites 18 Mar 04 - 6:49 AM

developed duringa Steno UME – did this procedure on at least 10 carcasses – objective being to find bone marrow and joint sites for evidence of emboli



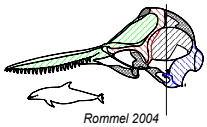
Ventral view w/ mandible??

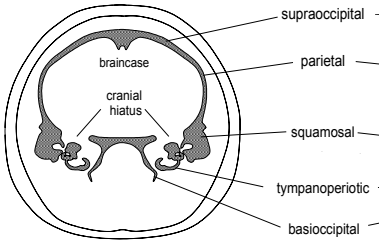
Fig 9

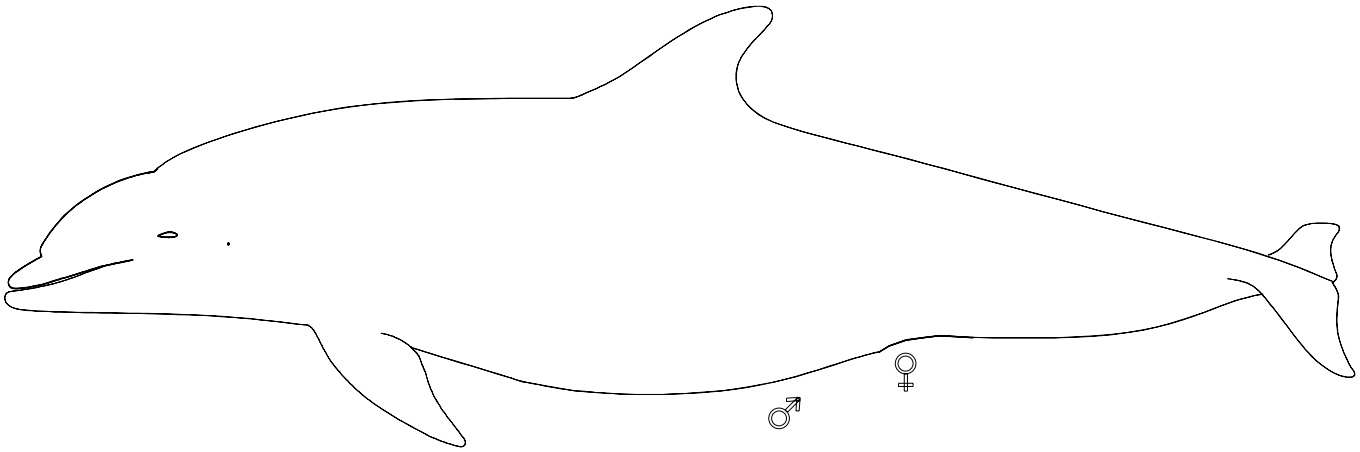
Fix numbers to fit histo check list

AMI internal maxillary artery
PTS pterygoid sinus
TB tympanic bulla
peribullar sinus

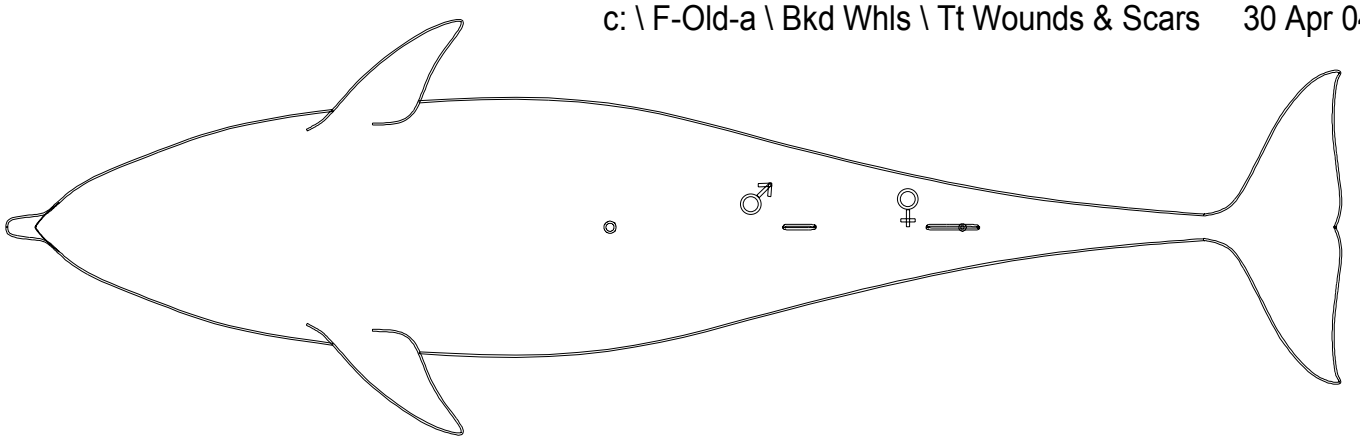
AM mandibular artery
APT pterygoid artery
FVP fibro-venous plexus

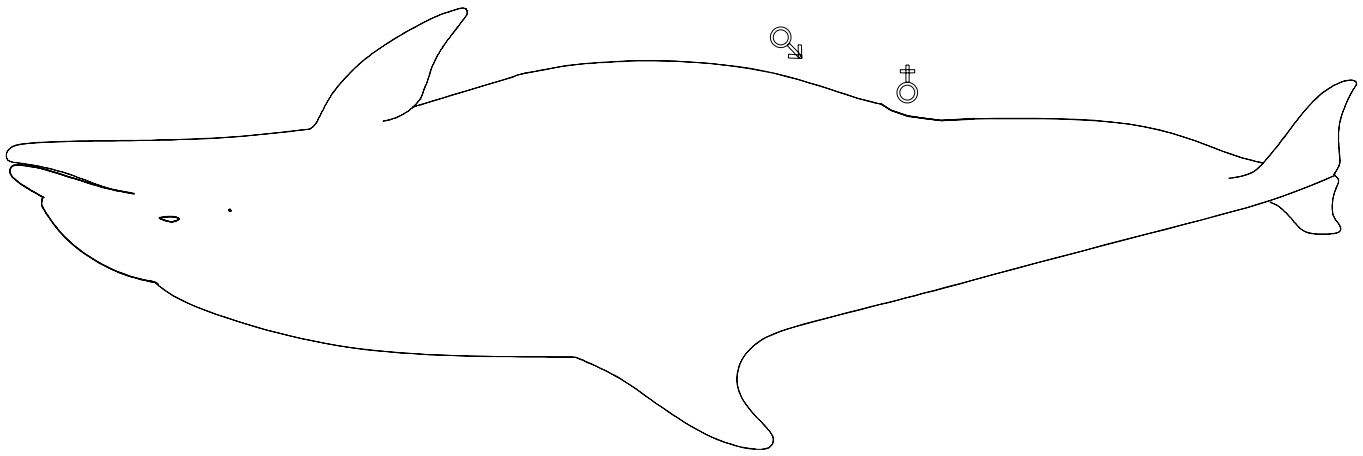

Rommel 2004





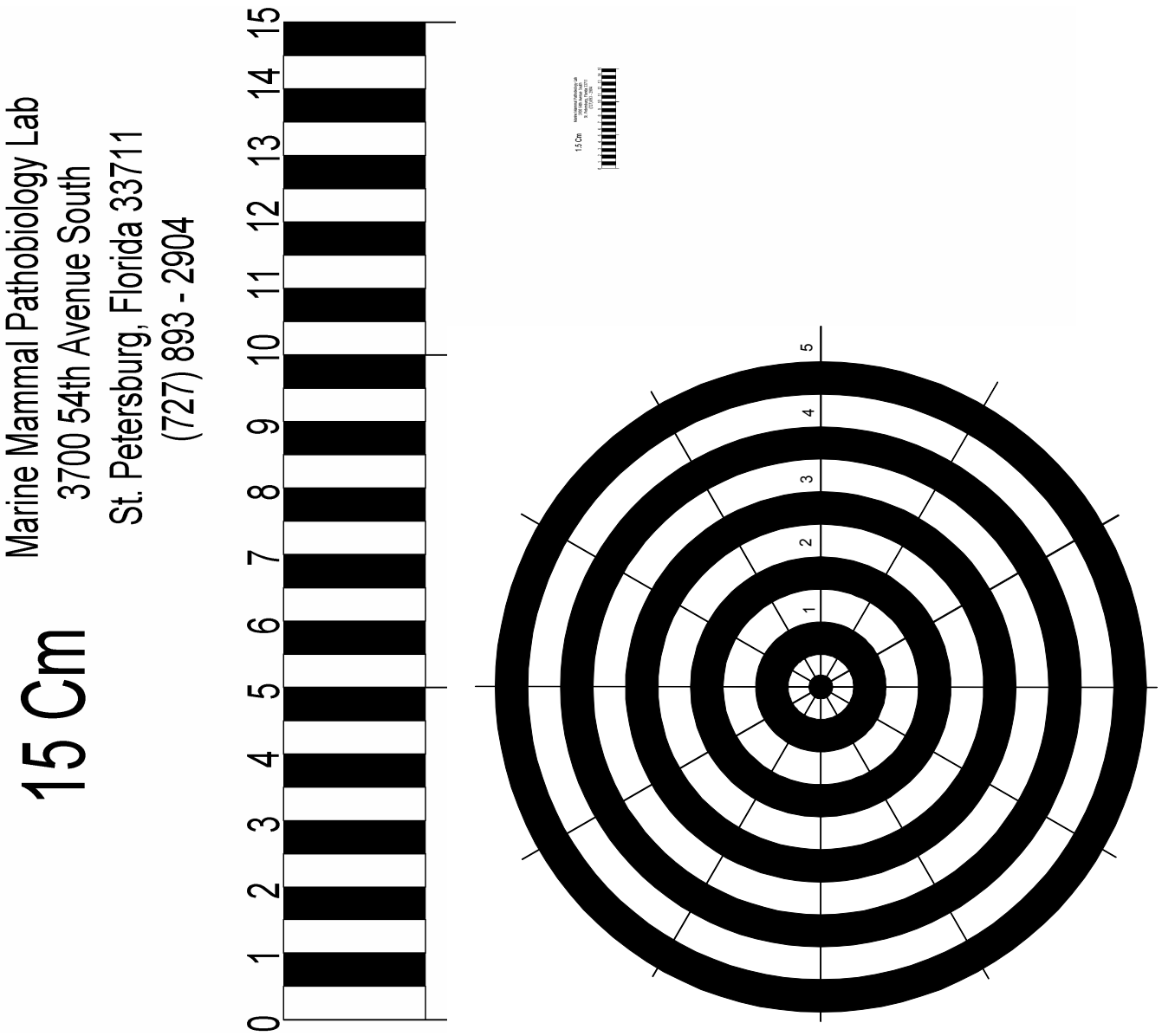
c: \ F-Old-a \ Bkd Whls \ Tt Wounds & Scars 30 Apr 04





11

Appendix 2 Blank wounds and scars report form – combine with Human Interaction (HI)?



Periodically check scale for copying errors

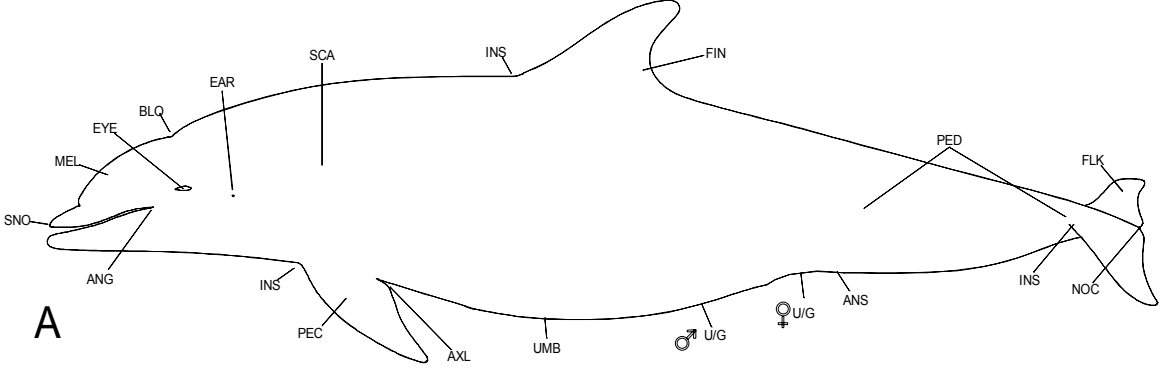
Appendix 3 Paper scale templates, 15 & 1.5 cm lengths - idea is from Frannie's lab – this may seem trivial, but its one of the best suggestions – customize for each lab – just make sure the copies of the scale that are used are really the correct size

Appendix 5 Histology Check List Appendix to be consistent with gross anatomy of the bottlenose dolphin from CRC handbook nad Lymph Node diagram

	FieldID: Da	TBL:	M / F	Tissue Trimmed By:
	Lymph Nodes	**Left**	**Right**	**Comments:**
1	Axillary (AXL)			
2	Superficial Cervical (SPC)			
3	Ext. Auditory Meatal (EAM)			
4	Retropharyngeal (RPH)			
5	Tonsils w/ pharyx (TNL)			
6	Tracheobronchial (TRB)			
	Pulmonary (PLM)	**7**. Marginal	**8**. Hilar	
	Mediastinal (MDS)	**9**. Cranial	**10**. Caudal	
	Sternal (STN)	**11**. Cranial	**12**. Caudal (L/R)	
	Aortic (ART)	**13**. Thoracic	**14**. Lumbar	
15	Mesenteric (MES)			
	Gastric (GTR)	**16**. Hepatic **17**. Pancreatic **18**. Gastric		
19	Abd. Diaphragmatic (ABD)			
20	Colic (CLC)			
	Iliac (ILC)	**21**. Medial	**22**. Lateral (L/R)	
23	Anorectal (ANR)			
24	Other			
25	Other			
	Internal Organs	**Left**	**Right**	
26	Heart Atria (HTA)			
27	Heart Ventricles (HTV)			
	Vascular Structures	**28**. Aorta **29**. Epidural rete **30**. Thoracic rete		
	Tongue	**31**. Tip (TGT)	**32**. Root (TGR)	
	Liver (LVR)	**33**. Proximal	**34**. Distal	
	Stomachs (STM)	**35**. 1st **36**. 2nd **37**. 3rd		
38	Duodenal Ampulla	additional chambers (beaked w hales)		
	Intestine	**39**. Proximal (PIN) **40**. Mid (MIN) **41**. Distal (DIN)		
42	Kidney (KID)			
43	Lung (LNG)			
44	Gonads (GND)			
45	Uterine Horns (UTH)			
46	Vas Deferens (VDF)			
	Epididymis	**47**. Proximal (PED)	**48**. Distal (DED)	
49	Urinary Bladder (BLD)			
	Central Nervous System (CNS)			
		50. Cerebrum (CBR)	**51**. Cerebellum (CBL)	
		52. Brainstem (BST)	**53**. Thoracic spinal cord (TSC)	
	Glands	**Left**	**Right**	
54	Pituitary (PIT)			
55	Adrenal (ADR)			
56	Pancreas (PAN)			
57	Mammary (MAM)			
58	Spleen (SPL)			
59	Thymus (THM)			
60	Thyroid (THR)			
	Other Tissues	**Left**	**Right**	
61	Diaphragm (DIA)			
62	Rib marrow			
63	Goosebeak/Larynx (LRX)			
64	Trachea (TRA)			
	Skin (LIP) (UGN) (MLT)	**65**. Lip **66**. Urogenital **67**. Mediolateral		
68	Conjuctiva of the Eye (CNJ)			
	Head Sections	**69.** Pterygoid sinus (PTS) **70.** Jaw fat w / FVP (FVP)		
		71. Brain case w / dura @ hiatus (BC@H)		
	Synovial Joints	**72.** Occipital condyle (OCC) **73.** gleno-humeral joint (GHJ)		
		74. Costo-vertebral joint @ T-2,3 (CVJ) **75.** Chevron-vertebral joint @ anus (CHJ@A)		

Fix numbers to fit illustrations & text

To get undistorted spreadsheet use: insert / object / create from file / browse


SCA
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ANS
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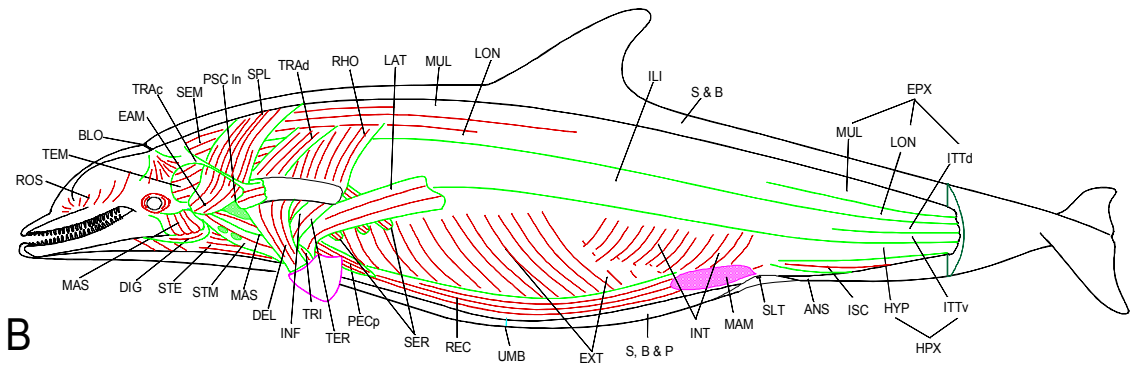
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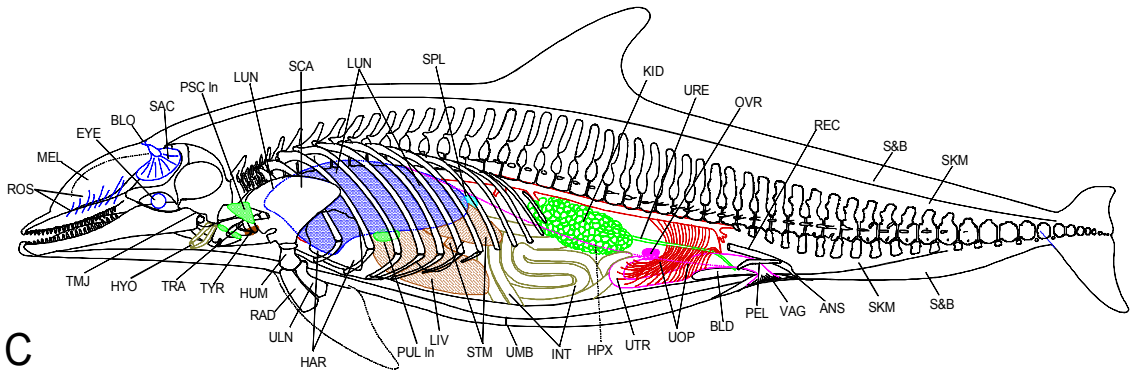
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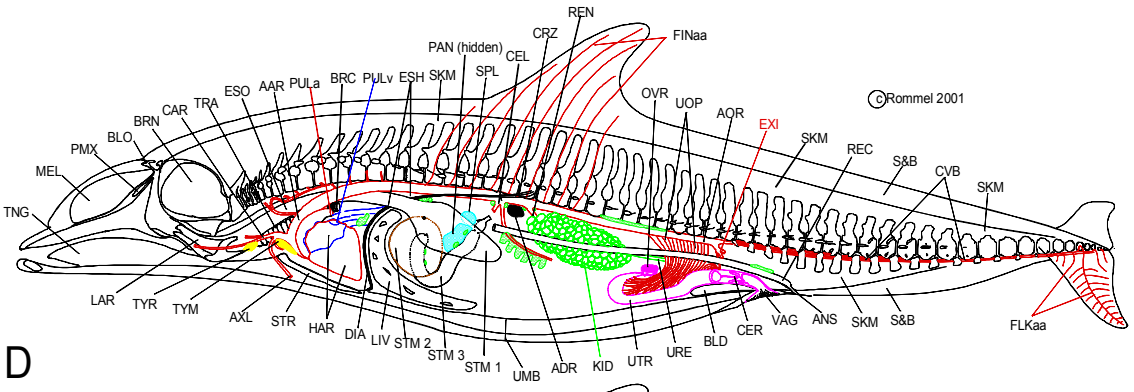
REN
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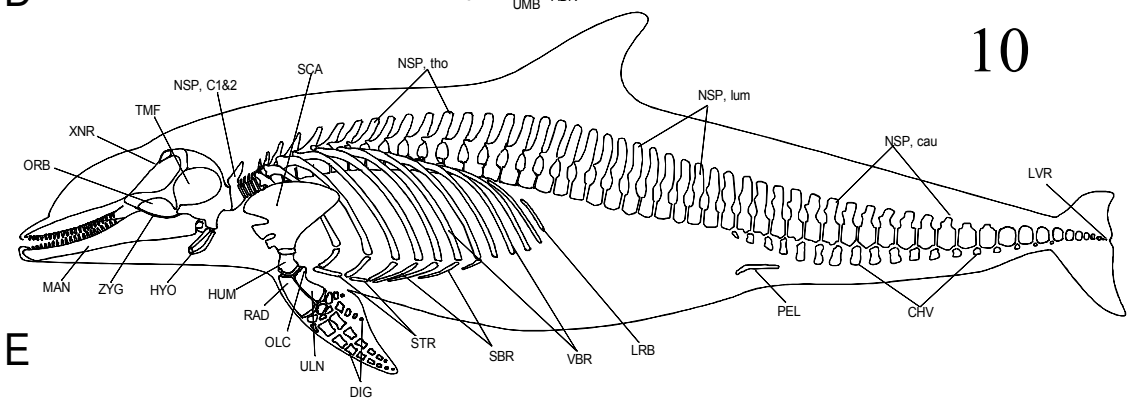

NSP, C1&2
SCA
NSP, tho
NSP, lum
TMF
XNR
NSP, cau
LVR
ORB
MAN
ZYG
HYO
HUM
RAD
OLC
ULN
DIG
STR
SBR
VBR
LRB
PEL
CHV
E

Left lateral illustrations of a healthy **bottlenose dolphin** (*Tursiops truncatus*). After: Rommel, S.A. and L.J. Lowenstein. 2001. Gross and microscopic anatomy of marine mammals. Pp. 129-163 in: L.A. Dierauf and F.M.D. Gulland, Eds., CRC Handbook of Marine Mammal Medicine, 2nd Edition, CRC Press, Boca Raton, FL.

Layer A - External features. The following abbreviations are used as labels: **ANG** - angle of mouth; **ANS** - anus; **AXL** - axilla; **BLO** - blowhole, external naris in dolphin; **EAR** - external auditory opening, ear; **EYE** - eye; **FIN** - dorsal fin; **FLK** - flukes - entire caudal extremity in cetaceans; **INS** - cranial insertion of the extremity; flipper, fin, and/or fluke; **NOC** - fluke notch in dugongs and in most cetaceans; **PEC** - pectoral limb, flipper; **PED** - peduncle, base of tail, between anus and flukes; **MEL** - melon; **SCA** - dorsal border of the scapula, palpable bony feature in emaciated dolphins; **SNO** - snout, cranial tip of upper jaw; **UMB** - umbilicus; **U/G** - urogenital opening.

Layer B - The superficial skeletal muscles. The layer of skeletal muscles just deep to the blubber and panniculus muscles. Note that the large muscles ventral to the dorsal fin are surrounded by a tough connective tissue sheath (Pabst, 1990). The following abbreviations are used as labels: **ANS** - anus; **BLO** - blowhole; **DEL** - deltoid; **DIG** - digastric; **EAM** - external auditory meatus; **EPX** - epaxial muscles, upstroke muscles; **EXT** - external oblique; **HYP** - hypaxialis; **HPX** - hypaxial muscles, down stroke muscles; **ILI** - iliocostalis; **INT** - internal oblique; **ITTd** - intertransversarius caudae dorsalis; **ITTv** - intertransversarius caudae ventralis; **LAT** - latissimus dorsi; **LEV** - levator ani; **LON** - longissimus; **MAM** - mammary gland; **MAS** - masseter; **MUL** - multifidus; **PECp**- deep (profound) pectoral; **PSC ln** - presacpular lymph node (superf. cerv.); **REC** - rectus abdominous; **RHO** - rhomboid; **ROS** - rostral muscles; **S&B** - skin, blubber, and panniculus muscle (where present) cut along midline; **SER** - serratus; **SLT** - mammary slit, nipple; **SPL** - splenius; **STE** - sternohyoid; **STM** - sternomastoid; **TER** - teres major **TMP** - temporalis; **TRAd** – trapezius dorsalis; **TRAc -** trapezius cranialis; **TRI** - triceps brachii; **UMB** - umbilicus.

Layer C - The superficial internal structures with "anatomical landmarks". The relative sizes of the lungs represent partial inflation -- full inflation would extend margins to distal tips of rib). The following abbreviations are used as labels: **ANS** - anus; **BLD** - urinary bladder; **BLO** - blow hole; **EYE** - eye; **HAR** -heart; **HYO** - hyoid apparatus; **INT** - intestines; **KID** - left kidney; **LIV** - liver; **LUN** - lung (note that it extends beneath the scapula); **MEL** - melon; **OVR** - left ovary; **PEL** - pelvic vestige; **PSC ln** - prescapular lymph node; **PUL ln** - pulmonary lymph node, unique to cetaceans; **RAD** - radius; **REC** - rectum; **ROS** - rostral muscles, to manipulate the melon; **SAC** - lateral diverticulae, air sacs in dolphin; **S&B** - skin and blubber; **SCA** - scapula; **SKM** - skeletal muscle; **SPL** - spleen; **STM** - stomachs; **TMJ** - temporomandibular joint; **TRA** - trachea; **TYR** - thyroid gland; **ULN** - ulna; **UMB** - umbilical scar; **UOP** - uterovarian plexus; **URE** - ureter; **UTR** - uterine horn; **VAG** - vagina.

Layer D – A view slightly to the left of the mid-sagittal plane illustrates the circulation, body cavities, and selected organs. Note that the diaphragm separates the heart and lungs from the liver and other abdominal organs. The following abbreviations are used as labels (structures on the midline are in bold, those off-midline are in italics): **AAR** - aortic arch; *ADR* - left adrenal gland; **ANS** - anus; **AOR** - aorta; *AXL* - axillary artery; **BLD** - urinary bladder; **BLO** - blowhole; *BRC* - bronchus; **BRN**- brain; *CAR* - carotid artery; **CEL** - celiac artery; **CER** - cervix; CRZ - left crus of the diaphragm; **CVB** - caudal vascular bundle; **DIA** - diaphragm, cut at midline, extends from crura dorsally to sternum ventrally; **ESO** - esophagus (to the left of the midline cranially, on the midline caudally); *ESH* - esophageal hiatus; *EXI* - external iliac artery; **FINaa** - arteries arrayed along the midline of the dorsal fin; *FLKaa* - arterial plexus on dorsal and ventral aspects of each fluke; **HAR** - heart; *KID* - right kidney; **LAR** - larynx or goosebeak; **LIV** - liver, cut at midline; **MEL** - melon; *OVR* - right ovary; *PAN* - pancreas (hidden behind 1st stomach); *PMX* - premaxillary sac; *PULa* - pulmonary artery, cut at hilus of lung; *PULv* - pulmonary vein, cut at hilus of lung; **REC** - rectum; *REN* - renal artery; **S&B** - skin and blubber, panniculus where appropriate cut at midline; *SKM* -skeletal muscle; *SPL* - spleen; *STM1* - forestomach; *STM2* - main stomach; *STM3* - pyloric stomach; **STR** - sternum, sternabrae; **TNG** - tongue; **TRA** - trachea; **TYM** - thymus gland; *TYR* - thyroid gland; **UMB** - umbilicus; *UOP* - right uterovarian vascular plexus in dolphin; *URE* - right ureter; *UTR* - uterus; **VAG** - vagina.

Layer E - The skeleton. Regions of the vertebral column (cervical, thoracic, lumbar, sacral, and caudal) are abbreviated (in lower case) as **cer, tho, lum, sac**, and **cau**, respectively, and are used as modifiers after an abbreviation in caps and a comma. If a specific vertebra is labeled, it will be represented by a capitalized first letter (for caudal **Ca** will be used) and the vertebral number, i.e., first cervical = **C1**, tenth thoracic = **T10**. The following abbreviations are used as labels: **CHV** - chevrons, chevron bones; **DIG** - digits; **HUM** - humerus; **HYO** - hyoid apparatus; **LRB** - last, or caudalmost, rib; **LVR** - last, or caudalmost, vertebra; **MAN** - mandible; **NSP** - neural spine; e.g., thoracic neural spines = **NSP, tho**; **OLC** - olecranon; **ORB** - orbit; **PEL** - pelvic vestige; **RAD** - radius; **SCA** - scapula; **STR** - sternum; **SBR** - sternal ribs, costal ribs; **TMF** - temporal fossa; **ULN** - ulna; **VBR** - vertebral ribs; **XNR** - external (bony) nares, nasal aperture of the skull; **ZYG** - zygomatic arch.

Appendix 6 Blank necropsy narrative / report form

Ventral views of skull for earbone extraction?

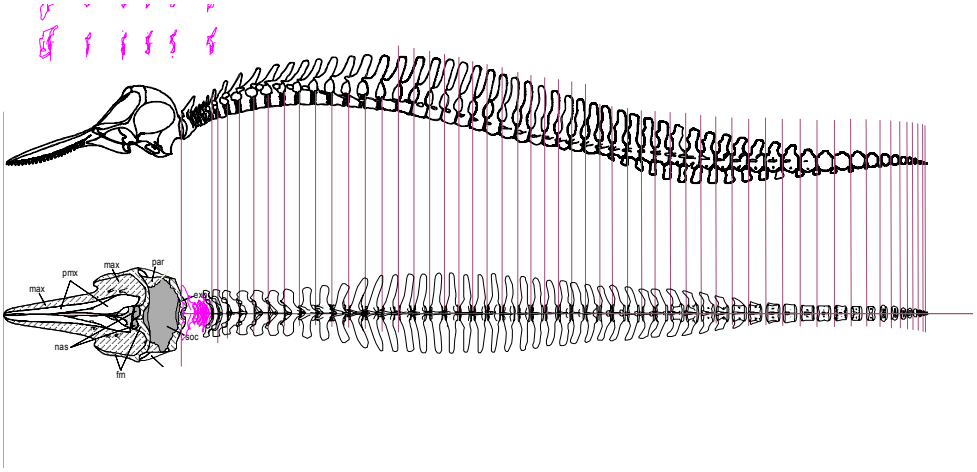
Carcass condition

Add – figs for guidance in region of ear – FVP – include or just refer to Fraser & Purves? – Ted Cranford, any progress on his description –of FVP – use his photo?

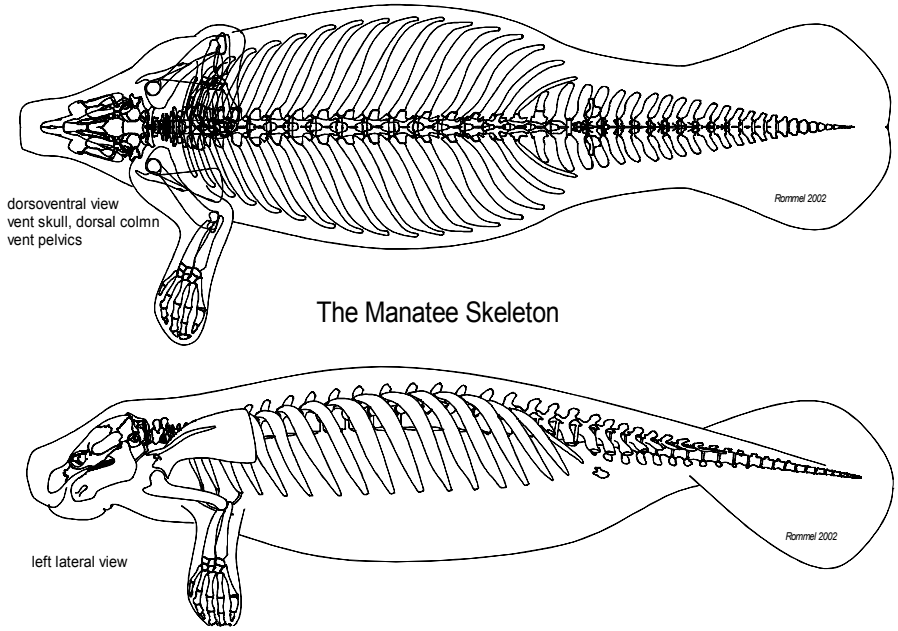
Data sheets for characterizing poorly known structures – e.g., Inn dimensions data, FVP, lipids

Cuts for extracting the brain in med-large and small odontocetes

Should we have a skeleton lesion sheet like in manatees? A perinatal one?



Skeleton illustration for describing osteo lesions

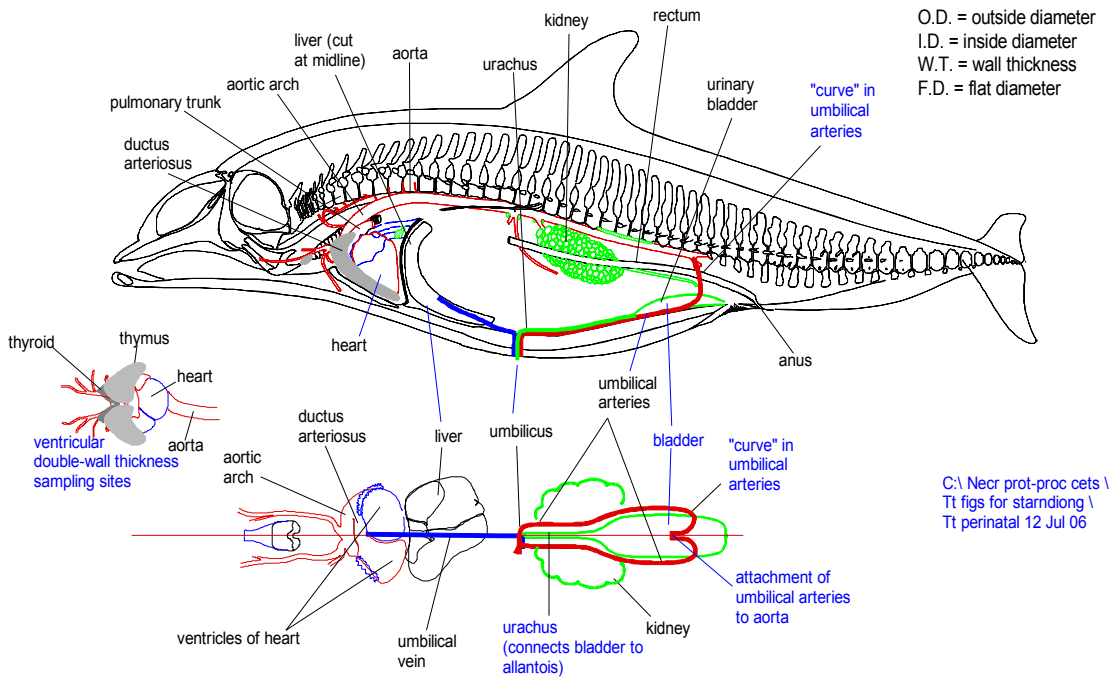


Draft Tt Perinatal Protocol Condition _____
ID# _____ Date_____ / _____ / _____ TBL _____ Gender M / F Wt _____

__Photograph the intact umbilicus, include reference scale _____
__Umbilicus - healed / raw --- open / closed cord / placenta attached / skirt_____
__Urachus (bladder to umbilicus to allantois) - open / closed / closed but probe patent_____
_____
__Umbil Art - open / closed / closed but probe patent_____
_____
__Umbil Vein - open / closed / closed but probe patent_____
_____
__Ductus Arteriosus - open / closed / closed but probe patent_ diameter _____
_____
__Pulmonary Artery diameter _____
_____
__Foramen Ovale, # openings _____ overall % open_____
__GI tract: intestine length _____
__Thymus _____
__Thyroid _____
__Lungs __consolidated / inflated / gassey (decomposition)_____

__Meconium: where in GI tract / texture / genl appearance_____
__Stomachs contents __I _____II_____III_____
__REPRO - gonad wt and dimensions:_____
_____
__Fetal body folds - fluke folds_ body / peduncle / fluke _____
__ Ventricular double-wall thickness: Left _____ Right _____
__signs of cerebral birth injury_____

tooth count - note if skin over each tooth is intact UR ___ UL ___ LR____ LL _____



Umbilical arteries parallel the urachus from umbilicus to the bladder "tip" and from then lateral to the margins of the bladder to the "curve" where they join the aorta at the dorsal midline of the abdominal cavity, caudal to the kidneys.

Examine the umbilical arteries by palpation and then by dissection -- determine if the wall thickness & inside diameter is reduced -- measure the distance between the umbilicus and the transition from 'full' to 'reduced' diameter; describe with words and numbers.

Record the occurence of frank or residual blood, either in the vessels and or the body cavities. This may be an artifact of normal trauma of birth or an indication of some problem with the fetal circulation or possibly some injury to the calf.
Examine and measure the ductus arteriosus -- compare its diameter with diameters of the pulmonary arteries after they branch from the pulmonary trunk. The region where the ductus arteriosus joins the aorta is usually obscured by the thymus and the cranial margin of the pericardium.
Dissect and weigh the thyroid and thymus if in good condition.
Careful dissection of the thymus adds little time since the ductus arteriosus has to be exposed anyway.

To evaluate the foramen ovale, open both atrial walls and place a finger against the foramena in the left atrium and examine them from the right atrium. Examine the membrane for % closure of the foramena and estimate the number and size of the openings (resembles Swiss cheese).

O.D. = outside diameter
I.D. = inside diameter
W.T. = wall thickness
F.D. = flat diameter