SMALL CETACEAN DISSECTION AND SAMPLING:
A FIELD GUIDE

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INTRODUCTION

The collection of biological data is critical to the management and monitoring of cetacean populations impacted by fisheries. Shipboard observer programs were established, as specified in the Marine Mammal Protection Act of 1972, to document the incidental take of marine mammals during fishing operations and to collect biological data from individual animals. Initially, the sampling program for the National Marine Fisheries Service, Southwest Region, focused on monitoring the take of dolphins incidental to the eastern tropical Pacific yellowfin tuna purse-seine fishery. The observer program was expanded in 1990 to document the incidental take of marine mammals by gillnet vessels fishing off the coast of California and to collect biological data from individual dead animals. Regional networks were also established under the Marine Mammal Protection Act of 1972 to monitor strandings of cetaceans, and the same procedures are used to standardize the collection of data. The biological data are used to: (1) learn about a species' natural history, (2) determine the age and sex structure of the take, (3) estimate life history parameters, and (4) interpret population structure. These categories are not mutually exclusive in their use of biological data.

Descriptions of a species' natural history include its distribution and seasonal movement patterns, estimates of herd size and composition, maximum body size, longevity, calving interval, and prey species. Observers provide a source of data for some of these parameters.

A fishery may selectively take certain age classes, and thereby, affect the reproductive potential of the population impacted. Data collected from individual animals can be used to describe fishery selectivity. Specifically, the data on species, sex, total body length, and age are of primary interest. Similarly, the susceptibility of animals to events that lead to stranding may differ by age class and reproductive condition.

Life history parameters estimated from biological data collected include pregnancy rates, calving interval, individual growth rates, asymptotic length, average age at attainment of sexual maturity, longevity, and breeding seasonality (Perrin and Reilly 1984). A number of papers have presented analyses of the life history data collected from eastern tropical Pacific dolphins (these include: Barlow 1984, 1985; Perrin et al. 1976, 1977, 1991a, 1991b; Perrin and Henderson 1984; Hohn and Hammond 1985; Hohn et al. 1985; Myrick et al. 1986; Bright and Chivers2; Chivers and Myrick 1993).

Population condition, which is a measure of the effect of population density relative to carrying capacity, can be monitored using biological indices, including the proportion of mature females in the population and the percentage of sexually mature females pregnant, lactating, or simultaneously pregnant and lactating (Perrin and Donovan 1984, Barlow 1985, Chivers

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Changes in population size due to exploitation or changes in carrying capacity can be distinguished when appropriate biological indices are identified (Gerrodette and DeMaster 1990).

The population or management unit (stock), of a species is the basic unit recognized to maintain genetic diversity, and is defined on the basis of distribution, morphology, life history, and genetics (Dizon et al. 1992). Historically, identification of populations within a species has been based on hiatuses in distribution and differences in morphological characteristics. Differences in the life history parameters within a species, may also reflect environmental adaptation and identify potentially unique populations. More recently, analyses of genetic sequences from mitochondrial DNA (mtDNA), extracted from samples of skin, have been used to characterize a population’s discreteness.

The purpose of this manual is to provide a practical, easy reference guide for standardized collection of biological data from cetaceans. This guide is more general than the manual by Myrick (1986) which specifically describes sampling procedures for the tuna purse-seine vessel observer program. Following a description of the sampling equipment, we describe the methods for collecting each data element. We first explain the external measurements and samples to be collected, and then, the collection and storage methods for sampled internal organs. For each sample collected, we also provide a brief description of research that uses the data. Additional information on methods for sample collection, storage, and preparation can be found in Heyning (1991), Akin et al. (1993), and Geraci and Lounsbury (1993). Pinnipeds are also sampled from gillnet vessels and, although the sampling procedures are similar, there are some differences in anatomy and collection methods from those for cetaceans (Winchell 1990). The specific sampling protocols for each fishery are provided in the "Purse Seine Observer Field Manual" and the "Gillnet Observer Field Manual." 

**SAMPLING EQUIPMENT**

A list of the sampling equipment issued to observers is included as Appendix 1. The use and care of each piece of equipment is described below in alphabetical order.

**Adrenal kits**

Pre-packaged kits are provided for collecting adrenal glands. Each kit consists of a Whirl-pac containing approximately 10 cc of formalin, an outer ziplock bag, and small tags for specimen labels.
Ten-percent (10%) formalin solution is used to fix tissues for histological examination, because freezing ruptures cells and reduces their value in histological studies. Concentrations >13% usually result in formalin "burn," a bleaching and hardening of the tissues, while concentrations of <10% inadequately preserve tissues, resulting in decomposition.

To prepare the fixative, mix one part formalin to nine parts water, preferably fresh water, but seawater may be used as a substitute. Prepare enough fixative to fill one bucket about 1/3 full. A bucket can be filled with freshly collected samples until the samples can no longer move freely in the bucket; adequate coverage of all materials is essential for proper fixation of all tissues. If the tissue sample is large, it should be sliced to the center with a knife to allow the formalin to penetrate through the sample.

Buckets and bags

Buckets (5 gallon) are used for collecting samples during dissections, fixing samples in formalin, and storing and transporting samples. Air-tight lids provide a seal to prevent formalin leakage and evaporation.

Plastic and burlap bags, in assorted sizes, are provided for packaging samples for frozen storage. Zippered body bags are sometimes provided for collecting whole carcasses, and Whirl-pac bags are provided for storage of small samples. To make the packages of frozen samples more manageable, wrap the bags tightly around the sample material with freezer or masking tape, and then label the outside of the package with a specimen label, before freezing.

Calipers

Calipers are used for measuring total body length and also can be used to provide scale in photographs. The calipers are 2-m wooden sticks with 2 plastic jaws. One of the jaws is stationary at one end, and the other is adjustable and can slide the length of the meter stick. To minimize the risk of warping, keep them dry and lubricated with silicone spray, and store them flat when not in use. Before using the calipers after they have been stored, check their accuracy by measuring a known-length object, preferably a section of a meter stick or measuring tape. They can be re-calibrated by loosening the set screw on the stationary jaw and adjusting the jaw's position until an accurate reading is made.

Cameras

The Kodak Fling 35 disposable camera is used to photograph animals for which species identification is uncertain and to document unusual markings or conditions. In each photograph, include a label that is easy to read with at least the specimen number, and the calipers or tape measure for scale. Preferably, the cruise number or collection locality is also included with the specimen number on the label. Record the roll and frame numbers of the specimen photographed on the life history form. A "blank" frame between photographs of different specimens helps to delineate separate specimens. These cameras are fragile and easily damaged by saltwater and organic materials, and should be handled with care.

Fixative

Ten-percent (10%) formalin solution is used to fix tissues for histological examination, because freezing ruptures cells and reduces their value in histological studies. Concentrations >13% usually result in formalin "burn," a bleaching and hardening of the tissues, while concentrations of <10% inadequately preserve tissues, resulting in decomposition.
Formalin is considered a potential carcinogen, and is an irritant to skin, eyes, and nasal membranes. It is best to minimize your exposure and avoid skin contact by wearing rubber gloves and protective eye-wear when preparing or handling fixative or fixed specimens. Work with this fixative only in well-ventilated areas.

**Foil**

Aluminum foil is provided for wrapping blubber samples for storage.

**Gloves**

Cotton and rubber gloves are provided to protect your hands when handling carcasses, samples, or fixatives. The cotton gloves are used for moving carcasses around the deck, and rubber gloves are used during specimen sampling or processing and when handling fixative or preserved samples.

**Knives**

Knives are provided for animal dissections. For safety, keep the knives sharp and cut away from yourself. A whetstone or steel is provided for sharpening knives. During dissections, we recommend embedding the knife in the heavy back musculature of the carcass when it is not being used.

**Labels and cable ties**

A specimen label is made of cotton rag with reinforced holes. The unique specimen number identifying the animal collected or sampled is written on the label with a permanent marker or soft lead pencil. Other identifying information also should be included on the label, i.e., species and the cruise number or collection date and location of the specimen material collected. Keep tags dry. All tags needed to label samples for one specimen may be made up prior to beginning that dissection. But to avoid mislabelling specimens, only prepare specimen labels for one specimen at a time. Small cotton rag tags are also supplied for labelling sample material stored in Whirl-pacs or ziplock bags (e.g., the adrenal and skin sample kits).

Cable ties, rather than string, are used for attaching specimen labels, because they do not break or loosen during freezer storage. Approximately 15-cm cable ties are used for attaching specimen labels to samples, closing the openings to the stomach and closing sample storage bags. Longer cable ties, approximately 60-cm, are used to attach specimen labels to the peduncle of a carcass. Always ensure that cable ties are cinched tightly.

**Measuring tape**

Flexible tapes are for measuring the maximum girth of the specimen. Because the tapes tend to sag and follow body curves when measuring, they should not be used to measure total body length of the specimen, unless calipers are unavailable (i.e., broken or lost). If the tape measure was used, however, note this on the life history form.
Pruning shears

Pruning shears or loppers are provided for collecting jaw sections with teeth. The blades will corrode in salt water, and therefore, after processing specimens, the blades should be cleaned, dried, and sprayed with silicone. The cutting edges will become dull after shearing bone and connective tissue, but can be sharpened with a whetstone, bench grinder, or flat metal file.

Skin-sample kits

Skin-sample kits, which have occasionally been referred to as biopsy kits, are provided for preserving samples of skin for genetic studies. The kits include plastic vials with DMSO (dimethyl sulfoxide). DMSO is not toxic, but one should avoid contact with this solution. An EXACTO\textsuperscript{\textregistered} knife, blades, gloves, a permanent marking pen, and small tags for specimen labels are also included in the kit. To avoid contamination of the samples, use a new EXACTO\textsuperscript{\textregistered} blade and clean gloves for each sample. Do not use dissection knives to take these samples.
To ensure standard collection of data and samples, we recommend establishing a uniform routine for processing specimens. Begin the processing sequence by positioning the carcass on its right side, with the belly towards you and the head pointing to your left. If all specimens are aligned in the same orientation, and the data elements are collected in the same order, fewer errors will be made and work-up time will be appreciably shortened.

If more than one animal is sampled from a set, collect specimens for processing as they become available to ensure random sampling of the incidental kill. This will avoid introducing species, sex, or size bias in the collected samples. We suggest that you work up each specimen as you come to it; discarding the remains as you go to avoid the potential for mislabelling samples. A summary of preservation techniques for the biological samples collected is provided in Appendix 2.

**Specimen number**

The specimen number consists of a three-letter group, followed by a four-digit number. A unique three-letter code is assigned to each observer during the Southwest Region’s observer training program, and the observer assigns a sequential four-digit number to each specimen sampled. For example, if your observer prefix is ABC, your first specimen number will be ABC0001, and the subsequent specimen will be ABC0002. Specimen numbers are allocated sequentially, regardless of whether they are assigned on more than one trip, to ensure that every specimen processed has a unique field number.

**Data forms**

Data forms (Appendices 3 and 4) facilitate standardized data collection. However, generally more measurements are made on stranded cetaceans usually because more time is available. These are listed on the "Cetacean Data Record" (Appendix 5) and are illustrated in Geraci and Lounsbury (1993; their Fig. 10.7, page 188). Fill out the forms with a soft-lead pencil, and keep them as clean and dry as possible. If you make a mistake, do not erase it. Line through the error and write the correction above or beside it, because records of errors can be used during data editing to correct discrepancies in the data.

**Collecting the carcass**

When the entire carcass is collected, tag it before freezing. Use a long cable tie and a specimen label to tag the carcass around the tail stock and rostrum. Place additional specimen labels in the mouth, blowhole, and genital opening.

If a body bag is used for storage, label the carcass as previously specified, and attach a specimen label that includes specimen number, species, and cruise number or collection locality to the outside of the bag.
External anatomy

Sex

To determine sex, examine the mid-ventral line of the animal (Fig. 1). Similar to other mammals, female cetaceans have genital and anal openings lie in close proximity to each other within a common ventral depression; generally, there is <10-cm between the centers of the two openings in adult female dolphins. These two openings are generally separated by >10-cm in males and are found in separate ventral depressions. There is inter-species variability in the placement of these openings, and the measurements given are a guideline for small cetaceans. Females also have a single short mammary slit along either side of the urogenital slit. This characteristic should not be used alone to determine the sex of the specimen, however, because males sometimes develop skin folds that resemble mammary slits.

The "pencil test" can be used to unequivocally determine the sex of a specimen (Fig. 2). To do this, insert the eraser end of a pencil (or any blunt probe) into the genital opening. Try to push the pencil forward, then backward. If it goes forward, the specimen is a female, and the pencil has entered the vagina which is angled forward and upward. If the probe goes backward, the specimen is a male, and is entering the sheath of the penis.

Total body length

Total body length is one of the most important data elements and is used to study growth rates and population structure (Perrin 1975; Perrin et al. 1985, 1991a; Hohn and Hammond 1985; Bright and Chivers²).

The standard total body length measurement is taken with the specimen lying on its belly and the long axis of the body straight (Fig. 3). The standard measurement is taken with the calipers from the tip of the upper jaw to the center of the notch between the tail flukes. The calipers should be above the midline of the body and parallel to the body's axis. Place the stationary jaw into the notch in the flukes, and slide the non-stationary jaw along the stick to the tip of the upper jaw. Record the measurement to the nearest centimeter.

When measuring a carcass close to or more than 2-m long, the non-stationary jaw will not reach to the tip of the upper jaw, and two measurements must be made and added together. To do this, measure from the fluke notch to 1-m or 2-m, and mark the carcass with a vertical cut using a knife. Then, measure from the mark to the tip of the upper jaw keeping the stick as horizontal as possible. Add the two measurements together, and record the total body length to the nearest centimeter.

If the calipers break or are unavailable for use in measuring total body length, the flexible tape measure may be used instead. Record on the life history form how the measurement was made. If the "Marine Mammal Life History Form" (Appendix 4) is used, there is a curvilinear measurement check box which should only be marked, if the total body length measurement is curvilinear rather than parallel to the body axis. Otherwise note that the meter stick measurement was approximated with the measuring tape. Duplicate the caliper measurement as nearly as possible by keeping the tape taut and parallel to the body.
Figure 1. Schematic drawing of the ventral surface of a male and female small cetacean for sex determination. In males, the genital and anal openings are located in two well-separated depressions, while in females, the openings are located in the same depression, i.e., a nearly continuous depression.
Figure 2. The "pencil test" for determining the sex of a small cetacean. If the blunt end of a probe can be pushed forward, it is female; if it can be pushed backward it is male. The animal shown in the photo is male.
Figure 3. The standard measurement of total body length is taken from the tip of the upper jaw to the notch in the flukes, parallel to animal's straight body axis. Maximum girth is measured just anterior to the dorsal fin. For *Lissodelphis borealis*, however, girth is measured at the posterior margin of the pectoral fins, i.e., axillary girth.
axis. Straight sticks or knives can be used in place of the caliper jaws for alignment at the fluke notch and tip of the upper jaw.

Girth

The maximum girth of the animal is taken to the nearest centimeter with the flexible tape measure. The standard measurement is made just anterior to the dorsal fin for all species of small cetacean. For the northern right whale dolphin (Lissodelphis borealis), however, axillary girth is measured; this measurement is taken posterior to the pectoral fins (Fig. 3).

Mammary gland examination

The presence of milk indicates that a female was nursing a calf, and data on lactation are used to estimate the length of the lactation period, a component of the calving or reproductive interval for small cetaceans (Perrin et al. 1976, 1977; Myrick et al. 1986).

To determine whether a female is lactating, locate the mammary slits on either side of the urogenital slit (Fig. 4). Using your thumb and forefinger, pinch each firmly several times. If the pinch test fails to produce milk, slice across the center of each mammary slit to a depth of about 2-cm and pinch again several times. If fluid is produced, check the color and inspect the incised area for cestode parasites. Milk will be cream colored, but cestodes sometimes encyst in the genital area, encasing themselves in an easily detectable membranous sac filled with yellowish-white fluid that can be mistaken for milk. If the fluid is milk, answer "yes" to the lactation question on the data sheet.

If a female is in the late stages of pregnancy or has just given birth, the mammary glands may contain colostrum, a product produced during the early stages of lactation. Colostrum looks like maple syrup or honey (Best and Robertson 1971). If colostrum is present, the female is considered to be lactating, if she is not also pregnant. The presence of colostrum, rather than milk, should be noted on the life history form in either case.

Head

Measurements made from prepared skulls provide the morphological data for distinguishing species and populations. When a new species is described, a type specimen, consisting of at least a skull, must be deposited in a museum. Morphometric data gathered from the skulls and carcasses have been used to study phenotypic variability within a species (Perrin 1975; Perrin et al. 1979, 1985, 1991b; Douglas et al. 1992).

To remove the head, locate the rear of the braincase, which slopes downward at an abrupt angle, by feeling with your hand behind the blowhole (Fig. 5). Make an incision slightly behind this sloped region, about 10-cm behind the blowhole, down to a point just anterior to the insertion of the flipper. Deepen and lengthen this cut as you pull the rostrum toward the animal’s belly. Once you have cut nearly to the center of the animal’s neck, locate the occipital condyles at the back of the skull. The occipital condyles are paired, smooth and shiny, and articulate with the atlas vertebrae. As you continue to cut and pull the rostrum toward the animal’s belly, the tension will suddenly release causing the occipital condyles to
Figure 4. To check whether a female dolphin is lactating, (A) pinch each mammary slit several times. (B) If no milk comes out, each slit is cut transversely and pinched again. The color of any discharge from the mammary glands should be noted to distinguish between milk, colostrum, and cestode infestation.
Figure 5. Collecting the head of a small cetacean. (A) To collect a head, first make a cut at the base of the skull, about 10-cm behind the blow hole, and deepen it while pulling the rostrum towards the animal's ventral surface. Locate the occipital condyles when the cut is about halfway through the body. (B) Continue cutting and applying pressure by continuing to pull the head towards the belly to separate the occipital condyles (arrows) from the atlas, or first vertebra.
separate from the atlas. Finally, complete removal of the head by cutting through the remaining muscle and connective tissues. After attaching a specimen label, heads are wrapped in a plastic or burlap bag, and frozen for storage. The plastic or burlap bag should be closed with a cable tie that has a specimen label attached.

**Teeth**

The age composition of the kill is determined by counting appropriate layers in prepared sections of teeth. To standardize the methods for age determination, a section of jaw with 8-10 teeth is collected from the center of the tooth row of the lower left mandible. The teeth are prepared and cut into 25 μm thin sections that are stained and mounted on a slide for aging under a microscope (Myrick et al. 1983). Age is essential for estimating several life history parameters, including growth rates and the average age at attainment of sexual maturity. Indicators of stress and parturition have also been identified in teeth (Klevezal' and Myrick 1984; Myrick 1988, 1991).

With the carcass on its right side, open the mouth slightly, making an approximately 15-cm slit between the mandibles along the left side. Your knife will protrude into the mouth along the left edge of the tongue (Fig. 6). Inspect the tooth row of the left mandible and note where to collect the center section of jaw with 4-5 teeth on either side of the center tooth. Insert one blade of the pruning shears into the slit, and sever the jaw transversely on either side of the selected sample. After attaching a specimen label, store the jaw sample in formalin. If the head is collected, a section of the jaw should not be removed.

**Blubber**

Blubber samples are collected for studies of contaminant levels, because many types of pollutants concentrate in the blubber. In addition to simply describing levels of environmental contaminants in a population, the data provide information about the movement patterns of animals, which can be used to discriminate populations (Calambokidis and Barlow, 1991).

To collect a blubber sample from the dorsal, mid-thoracic area of the specimen (Fig. 7), make a cut approximately 10-cm X 10-cm square into the skin and blubber of the animal. Then, lifting one corner, cut the sample of skin and blubber layers away from the underlying red muscle tissue. Wrap the sample in foil, shiny side out, with a specimen label on the inside and another taped to the outside of the foil. Freeze the sample for storage.

**Skin sample**

Comparison of genetic sequences extracted from skin samples have been used to identify population units of a species. By sequencing portions of the mitochondrial DNA (mtDNA), genetic variability can be analyzed for a sample of animals to determine whether they are likely to be from one or more populations (Dizon et al., 1991; Rosel, 1992).

Collect a 2-cm x 4-cm section of skin tissue from the dorsal mid-thoracic area, minimizing the amount of blubber attached to the skin by cutting away the blubber with the EXACTO knife (Fig. 7). Put the sample into one of the vials provided in the skin sample kits.
Figure 6. Collecting teeth. (A) Remove a jaw sample containing 8-10 teeth from the center of the lower left mandible, by first making a slit along the inside of the left mandible. (B) Then sever the jaw with the pruning shears or loppers.
Figure 7. Collecting blubber and skin samples. Diagram illustrates the standard dorsal location for taking these samples.
(see p. 5), making sure the tissue is submerged in DMSO. Place a specimen label in the collection vial, and label the outside of the vial and the cap with the specimen number and species using a permanent marker. To avoid contamination of the samples, use a new blade in the EXACTO knife and wear clean rubber gloves for each specimen collected.

**Internal anatomy**

In general, position and structure of organ systems in cetaceans is the same as in most placental mammals, or eutherians. However, proportions and specific anatomy of some organs differ. Some features that distinguish cetacean internal anatomy from other eutherians are the: (1) multi-chambered stomach, (2) small number of lobes to the liver, (3) lack of a gall bladder, (4) botryoidal (lobulated) kidneys, (5) presence of numerous retia mirabilia, (6) remarkably long and undifferentiated intestine, (7) internal testes, and (8) complete cartilaginous rings supporting the trachea and primary bronchi, with at least partial cartilaginous rings extending into the secondary and tertiary bronchioles. More detailed discussions of cetacean internal anatomy can be found in Fraser (1952), Green (1972), Harrison (1972, 1974, 1977) and Winchell (1982).

The body cavity is partitioned transversely by a muscular diaphragm into a thoracic and abdominal portion. The thoracic cavity contains the 4-chambered heart, trachea and paired lungs, esophagus, and various major blood vessels, and the abdominal cavity contains the liver, 3-chambered stomach, spleen, pancreas, intestines, reproductive organs, and urinary bladder. Retroperitoneally, the abdominal cavity also contains the kidneys and adrenal glands.

**Opening the body cavity**

To collect the remaining data and samples, the body cavity must be opened. There are several techniques for opening the body cavity; use the one that works best for you. We suggest the technique below which has been used many times at sea and has been found to be easy, safe, quick, and efficient.

To open the abdominal cavity, position the animal on its right side. Kneeling alongside the animal with its head to your left, make a shallow longitudinal cut starting just behind the dorsal fin and below the bony transverse processes of the adjacent vertebrae, which you can feel by pushing on the side of the animal's body. Angle the cut down to the anal opening (Fig. 8). Make a second cut vertically to the ventral midline of the animal, starting in the same place as the first cut. Once the length of the cuts has been established, deepen them, cutting through the blubber and muscle layers but not so deep into the body cavity to sever organs.

The knife will pass through the thin layer of skin, the white blubber layer, and the dark red abdominal muscle before reaching the peritoneum. The peritoneum is the transparent outer lining of the abdominal cavity surrounding all of the organs. Once you have cut to the internal lining, you will be able to fold the skin, blubber and muscle layer away from the body to expose the abdominal cavity.
Figure 8. Opening the body cavity. Dotted lines show where to make incisions. Cuts 1 and 2 are for opening the abdominal cavity, and cuts 3 and 4 are for opening the thoracic cavity. The numbers mark the beginning of each incision.
To open the thoracic cavity, make a mid-ventral cut anterior to the abdominal opening, and then a vertical cut to the posterior margin of the pectoral fin (Fig. 8). Separate the ribs from the sternum by cutting the cartilage between the ribs and the xiphoid, or posterior, process with your knife or loppers. Then, cut through the muscle between each rib with a knife; this will increase the flexibility of the ribs and ease access to the thoracic cavity.

Alternatively, the final opening of the thoracic cavity can be made by using the pruning shears to cut each rib, rather than separating the ribs by cutting the muscle between each rib. Then, use your knife to cut vertically down to the ventral mid-line of the body between any two of the anterior ribs. Open the thoracic cavity by folding the layer of skin, blubber, muscle, and ribs toward the animal's ventral surface. Use care while sampling from the thoracic cavity using this method, because the cut edges of the ribs are sharp.

**Sampling from the abdominal cavity**

Before sampling the specified organs, removal of most of the intestine will improve accessibility to the abdominal cavity (Fig. 9, 10). If intestine is removed, be sure to leave a short length of intestine just below the pyloric sphinctor of the stomach.

**Female reproductive tract.**—The ovulation history of a female dolphin is recorded in her ovaries. A female is considered sexually mature when one corpus or more (plural: corpora) are present on either ovary. A corpus luteum on the ovary indicates that a female is pregnant or has just ovulated (for descriptions of the female reproductive cycle, see Perrin and Donovan 1984, Akin et al. 1993). A regressed corpus luteum remains as a scar, or corpus albicans, on the ovary, and apparently the scars remain for life (Marsh and Kasuya 1984, Perrin and Reilly 1984).

Data from the examination of the female reproductive tract, and the determination of whether a female is lactating, are used to estimate the calving interval. The calving interval is the sum of the time pregnant, lactating, and resting (Perrin and Reilly 1984). Determination of the state of sexual maturity is critical in calculating the average age and average length at attainment of sexual maturity (Frick et al. 1986, Chivers and Myrick 1993).

The female reproductive tract is held in place by the broad ligament, a sheet of peritoneal tissue dorsal to the sheet holding the more ventral urinary bladder. The tract includes the uterus which is oriented along the midline of the body cavity, and the right and left uterine horns which branch laterally from the anterior portion of the uterus. Anterior to each uterine horn, an ovary is attached. The ovaries are light gray to tan in color and are bean-shaped. The uterus opens posteriorly into the vagina, from which it is separated by the cervix (Fig. 11).

More ovulations usually originate from the left ovary, and the left ovary is generally larger than the right ovary. However, once removed from the uterus, they cannot be easily distinguished without specialized training. Therefore, it is important to tag the left uterine horn before removing the reproductive tract. Both ovaries must be
Figure 9. Line drawing of the internal anatomy of a female small cetacean.
Figure 10. Line drawing of the internal anatomy of a male small cetacean.
Figure 11. Collecting the female reproductive tract. (A) To remove, hold the left uterine horn and ovary away from the body cavity wall and cut the peritoneum. (B) Attach a specimen label around the left uterine horn. Detach the right uterine horn and ovary in the same manner. Cut the uterus at the cervix to complete removal of the reproductive tract. (C) Photo shows location of the reproductive tract relative to other major organs in the body cavity.
collected and examined to accurately determine the number or lack of ovarian corpora, and thus determine the state of sexual maturity. Cut away the ventral peritoneal sheet containing the bladder, then cut a small slit in the broad ligament along the left horn and attach a specimen label around the horn through this slit with a short cable tie. Continue cutting the broad ligament to remove the reproductive tract, holding the left ovary and horn in your free hand to keep the tissue taut and protected, and pulling away from the wall as you cut. Free the right ovary and uterine horn in the same way (Fig. 11).

To complete the removal of the reproductive tract, locate the cervix by feeling along the vaginal canal for this firm, muscular barrier, and make a cut through the vagina posterior to the cervix. After making sure the left uterine horn is tagged, check for evidence of a fetus.

**Sampling a fetus.**— Inspect the reproductive tract visually and with your hands to determine whether a fetus is present. The presence of a corpus luteum, a large, light-colored bulge on the ovary, usually indicates that a female is pregnant. The early stages of pregnancy are easy to miss. However, careful examination of the uterus in the lab can distinguish the corpus luteum of pregnancy and ovulation (Akin et al. 1993).

If a fetus is detected and is <25-cm long, collect the reproductive tract as specified in the previous section. Tag the left horn of the uterus and do not remove the fetus. Preserve the entire reproductive tract in formalin. If a large, >25-cm long, fetus is present, open the uterus and remove the fetus (Fig. 12). Sex and measure the fetus with your calipers, as for adults (see pg. 7). If a skin sample is also required (sampling instructions are issued during training), follow the directions beginning on p. 14. Discard the fetus when you have collected all the data, and collect the entire reproductive tract, as previously described.

**Male reproductive tract.**— Histological preparations of testes are necessary to definitively determine the state of sexual maturity and seasonality in males. Sexual maturity is defined, in part, by which products of spermatogenesis are present, and the diameter of the seminiferous tubules. The recognized categories for the state of sexual maturity are immature, pubertal or maturing, and mature (Collet and Saint Girons 1984, Hohn et al. 1985, Akin et al. 1993). Substitute criteria (e.g., the average total body length and weight of testis at attainment of sexual maturity) are often used to identify sexually mature males when histological preparations are not available. These data are used to study breeding seasonality and, with ages estimated from tooth layers, to calculate the average age at attainment of sexual maturity (Perrin et al. 1976, 1977; Perrin and Henderson 1984; Hohn et al. 1985).

The testes (singular: testis) are paired, sperm-producing organs (Fig. 10, 13, 14), located in approximately the same position as the female reproductive tract (Fig. 9). The testes are surrounded by peritoneum which attaches them to the dorsal wall of the abdominal cavity. Testes of small cetaceans are sausage-shaped and range in length from a few centimeters in calves, to >25-cm in adults of some species.
Figure 12. Collecting a fetus. (A) A near-term dolphin fetus and uterus is shown after it slid out through the opening in the body cavity. (B) The fetus is removed by cutting the uterine wall. Large fetuses (>25-cm) are sampled in the field and discarded.
Figure 13. Location of organs in the abdominal cavity of a male dolphin. (A) The testes and epididymis in the abdominal cavity of a male dolphin. The arrows on the photograph indicate the ends of the epididymis. (B) The location of the kidneys and adrenal glands. The adrenal glands are located just anterior to the kidneys held against the dorsal wall of the abdominal cavity by a double thickness of peritoneum. (C) In some species of small cetacean, the testes of mature males may be as large or larger than the kidneys.
Figure 14. Collecting the male reproductive tract. (A) To remove the right testis and epididymis, the testis is pulled away from the body cavity wall, and (B) the connecting peritoneum is cut. (C) After removal, testes >15-cm in length are slit with a longitudinal incision to their center, to ensure that specimen samples will be properly preserved.
The epididymis is a long, narrow, vermiform organ which lies dorsal to the testis. The epididymis is longer than the testis and curves around both ends of the testis. The border between the epididymis and vas deferens, which appears as an abrupt thickening of the tissue, is difficult to distinguish.

The urinary bladder is occasionally mistaken for the testis, because the bladder is nearly the same shape and size, particularly in immature specimens (Fig. 10). But the urinary bladder is a muscular organ which lies along the body’s ventral midline, and the paired testes lie on either side, about half-way up the abdominal wall.

For uniformity, the right testis and epididymis are collected. To remove the testis, cut the peritoneum while holding it taut and away from the dorsal wall. When you reach the posterior end, you must identify the transition between the epididymis and vas deferens. Cut the epididymis posterior to this point, to ensure that the entire epididymis is collected with the testis. If a portion of the vas deferens is collected, it can easily be trimmed away during processing in the lab (Fig. 14).

If the testis is >15-cm long, slice along the long axis of the organ to its center. This will allow the fixative to penetrate through the organ, to adequately preserve the tissue and prevent it from rotting. Attach a specimen label to each organ using a cable tie inserted through a small slit in the peritoneum between the testis and epididymis. Identify the organ as left or right on the specimen label. Preserve testes and epididymides in formalin.

**Adrenal glands**—Adrenal glands are collected to study the impact of stress on populations of small cetaceans. These glands are endocrine organs that secrete glucocorticosteroids which are hormones produced when animals are stressed. If an animal has been under chronic stress (such as occurs with continuous or frequent harassment, crowding, heavy pollution, or habitat degeneration), the cortex of the adrenal gland tends to show microscopic histopathology. If an otherwise normal animal is acutely stressed before death, adrenal cortices often show signs of depletion of metabolic precursors and blood engorgement (Majors and Myrick 1994).

The retroperitoneal adrenal glands are paired organs that are small, firm, and oval in shape. Because of their small size and a double thickness of peritoneal tissue holding them tightly against the dorsal body wall, they are not easy to find. The best way to locate the organs is with the kidneys in place. An adrenal gland is located just anterior to each of the kidneys on the body wall. The glands are 2- to 10-cm in length depending on the species, and they are light yellow to dark red (Fig. 13). Collect both glands. Grasp one gland at a time, and simultaneously pull and cut the peritoneum away from the body wall until the gland is free.

Once removed, the adrenal glands should be placed in an adrenal kit (see p. 2). Place a specimen label inside the Whirl-pac, and remove the air from the bag by gently squeezing from the bottom. Then roll down the top to seal the Whirl-pac closed. Whirl-pacs should not be “whirled” closed, because of the potential danger of spraying yourself with formalin. After sealing the Whirl-pac, place the bag and a second specimen label, in the larger bag of the kit for storage.
Stomach.-- Prey remains recovered from collected stomachs can be identified to species using the undigested hard parts. For most species of small cetaceans, the prey remains consist primarily of fish otoliths and squid beaks. The presence of fleshy remains provide information about the time of day animals' feed, if the time of death is known (Perrin et al. 1973, Bernard and Hohn 1989).

The cetacean stomach has three compartments: (1) the highly muscular, non-secretory forestomach, or esophageal stomach, (2) the enzyme-secreting midstomach, or main stomach, and (3) the thin-walled, non-muscular pyloric stomach. Most prey remains are recovered from the forestomach. The stomach is immediately behind the diaphragm and liver which are in the anterior portion of the abdominal cavity (Fig. 9, 10). The esophagus leads through the diaphragm into the forestomach, and the final products of digestion pass from the pyloric stomach, through the pyloric sphincter, to the duodenum or small intestine (Fig. 15).

To remove the stomach, begin by locating the esophagus and the duodenum. The esophagus can be distinguished from the trachea by the latter's cartilaginous rings. Expose the esophagus by cutting through the diaphragm and peritoneum, clearing away enough tissue to insert a cable tie around the esophagus and the duodenum. Use the cable tie to attach a specimen label and close the esophagus about 10-cm above the opening to the forestomach. The pyloric sphincter is a firm, wide knot located behind the stomach, anterior to the duodenal ampulla. Use a cable tie to close off the duodenum approximately 10-cm posterior to the pyloric sphincter (Fig. 15). The cable ties should be cinched tightly to ensure that all stomach contents are retained in the stomach. Next, clear away the remaining tissue holding the stomach in place being careful not to puncture the compartments. The pyloric stomach is usually the most difficult to remove; its walls are thin and flaccid, and are closely associated with the pancreas. Pull gently on the pyloric stomach and cut away connecting mesenteries, ducts, and blood vessels. The spleen, pancreas, and mesenteric lymph node are closely associated with the stomach (Fig. 15) and should be collected with the stomach. The stomach can now be removed by severing the esophagus anterior to the cable tie and the duodenum posterior to the cable tie.

When the stomach has been removed, place it in a plastic bag and attach a specimen label to the bag with a cable tie. Freeze the stomachs for storage; formalin can dissolve the fish otoliths used to identify prey species.

Figure 15. Collecting the stomach. (A) The esophagus (arrow) is exposed after cutting away the diaphragm and peritoneum. (B) The tissues covering the three stomach chambers are removed. (C) The excised stomach.
Currently, there are no specific requirements for collecting parasites. However, if parasites are observed, make notes on the life history form and collect a sample, if possible. To collect a sample, cut around the area with the parasites. In the note on the data form, include a description of where in the body the parasites were found and the extent of the infestation. Use formalin to preserve parasites in a Whirl-pac bag. The specimen label inside the bag should also include the parasite type and region of the body sampled.

Other major organs

In this section, we complete the anatomical description of small cetaceans by describing the location of the other major organs in the body (Fig. 9, 10). Some of these have been part of the biological sampling program operated by the Southwest Region for specific research projects. No specific sampling methods for these organs are presented. Special instructions will be issued when their collection is requested.

Abdominal cavity.-- Samples of spleen, mesenteric lymph nodes, and liver have been collected for studies of the immune system. Prior to development of techniques to extract mtDNA from skin samples, liver was collected for studies of genetics.

The spleen is a small, nearly spherical, dark gland located near the pancreas at the posterior end of the stomach (Fig. 15). There are several lymph nodes associated with the intestinal mesenteries, the largest of which is called the mesenteric lymph node or "pseudopancreas" (false pancreas). The pseudopancreas is long and irregularly-shaped, positioned at the root of the mesentery just caudal to the stomach complex. This organ's color and irregular shape make it difficult to discern from the pancreas. The pancreas is a large, flattened organ with uneven margins which secretes various enzymes and hormones. It is generally light pink and is connected to the border of the pyloric stomach and the liver (Fig. 15). Generally, these are collected with the stomach (see pg. 28), but particular studies may require different collection protocols.

The intestine is unmistakable (Fig. 15). In cetaceans, it is extremely long and poorly differentiated into large and small segments. When sampling within the abdominal cavity, its removal improves accessibility to the abdominal cavity.

Cetacean kidneys are unusual in that they are botryoidal, that is, they resemble a bunch of grapes (Fig. 13). This structure is thought (but not proven) to increase their efficiency and result in the excretion of highly concentrated urine. They are retroperitoneal, i.e., located outside the peritoneum, but inside and along the dorsal wall of the abdominal cavity.

The urinary bladder is a small, slender, cigar-shaped organ connected to the ventral wall of the abdominal cavity by two sheets of peritoneum. The bladder lies below the uterus in females, and between the paired testes, and slightly anterior to the penis, in males.

**Thoracic cavity.**—The heart or samples of blood may be collected for immunological and blood chemistry studies. The heart lies between the lungs and is encased in the cardiac sac, the pericardium. The heart of an adult small cetacean is heavy and muscular, being nearly spherical and about the size of a softball. The pericardium must be cut to access the heart. The largest and thickest of the four chambers is the left ventricle. Aerated blood is pumped from the left ventricle to all parts of the body via the aortic arch. Blood travels dorsally and posteriorly through the dorsal aorta, the largest blood vessel in the body, which is located on the dorsal wall of the body cavity. Blood samples may be collected from the left ventricle or from the dorsal aorta.

The heart is the largest organ in the body cavity. Lying in close proximity to the liver, are the diaphragm and lungs (Fig. 15). The diaphragm separates the thoracic and abdominal cavities. When the diaphragm is cut away, the lung and liver tissue may be confused. The two organs resemble each other in gross shape and sometimes color, but differ in texture. The liver tissue is more compact and muscle-like when compared to the lung, which has a spongy consistency. Although the color of these organs is variable, generally, the color of liver is a darker red.

Cetacean lungs are unique in that they contain tiny myoelastic sphincter muscles that contract around and separate the lung bronchiole. Future studies of stress tetany and involuntary closure of these muscles in strandings are envisioned that may require samples from the lungs.

**FINISHING UP**

Complete the life history form and check to ensure that the requested samples were collected. Finally, discard the specimen, retrieve your equipment, and store the samples appropriately. The methods for storing each type of sample are summarized in Appendix 2 as well as in a matrix in the observer field manuals.

**ACKNOWLEDGMENTS**

We thank M. Henshaw, J. E. Heyning, K. M. Peltier, W. F. Perrin, R. J. Tarpley, and the staff of the National Marine Fisheries Service, Southwest Region, Observer Branch for reviewing earlier drafts of the manuscript. R. Allen and H. Orr drafted the figures.
REFERENCES


APPENDICES
APPENDIX 1. Equipment and supply list for dissecting and sampling small cetaceans.

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**Adrenal Kits**

- Bags, plastic (assorted sizes)
  - Body bags
  - Whirl-pacs
  - Ziplock

- Buckets (5 gallon)

- Cable ties (ca. 15-cm and 60-cm)

- Calipers

- Camera, disposable

- Foil

- Formalin

- Gloves
  - cloth
  - rubber

- Knives

- Labels, cotton rag

- Measuring tape

- Pruning shears

- Skin sample kits

- Tape
  - freezer
  - masking

- Whetstone
### APPENDIX 2. Summary of preservation and storage techniques for biological samples.

<table>
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<th>Second Choice</th>
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<tr>
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<td>Cold storage</td>
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<td>Carcass</td>
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<td>Cold (&lt;3 days)</td>
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APPENDIX 3. Cetacean Life History Form. This is the standard data collection form for the eastern tropical Pacific tuna purse-seine fishery observer program.
APPENDIX 4. Marine Mammal Life History Form. This is the standard data collection form for the California gillnet fisheries observer program. The form is used for the collection of biological data from both cetaceans and pinnipeds.
**MARINE MAMMAL LIFE HISTORY FORM**

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<th>SET #</th>
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**SPECIES:**

**SEX:** [ ] M [ ] F [ ]

**LENGTH (cm):** [ ] CURVILINEAR [ ]

**CURVILINEAR? [ ] Y [ ] N [ ]**

**GIRTH (cm):** [ ]

**FLIPPER LENGTH (cm):** [ ]

**LACTATING?: [ ] Y [ ] N [ ]**

**FETUS?: [ ] M [ ] F [ ]**

**FETUS LENGTH (cm):** [ ]

**CURVILINEAR? [ ] Y [ ] N [ ]**

**WERE THESE COLLECTED?:**

[ ] YES [ ] NO

**YES NO**

| [ ] ] CARCASS 60 [ ] | [ ] ] HEAD 61 [ ] | [ ] ] TEETH 62 [ ] |
| [ ] ] STOMACH 63 [ ] | [ ] ] BLUBBER 64 [ ] | [ ] ] BIOPSY 65 [ ] |
| [ ] ] OVARIES 66 [ ] | [ ] ] FETUS 67 [ ] | [ ] ] FETUS BIOPSY 66 [ ] |
| [ ] ] TESTIS 69 [ ] | [ ] ] ADRENALS 70 [ ] | [ ] ] OTHER 71 [ ] |
| [ ] ] PHOTOS 72 [ ] |

**COMMENTS:**

**DIAGNOSTIC CHARACTERISTICS:**

**SKETCH THE ANIMAL:**

**IN FIELD**

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<table>
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<th>19 FETUS WEIGHT (gm)</th>
<th>26 GLGs</th>
<th>30 ADRENAL WTS (gm)</th>
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<tr>
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</table>

**C.A. diams. (mm) by Type**

| 1 | 2 | 3 | 4 | 5 | 6 |

**NOTES:**

[ ]

[ ]

43
Steller Sea Lion
(*Eumetopias jubatus*)
M. to 13'/1800#; F. to 9'/600#; external ear flaps; long foreflippers; gap between 4th and 5th post canine teeth; short, stiff hair, brown to blonde; no distinct sagittal crest.

California Sea Lion
(*Zalophus californianus*)
M. to 8'/800#; F. to 6'/600#; external ear flaps, long foreflippers; M. prominent sagittal crest-light top knot; short, stiff hair, dark brown to light tan.

Northern Elephant Seal
(*Mirounga angustirostris*)
M. to 16'/4000#; F. to 10'/2000#; M. large, pendulous nose; F. "roman nosed"; short foreflippers; hind flippers angled backwards; 1st and 5th hind toes noticeably longer than others; minute ear hole; 4 incisors upper jaw.

Northern Fur Seal
(*Callorhinus ursinus*)
M. to 7'/650#; F. to 5'/130#; very long hind flippers; short, pointed snout; fur on foreflippers stops abruptly at wrist; soft underfur/course guard hairs; external ear flaps.

Guadalupe Fur Seal
(*Arctocephalus townsendi*)
M. to 8.5'/650#; F. to 6'/270#; very long hind flippers; fur extends onto foreflippers; "collie-like" face-dished in profile; soft underfur/course guard hairs; external ear flaps.

Harbor Seal
(*Phoca vitulina*)
M./F. to 6'/300#; spotted/blotchy coat-variable coloration; short foreflippers; hind flippers angled backwards; large ear hole; sharp nails near ends of toes; round head; 6 incisors upper jaw.

ADDITIONAL COMMENTS: ____________________________
APPENDIX 5. Cetacean Data Record. This is the standard data collection form for collecting data from cetaceans dissected in the laboratory. Animals may have been stranded or collected by fisheries observers.
**CETACEAN DATA RECORD**

**Catalog no**

**Field no**

**Species**

**Sex**

**Length**

**Condition**

**Observer**

**Date, occurrence**

**Locality**

**Lat. and Long.**

**Reported by**

**Photographs/Drawings**

**Circumstances, cause of death**

**External description**

**Tooth/baleen count:** erupt total up L up R low L low R

**Diameter largest tooth/length longest baleen plate** baleen color

**MEASUREMENTS** *(specify units) (* not parallel to body axis)

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</tr>
<tr>
<td>7 snout to ant. dorsal fin</td>
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<td>9 snout to ear</td>
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<td>11 snout to gape</td>
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<td>12 snout to blowhole(s)</td>
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<td>13 snout to melon apex</td>
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<td>14 eye to ear*</td>
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<td>15 eye to gape*</td>
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<td>16 eye to blowhole edge,L*</td>
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<td>17 eye to blowhole edge,R*</td>
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<td>18 blowhole length *</td>
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<td>19 diameter ear opening</td>
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<td>20 head diameter at eyes*</td>
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<td>21 length of eye opening</td>
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<td>22 rostral width,melon apex*</td>
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<td>23 projection up/lower jaw</td>
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**REPRODUCTIVE SYSTEM**

**Female**

ovaries: weight R L, dimensions(LxWxD) R L

number corpora albicantia R L, corpora lutea R L

uterus: immature R L, mature R L, uterine horn width R L

mammary gland: color R L, length R L, width R L, depth R L, milk R L

pregnant R L, fetus: length R L, sex R L, weight R L

vagina length R L, number of vaginal folds R L

**Male**

testes: weight with epididymis R L, without R L

dimensions (LxWxD) R L, R L

penis length R L

sperm in epididymis R L

**STOMACH CONTENTS**

fore: volume fish bones otoliths squid beaks

main: volume fish bones otoliths squid beaks

pyloric: volume fish bones otoliths squid beaks

Contents, general remarks
AGE DETERMINATION
umbilicus healed, fetal folds present, teeth erupt
growth layer groups: cementum, dentine, earplug
vertebral epiphyses: open mm, closed visible, closed invisible

WEIGHTS (specify units, type of scales)
intact carcass, heart, stomachs, empty
viscera, lung, right, intestines
muscle: epaxial, lung, left, pancreas
hypaxial, liver, adrenal right
misc, spleen, adrenal left
total, kidney right, brain
bone, kidney left, thymus
blubber, stomachs, full, intestine length

PARASITE/PATHOLOGY CHECKLIST (X if present, NO if absent, NE if not examined)
eye, forestomach, mammary glands, muscle
mouth, mainstomach, liver, Phyllobothrium
genital slit, pyloric, bile duct, Monorhygma
anal slit, intestine, uterus, crassicaudid.
appendages, rectum, lungs, Braunina
barnacles, kidney, heart, Nasitrema
cyamids, kidney duct, brain, lymph nodes
Penella, pancreas, air sinuses, enlarged

SPECIMEN COLLECTION CHECKLIST
teeth/baleen, ear plugs, liver sample, epiphyses
stomach content, ectoparasites, kidney sample, frozen tissues
gonads, endoparasites, fetus, toxicology
mammary gland, blubber, skull, X-ray
uterine mucosa, muscle, skeleton, histology

MISCELLANEOUS
skull length, width; length tooth/baleen row up, low
vertebral count: total, cerv., thor., lumb., caud.
number ribs, dbl headed, single headed; number chevrons

REMARKS
atlas - The first vertebra
biopsy - Skin sample from a live animal
botryoidal - Shaped like a bunch of grapes
carrying capacity - Equilibrium population size determined by the available resources
cestodes - endoparasitic tape worms belonging to Class Platyhelminthes
colostrum - The first product of lactation which is high protein and antibodies
corpus albicans (pl. corpora albicantia) - An ovarian scar resulting from an ovulation
corpus luteum (pl. corpora lutea) - Endocrine tissue that forms around the ruptured Graafian follicle in the ovary; A corpus luteum is formed during ovulation and is sustained during pregnancy
duodenal ampulla - The sac at the beginning of the small intestine
fluke - tail of a cetacean
formalin - Saturated solution of 37% formaldehyde
histology - The anatomical study of the microscopic structure of tissue
histopathology - The pathologic changes in diseased tissues
mesentery - Double layer of peritoneum attaching stomach, intestines, spleen, etc., to the dorsal wall of the abdominal cavity
mtDNA - Mitochondrial deoxyribonucleic acid. This DNA is maternally inherited and is sequenced to study the population structure of species
occipital condyles - Rounded projecting facets of bone at the back of the skull that articulate with the first vertebra
otoliths - Granules of calcium carbonate that form in the ears of fish; Shape and size are unique to a species
pectoral fin - Paired appendages attached to the shoulder girdle
peritoneum - Connective tissue lining the body's cavities
population - The fundamental unit for management of a species; May also referred to as the
stock or management unit
retroperitoneal - Outside the peritoneum
stock - See population
tail stock - The laterally-compressed part of a cetacean's body between the dorsal fin and
flukes
transverse processes - The lateral extensions of the vertebrae
vermiform - Shaped like a worm; sinuous
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