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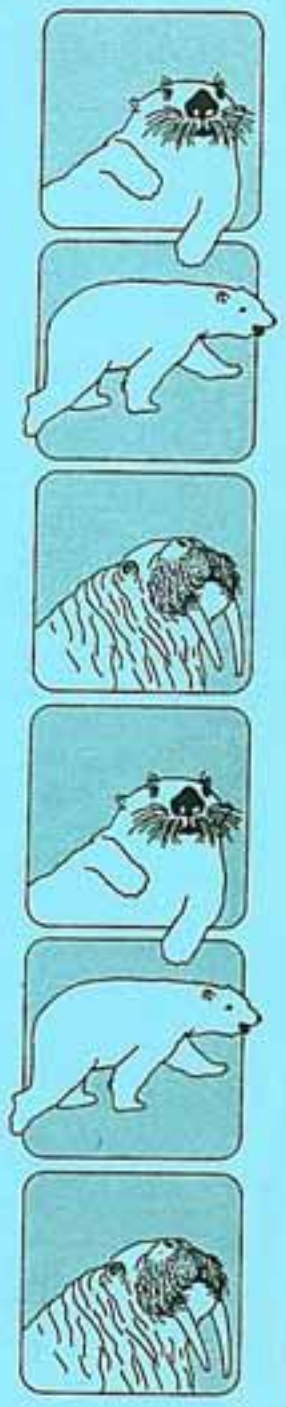

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Daniel Mulcahy

August 1997

Technical Report MMM 97-3

MARINE MAMMALS MANAGEMENT
Fish and Wildlife Service
Region 7, Alaska
U.S. Department of the Interior

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INTRODUCTION

The U. S. Department of Interior, Fish and Wildlife Service (FWS) has the responsibility for the management of sea otters in the United States. As part of the management of the sea otter in Alaska, we collect beach-cast carcasses to monitor natural mortality within the population by determining cause of death and collecting data on sex, age, and body condition. Beach-cast carcasses are obtained from a variety of sources including: 1) refuges and wildlife staff biologists, 2) National Marine Fisheries staff, 3) Alaska Science Center staff, 4) State Troopers, and 5) contacts in local coastal communities. Information gathered from beach-cast carcasses tends to be sporadic and limited to areas near coastal villages and towns, however, it provides an index to causes of natural mortality and contributes to our knowledge of the general life history of sea otters.

In addition to monitoring natural mortality, a biological monitoring program has been developed to sample subsistence harvested sea otters in Alaska. The program was developed cooperatively with the Alaska Sea Otter Commission ((ASOC), a statewide coalition of Alaska Natives), the Alaska Science Center (U.S. Geological Survey) and the FWS. Sea otters are harvested for subsistence use and the creation of handicrafts by coastal Alaska Natives. Many of the hunters are interested in the conservation and management of the sea otters which they harvest. A goal of this cooperative program is to provide biological information relevant to conservation and management of sea otters in Alaska. By working cooperatively within the Native communities, carcasses from subsistence hunted sea otters are necropsied and fresh tissue samples from healthy sea otters are obtained. The biological monitoring program collects sea otter tissues to address a wide variety of objectives, such as: food habits, reproductive characteristics, contaminant levels in tissues, sea otter genetics, body condition, and skeletal morphology.

The purpose of this field guide is to provide standardization of tissue collection techniques and sample handling procedures for the gross necropsy of sea otters as well as provide detailed information on simple data and tissue collection techniques for Alaska Native hunters and taggers, volunteers, and marine biologists. The use of a standardized methodology will aid in making data from a variety of sources comparable within Alaska. This protocol has been developed by the FWS and the Alaska Science Center in collaboration with the Alaska Sea Otter Commission.

ACKNOWLEDGMENTS

This work represents the collective support of many individuals over a four year period. The primary groups involved in the cooperative program were, the Fish and Wildlife Service, the Alaska Science Center, and the Alaska Sea Otter Commission. We thank the following people from FWS for their comments: C. Gorbics, W. Stephensen, L. Dickerson, J. Snyder, M. Cody, J. Garlich-Miller, D. Burn, and L. Slater. We thank the following people from Alaska Science Center: B. Ballachey and J. Bodkin. We thank the following people from the ASOC: P.Wheeler, K. Williams, L. Quakenbush, B.Bodnar, and B. Kelly. The following people provided reviews of the draft report and provided many valuable suggestions and comments: J. Ames, J. Bodkin, J. Garlich-Miller, and C. Gorbics. And most importantly, we thank the people trained in the necropsy procedure who have provided us with invaluable comments and suggestions for the improvement of the data collection form in the field: M. Bartels, J. Boone, J. Miller, B. Short, O. Totland, J. Webber, I. Williams, G. Shellikoff, P. Kompkoff, R. Vincler Sr., S. Hakala, J. Panamaroff, S. Virg-in, N. Ruhl, J. Peterson, M. Simeonoff, G. Norman, B. May, E. James, E. Lucas Jr., L. Broad Jr., J. Gregorio, R. Lind, H. Kalmakoff, J. Yagi, and C. Itumulria.

Note: Some illustrations included in this guide were taken from Marine Mammals Ashore A Field Guide for Strandings by Joseph R. Geraci and Valerie J. Loundsbury. 1993. Texas A&M University Sea Grant College Program. Publication TAMU-SG-93601. ISBN1-883550-01-7.

Sea Otter Necropsy Form

1. Sample Information:

MTRP Certificate Number: _____

Sample Number: _____

Hunter Name: _____

Kill Date (mm/dd/yy): _____

Kill Time (24 hr): _____

Kill Location: _____

Latitude (deg min sec): _____

Longitude (deg min sec): _____

Sample Collector: _____

Date Frozen (mm/dd/yy): _____

Necropsy Date (mm/dd/yy): _____

Necropsy Time (24 hr): _____

Sample Source: subsistence hunt___ beach-cast ___ other_

Specify other source: _____

Other Sample Collectors: _____

2. General Physical Condition:

Body: Thin ☐ Normal ☐ Fat ☐

Haircoat: Normal ☐ Slip ☐ Unknown ☐

Nose Scars: Y ☐ N ☐ U ☐

Whiskers Collected: Y ☐ N ☐

Estimated Age:

Old Adult ☐ Adult ☐ Subadult ☐ Pup ☐

Comments: _____

3. Body Measurements:

	Skinned	Unskinned
Weight:	_____	_____(Kg/Lbs)
Length:	_____	_____cm
Girth:	_____	_____cm

Rt. Forepaw Width: _____cm

Skull Length: _____cm

Skull Width: _____cm

Comments: _____

4. Sex Information:

Sex: Male ☐ Female ☐ Unknown ☐

If Female:

Lactating Y ☐ N ☐ U ☐

Pregnant Y ☐ N ☐ U ☐

Fetus sex M ☐ F ☐ U ☐

Fetus length _____cm

Uterus collected Y ☐ N ☐

If Male:

R Testicle weight _____g

R Testicle length _____cm

R Testicle width _____cm

Baculum length _____cm

5. Oral Cavity:

Oral Lesions: Y ☐ N ☐

If present, describe appearance:

_____

_____

Length: _____cm Width: _____cm

Tooth Wear:

Heavy ☐ Medium ☐ Light ☐ None ☐

Tooth Abcesses: Y ☐ N ☐

Tooth Loss: Y ☐ N ☐

If 'yes', describe location and appearance:

_____

_____

Teeth Collected:

(check which teeth are collected)

URP ☐ LRP ☐ URC ☐ R Canine Width: _____cm

ULP ☐ LLP ☐ ULC ☐

Comments: _____

6. Estimates of Body Fat Stores:

	None	Little	Average	Abundant
Subcutaneous	☐	☐	☐	☐
Groin _____cm	☐	☐	☐	☐
Kidneys	☐	☐	☐	☐
Mesenteric	☐	☐	☐	☐

Comments: _____

7. Respiratory System:

Lungs: Normal ☐ Abnormal ☐

Comments on respiratory system: _____

8. Circulatory System:

Heart: Normal ☐ Abnormal ☐

Spleen: Normal ☐ Abnormal ☐

Comments on circulatory system: _____

9. Liver:

Appearance: Normal ☐ Abnormal ☐

Histology sample Y ☐ N ☐

Contaminants sample Y ☐ N ☐

Weight of HM sample _____g

Weight of OC sample _____g

10. Gall Bladder:

Bile: Full ☐ Some ☐ Empty ☐

Parasites present Y ☐ N ☐

11. Stomach Collected: Y ☐ N ☐

Comments: _____

12. Kidney:

Appearance: Normal ☐ Abnormal ☐

Left Kidney:

Histology sample Y ☐ N ☐

Contaminants sample Y ☐ N ☐

Weight of HM sample _____g

Weight of OC sample _____g

Right Kidney:

Kidney collected Y ☐ N ☐

Weight of whole kidney _____g

Comments: _____

13. Gastrointestinal Tract:

Small Intestine: Normal ☐ Abnormal ☐

(list the number and type of parasites (THW=thorny headed worm, TPW=tape worm, NMT=nematode) observed in each of three 20 cm segments of the small intestine)

Segment 1: THW____ TPW____ NMT____

Other _____

Segment 2: THW____ TPW____ NMT____

Other _____

Segment 3: THW____ TPW____ NMT____

Other _____

Comments: _____

14. Parasites:

Were parasites collected? Y ☐ N ☐

If parasites are collected, list the type and where they occurred: _____

15. Other Tissues Collected:

Required for long-term study objectives:

Right Femur Y ☐ N ☐

Muscle (20g) Y ☐ N ☐

Genetics Vial Y ☐ N ☐

Other Tissues which may be collected but are not part of the long-term archival program:

Right Testicle Y ☐ N ☐

Urine Y ☐ N ☐

Blood Y ☐ N ☐

Bile Y ☐ N ☐

16. Additional Comments: _____

KEY TO THE SEA OTTER NECROPSY FORM

1. Sample Information

MTRP Certificate Number: For all sea otters hunted for subsistence purposes, record the Marking, Tagging, and Reporting Program (MTRP) certificate number. The certificate number is located in the upper right corner of the certificate and is printed in red ink. Work with the local tagger or hunter to obtain the hunt information. Note that in addition to the certificate number there are also numbers associated with skull and hide tags.

Hunter Name: Record the name of the person who killed the sea otter and provided it for sampling.

Kill Date: Record the date the sea otter was killed as the month/day/year (mm/dd/yy).

Kill Time: Record the time of day the sea otter was killed using the 24 hour military time convention, for example 1:00 pm = 1300 (hhmm). Note whether the hunter provided the actual time or an estimation of the time of kill. Your kit contains "toe tags" for the hunters to mark each animal with the date, location, and time of kill.

Kill Location: Record the geographic location where the hunting occurred.

Latitude and Longitude: Record the latitude and longitude of the kill location from a navigation chart in degrees, minutes, and seconds (deg min sec). Advise hunters using GPS to write the latitude and longitude directly on the toe tags.

Sample Number: The sample number is obtained from the pre-labeled sampling package in the kit provided.

Sample Collector: Record the name of the person who is conducting the sample collection.

Date Frozen: If the sea otter carcass is being frozen for sampling at a later date, record the date the animal is placed in the freezer as the month/day/year (mm/dd/yy).

Necropsy Date: Record the date the sea otter was necropsied as the month/day/year (mm/dd/yy).

Necropsy Time: Record the time of day the sea otter was necropsied using the 24 hour convention (hhmm).

Sample Source: Place a check mark behind the category which describes where the carcass was obtained (subsistence hunt, beach-cast, or other).

Specify Other Source: If the carcass is not from a subsistence hunt or a beach-cast source please explain the origin of the animal(s). Examples of other sources include: boat kill, research permit, oil spill.

Other Sample Collectors: Record the names of other people (if any) who participate in the sea otter necropsy.

2. General Physical Description

Place a check in the boxes which best describe the condition of the body, haircoat, nose scars, and estimated age.

Body: A thin body condition is characterized by readily visible, often protruding, ribs and back bone. In a normal body condition, ribs and backbone can be felt beneath the pelt but are not readily visible. In a fat body condition, the ribs and backbone are padded by a layer of subcutaneous fat.

Haircoat: A normal haircoat has a fluffy, down-like appearance in the under layer of the fur and glossy guard hairs; hair is firmly attached to the skin. Hair slip is determined by grasping the fur and pulling, if the hair pulls easily from the pelt, this is slip. For beach-cast carcasses, hair slippage can give the sample collector an indication of how long the sea otter has been dead. Check 'U' if the pelt was removed prior to sampling or you are uncertain which category applies.

Nose Scars: Examine the nose pad of the sea otter for fresh wounds or healed scars. For females, nose scars may indicate whether the animal has mated and perhaps how recently the mating occurred. Check 'U' if the nose pad was removed prior to sampling or you are uncertain which category applies.

Whiskers Collected: Collect several (5-7) whiskers by pulling them from either side of the muzzle and place in the pre-labeled envelope. It is important that the whiskers be pulled rather than cut.

Estimated Age: Sea otter age can be estimated by examining a combination of body size, pelt coloration, and degree of wear on the teeth. Old adult and adult animals weigh from 50 to 100 pounds, the fur ranges from dark brown to heavily grizzled (gray), and tooth wear is moderate to heavy. Subadult animals weigh approximately from 40 to 60 pounds, the fur is a fairly uniform dark brown, with light to no tooth wear. Pup weights range from 5 to 40 pounds, pup natal fur is a light 'buffy' brown and is replaced by adult fur by age 3 months, there is no tooth wear (however, check to determine whether there are teeth erupting). These are very general guidelines and overlap will occur especially in the size classifications. For example, in general adult males are larger, longer, and heavier than adult females and adult and subadult classifications may be difficult to determine.

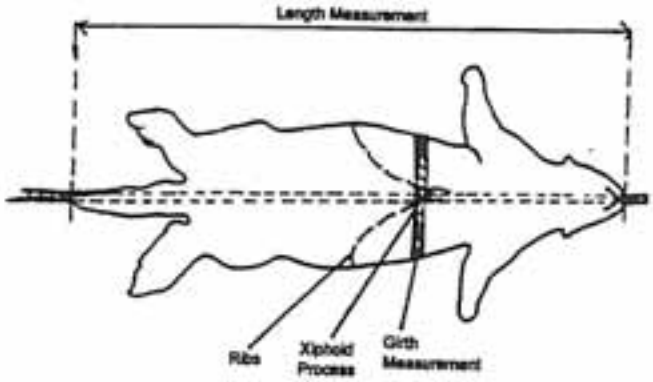
Comments: Describe anything unusual regarding the external condition of the animal, for example, coloration, pelt condition, physical abnormalities such as malformations, tumors, new or old wounds, and scars. Describe any unusual circumstances regarding the animal's behavior prior to death. If an animal has numbered plastic tags on the hind flippers, note which color and number combinations occur on the right and left flippers and collect the tags. Check the space between digits of the hind flippers for tears or scars that may indicate that the animal had been tagged. During the necropsy, examine the tagged animals for a radio transmitter in the abdominal cavity. Tagged sea otters have been part of research studies and any additional information gathered on such animals is very useful.

3. Body Measurements

Always note the units of measurement (pounds or kilograms), and always record the metric measures in centimeters as shown on the data form. In the following sections, we specify that measurements be taken from right or left organs, by this we mean the **animals** right or left not the person taking the samples. Where feasible, obtain the body weight, length, and girth before and after skinning. This information will be used to estimate body weight for animals for which only a skinned weight is available. For subsistence hunted animals, if the skull has been badly damaged then do not measure the length and width.

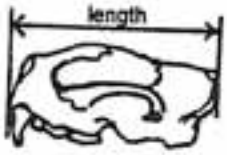
Weight: Place the body in the mesh bag and hook it on the large ($\geq$100 lbs) spring scale. Be certain to hold the scale completely vertical without allowing the bag to touch the ground or nearby objects. Record to the nearest pound or kilogram (circle which unit is used on the form).

Length: On a flat surface, place the animal ventral (belly) side up, and place a nylon measuring tape beneath it. Stretch the tape from the tip of the nose to the tip of the tail (not including the hair which extends past the skin). Measure the length and record to the nearest half centimeter.



Girth: On a flat surface, place the animal ventral (belly) side up and place a nylon measuring tape beneath it. Wrap the nylon tape measure around the otter at the xiphoid process (the tape should be snug but not pulled tight). The xiphoid process is located at the point where the front ribs connect at the bottom of the sternum (breast bone). Record the circumference to the nearest half centimeter.

Right Forepaw Width: Measure the width of right forepaw across the widest portion of the pad using a small plastic ruler or calipers in the kit. Record the measurement to the nearest 0.1 centimeter.



Skull Length: Cut away tissues so that the base of the skull is exposed where the neck vertebrae join the skull. Place the large calipers at the base of the skull and extend it to the gumline of the upper incisors. Measure the distance between the jaws of the calipers in centimeters on the plastic ruler or measuring tape to the nearest 0.1 centimeter.

Skull Width: Cut away tissues at the zygomatic arch to expose the bone. The zygomatic arch is the 'cheek bone' and it is an arch of bone that extends along the front side of the skull beneath the eye. Place the large calipers on the outermost point of each zygomatic arch. Measure the distance between the jaws of the calipers in centimeters on the plastic ruler or measuring tape to the nearest 0.1 centimeter.

Comments: Comment on the body measurements in the space provided. Note, for example, whether skull measures were not possible or other unusual circumstances.

4. Sex Information

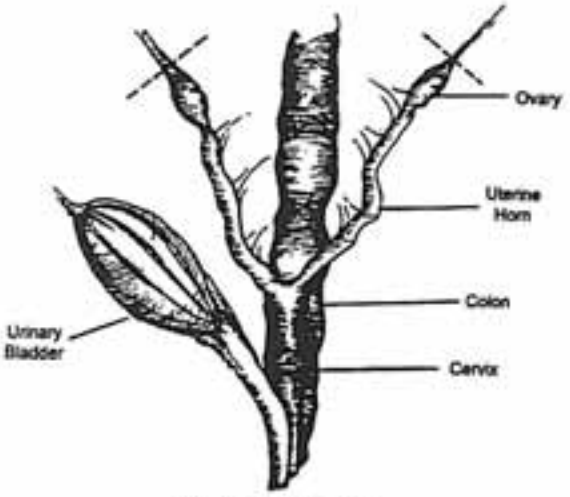
The primary indicator of sex for sea otters is the presence of a penis bone (baculum) on male animals. The penis sheath on male animals is relatively easy to locate and there is often discoloration of the long guard hairs around the sheath. Place a check mark in the boxes which best describe the sex and associated information regarding each sex.

Lactating: In the general physical examination of the carcass, if milk can be expressed from the nipple by squeezing, the female is lactating. If you are uncertain whether the female is lactating, check 'U' for unknown.

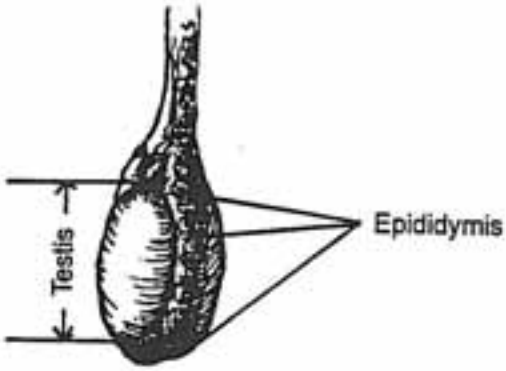
Pregnant: Only advanced pregnancy may be detected in the field. Reproductive tracts (the uterus and ovaries) will be examined in the laboratory to determine the reproductive status. Unless pregnancy is certain (for example, advanced enough to see the fetus), check 'U' for unknown.

Fetus sex and body measurement: Remove the fetus from the reproductive tract only if it is too large to be preserved with the uterus and ovaries in the nalgene bottles provided. If the fetus is removed, determine fetus sex by examining the genital area. If you are uncertain of the sex of the fetus, check 'U' for unknown. To measure the 'crown to rump' fetus length, place the nylon tape measure on the crown of the head and measure along the spinal column to the end of the sacrum (not including the tail). Measure to the nearest 0.5 centimeter.

Uterus collected: To locate the uterus (reproductive tract), follow the small intestine to the large intestine to the colon. The reproductive tract is located between the colon and the urinary bladder in the lower abdominal region. Remove the reproductive tract by cutting just below the cervix and above the ovaries (follow each horn to the ovary and detach the reproductive tract by cutting the tissues past the ovary). Be very careful to include the ovaries, they can be felt as small bean-shaped lumps within the tissues at the ends of the uterine horns. Preserve the reproductive tract in a large Nalgene bottle with 10:1 volume of 10%



Uterus - ventral view



Testis - medial view

buffered formalin to tissue. Do not freeze the formalin. If this is not possible, place the sample in a large sealable plastic bag and freeze the entire sample.

Testicle weight: Remove the right testicle after the carcass has been skinned and cut away the epididymis and tunic (the outer 'sack' that the testis is contained in) and weigh the right testicle to the nearest 0.1 gram on the digital scale.

Testicle length: Remove the right testicle after the carcass has been skinned and cut away the epididymis and tunic (the outer 'sack' that the testis is contained in). Measure the length with the dial calipers to the nearest 0.1 centimeter without compressing the testicle.

Testicle width: Remove the right testicle after the carcass has been skinned and cut away the epididymis and tunic (the outer 'sack' that the testis is contained in). Measure the width with the dial calipers to the nearest 0.1 centimeter without compressing the testicle.

Baculum length: Measure the straight line distance end to end of the baculum (penis bone), after it is removed from the carcass, to the nearest 0.1 centimeter with the calipers or plastic ruler.

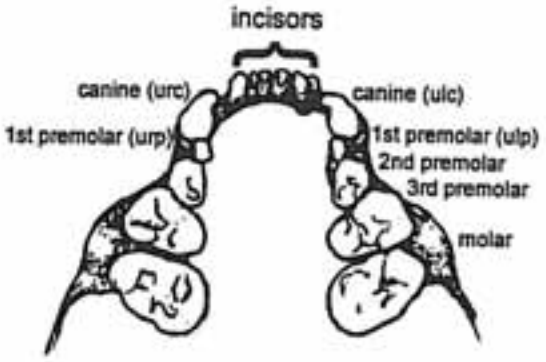
5. Oral Cavity

Examine the mouth carefully for the presence of oral lesions, degree of tooth wear, abscesses, and natural tooth loss. Use the space provided to make comments on lesions, abscesses, and tooth loss.

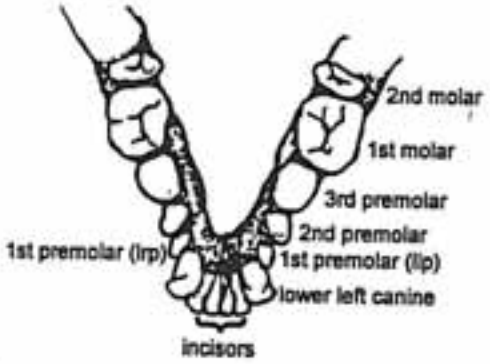
Oral lesions: Look under the tongue, on the roof of the mouth and inside the lips for circular or oval, reddened or whitened flat sores. Note the number, location, size (measure the lesion(s) in centimeters with the dial caliper or ruler), and describe the shape, color, and appearance of the lesion.

Tooth wear: This is a subjective description based on the degree of flattening of the incisors, rounding and blunting of the canines, and presence and extent of wear spots (pits) on the molars.

- Heavy wear - incisors and molars are worn to or near gumline, tips of canines are rounded, and molars are flattened with heavy pitting.
- Medium wear - incisors and molars show wear but the wear does not extend as far as the gumline. Moderate pitting in the molars may be observed.
- Light wear - slight rounding of the cusps of the molars and incisors, little or no pitting in the molars.
- None - fresh pointed canines, incisors, and cusps of molars, no flattening or pitting on the molars, usually observed in animals less than or equal to one year of age. Check to see if the molars are in the process of replacement with adult teeth.



UPPER JAW



LOWER JAW

Tooth abscesses: Note the location of any abscessed (infected) teeth.

Tooth loss: Note the **natural** absence of any teeth. Do not include teeth removed by the tagger or teeth damaged by gunshot. Record the type of tooth missing and its location in the mouth (for example, upper or lower jaw and right or left side) using the naming convention in the illustration.

Teeth collected: Note which teeth were removed during the necropsy from the animal by checking the appropriate box. Any of the four premolars may be collected for age determination provided the root of the tooth is intact. A premolar cannot be used for age determination if the root is broken. The abbreviations are as follows:

- URP - upper right premolar
- LRP - lower right premolar
- URC - upper right canine
- ULP - upper left premolar
- LLP - lower left premolar
- ULC - upper left canine

Measure the URC (upper right canine) width prior to collection of the tooth (if collected) with a dial calipers. Prior to measurement, scrape away any plaque from the tooth. Then measure the canine width at the widest part (a bit above the gumline) in centimeters, record the measurement to the nearest 0.1 centimeter.

Comments: Comment on the oral cavity or any of the measurements collected.

6. Estimates of Body Fat Stores

These are subjective evaluations which become consistent only with practice.

- None - no visible fat on the tissue
- Little - some fat visually detected on the tissue
- Average - fat is easily detected on the tissue
- Abundant - fat is ample and covers much of the surface of the tissue

Check the box which best describes your visual estimate of the quantity of fat on the various tissues. Measure the depth of fat only in the groin.

Subcutaneous: Subcutaneous fat refers to the fat located between the skin and muscle layers that is readily visible after the animal has been skinned.

Groin: The groin is the area on the inside of the thigh where the leg meets the body. Measure the fat thickness in the right groin by slicing the fat layer with the scalpel and inserting the plastic ruler. Measure to the nearest 0.1 centimeter.

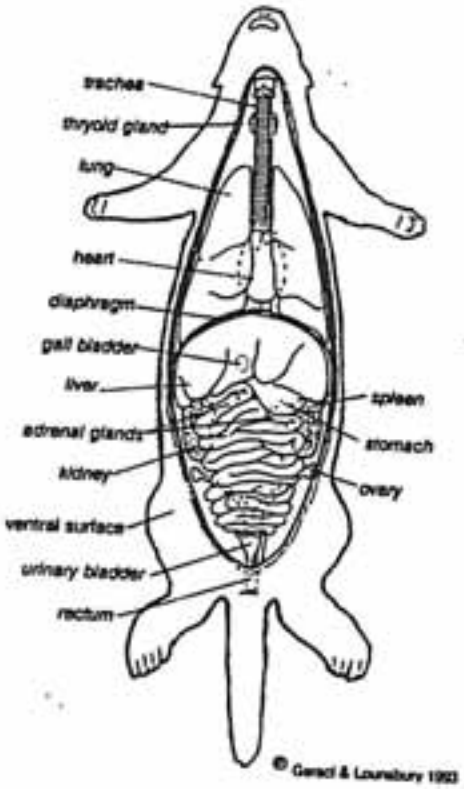
Kidneys: Kidney fat refers to the fat located on the top of the kidney and the fat occurring between the nodules of the kidney itself.

Mesenteric: Mesenteric fat refers to fat associated with the supportive membranes which supply the intestines with blood. Do not confuse the mesentery with the pancreas or the omentum.

Comments: Describe any notable details or abnormalities about the fat such as the color or texture.

7. Respiratory System

Examine the lungs and trachea for color, texture, and the presence of any abnormalities. Remove the lungs from the body cavity and examine color and texture and record if they appear normal or abnormal. Normal lung tissue is light pink (though sometimes dark after death) and feels soft and spongy. If the tissue appears abnormal, describe the abnormality in the comments section and collect a small sample of normal and abnormal tissue for histopathology (10:1 volume of buffered formalin to tissue for preservation).



Comments: Describe any notable details or abnormalities in the respiratory system in the space provided.

8. Circulatory System

Examine the heart, spleen, and vessels for color, texture, and the presence of any abnormalities. Record whether the tissue appears normal or abnormal. If the tissue appears abnormal, describe the abnormality in the comments section and collect a small sample of normal and abnormal tissue for histopathology (10:1 volume of buffered formalin to tissue for preservation).

Comments: Describe any notable details or abnormalities in the circulatory system in the space provided.

9. Liver

Examine the liver for color, texture, and the presence of lesions or other abnormalities. Remove the liver from the body cavity and record if the tissue appears normal or abnormal. If the tissue appears abnormal, describe the abnormality in the comments section and collect a small sample of normal and abnormal tissue for histopathology (10:1 volume of buffered formalin to tissue for preservation).

Histology sample: Collect approximately a one centimeter square sample (about the size of a thumbnail) and place in a 10:1 volume of buffered formalin in a pre-labeled nalgene bottle. Do not freeze this sample.

Contaminants sample: The field sampling procedure is to collect the entire liver and place in a pre-labeled sealable plastic bag. Freeze the sample as soon as possible after collection. Samples will be processed at a later date in the laboratory.

Weight of contaminants samples: This procedure is conducted only in the laboratory. Collect two, > 20 gram samples of liver from each animal. Mark one label for heavy metal analysis (HM) and the other for organochlorine analysis (OC). Tare the empty, chemically cleaned, sample jar and lid on the digital balance. Place the sample in the jar and record the sample weight to the nearest 0.1 gram. Repeat for the second sample.

10. Gall bladder

Note the volume of bile in the gall bladder and check the box which best describes your estimate of the volume: full (bladder turgid), contains some bile, or is empty. Examine the inside of the gall bladder for parasites. The most frequently observed parasites are small (2-3 mm)

white to transparent trematodes (flukes). If there are unfamiliar parasites in the gall bladder, collect them and place in 10:1 volume of buffered formalin to tissue for preservation.

11. Stomach

To collect the stomach, tie both ends off with a string (from above the esophagus to just below the duodenum). Cut above the upper tie and below the lower tie. Remove the stomach and place in the pre-labeled sealable plastic bag and freeze as soon as possible after collection.

Comments: Describe any notable details or abnormalities in the liver, gall bladder, or stomach in the space provided.

12. Kidney

Examine the kidneys for color, texture, and the presence of lesions or other abnormalities. Remove the kidneys from the body cavity (keep track of which is right and left) and examine color and texture and record if they appear normal or abnormal. It is normal for there to be a slight difference in size between the right and left kidneys. If the tissue appears abnormal, describe the abnormality in the comments section and collect a small sample of normal and abnormal tissue for histopathology (10:1 volume of buffered formalin to tissue).

Histology sample: Collect approximately a one centimeter square sample (about the size of a thumbnail) from the end of the **left kidney**. Place the sample in a 10:1 volume of buffered formalin in a pre-labeled nalgene bottle for preservation. Do not freeze this sample. **Note, do not take the histopathology sample from the center of the organ.**

Contaminants sample: The field sampling procedure is to collect the entire **left kidney** (minus the histology sample) in a pre-labeled sealable plastic bag. Freeze the sample as soon as possible after collection. Samples will be processed at a later date in the laboratory.

Weight of contaminants samples: This procedure is conducted only in the laboratory. Collect two, > 20 gram samples of kidney from each animal. Mark one label for heavy metal analysis (HM) and the other for organochlorine analysis (OC). Tare the empty, chemically cleaned, sample jar and its lid on the digital balance. Place the sample in the jar and record the sample weight to the nearest 0.1 gram. Repeat for the second sample.

Kidney collected: Remove the **right kidney** and examine and weigh it. Place the entire right kidney in a pre-labeled sealable plastic bag. Freeze the sample as soon as possible after collection.

Weight of the right whole kidney: Remove the **right kidney** and weigh on the digital balance. Record the weight to the nearest 0.1 gram, then store in a pre-labeled sealable plastic bag. Freeze the sample as soon as possible after collecting.

Comments: Describe any notable details or abnormalities in the kidneys in the space provided.

13. Gastrointestinal Tract

The gastrointestinal tract includes the stomach and gall bladder (see sections 11 and 12), small intestine, large intestine, and rectum. Before collecting tissues, examine the tissues from the whole system. If there are gross abnormalities observed, describe them in the comments section and collect samples of normal and abnormal tissues for histopathology (10:1 volume of buffered formalin to tissue). Note the gall bladder is examined and the stomach collected when the liver is collected.

Tapeworm (TPW) (size > 50 cm)

Thorny-headed worm (THW) (size 0.5 - 1.8 cm)

Nematode (NMT) (size <6 cm)

Small intestine: Thread through the small intestine with your hands, look at the health of the tissue and note if there are blockages in the intestine. Check the box which best describes the condition of the intestine. Then, randomly select a portion of the small intestine and remove approximately 20 centimeters in length. On the cutting board, slit the section of intestine open such that it lays flat. Move your scalpel blade through the contents of the section of intestine and count the number and type of parasites. Record the number of thorny headed worms (THW), tape worms (TPW), and nematodes (NMT) on the blank space provided on your data form by 'Segment 1'. Note if there are ulcerations

in the intestinal lining or any unusual food particles. Clean the cutting board surface and repeat the procedure for segments two and three. Some parasites may be too numerous to count, such as several large tape worms entangled together. If this is the case, simply describe what you observe.

Comments: Describe any notable details or abnormalities in the small and large intestine in the space provided.

14. Parasites

Record whether or not parasites were collected during the necropsy from any of the tissues examined. We ask that you only collect parasites that are unusual or are located in an unusual place in the body. Preserve parasites in formalin, 10:1 volume of buffered formalin to tissue. Keep the parasite samples separate from the histopathology samples. Do not freeze the formalin samples. Use the small nalgene bottles supplied in your kit.

15. Other Tissues Collected

Right femur: Collect the right femur from each animal necropsied. The femur should be dissected free of the hip and knee joints and cleaned of excess muscle and tendon. Place the femur in a pre-labeled sealable plastic bag and freeze and soon as possible after collection.

Muscle: Clean the cutting surface to remove any body fluids (such as bile) before collecting the muscle tissue. The muscle tissue will be used for genetic analysis and other bodily fluids could influence the laboratory results. Collect approximately 20 grams of muscle (approximately the size of a golf ball) and place it in a pre-labeled sealable plastic bag and freeze as soon as possible after collection.

Genetics vial: Clean the cutting surface to remove any body fluids (such as bile) before collecting the muscle tissue. The muscle tissue will be used for genetic analysis and other bodily fluids could influence the laboratory results. Collect a small piece of muscle tissue and place it into a pre-labeled vial containing a saline solution for tissue preservation. Be sure the entire tissue is surrounded by the solution. Freeze the sample with the other tissues collected.

Right testicle: This tissue is not, at this time, a part of our long term tissue archival program though we may request collection of the tissue during the course of the program. After completing the external measurements of the right testicle, slice the tissue in three to four sections and place in a 10:1 volume of buffered formalin to tissue. Keep the testis separate from the histopathology samples by using the small nalgene bottles supplied in your kit.

Urine collected: This tissue is not, at this time, a part of our long term tissue archival program though we may request collection of the tissue during the course of the program. There is no sample procedure for urine collection at this time.

Blood: This tissue is not, at this time, a part of our long term tissue archival program though we may request collection of the tissue during the course of the program. There is no sample procedure for blood collection at this time. However, when blood is collected, record what form the blood was preserved (for example, whole blood, plasma, serum) and for what purpose the blood was collected. Store appropriately.

Bile: This tissue is not, at this time, a part of our long term tissue archival program though we may request collection of the tissue during the course of the program. There is no sample procedure for bile collection at this time. However, when bile is collected, record the approximate volume collected and whether or not parasites are present. Store refrigerated.

16. Additional Comments

Include any additional information about the necropsy or circumstances of the hunt in the space provided here.

GLOSSARY OF TERMS

Abscess: A localized inflammation of tissue; a sore with accumulated pus.

Baculum: The penis bone in male sea otters (oosic).

Bile: A yellowish fluid secreted by the liver and stored in the gall bladder. Bile is passed into the small intestine to aid in the digestion of fats.

Cortex: The outer layer or superficial part of an organ or body structure (such as the kidney).

Duodenum: The first part of the small intestine extending from the opening of the stomach (pylorus) to the jejunum.

Esophagus: A muscular tube that extends from the throat (pharynx) down the neck between the trachea (a large, ribbed tube) and the spinal column and joins the stomach.

Femur: The thighbone which is the proximal bone of the hind or lower limb.

Gastrointestinal tract: Organs of or relating to the process of digesting food which includes the stomach, intestine, and gall bladder.

Groin: The fold or depression located at the juncture of the lower abdomen and the inner part of the thigh.

Heavy metal: The term heavy metal is generally used for metals with a specific gravity greater than 5 (the specific gravity of water is 1). Heavy metals bind with many organic molecules and are potent enzyme inhibitors. Specific heavy metals of interest include: cadmium, lead, nickel, zinc, and copper.

Histology: A branch of anatomy that deals with the small (microscopic) structures of animal tissues.

Histopathology: A branch of the study of pathology which examines the changes in animal tissue that occur when disease is present.

Lactate: Secretion of milk from the mammary gland and nipple.

Mesentery: Supportive membranes which supply the small and large intestines with blood.

Necropsy: A postmortem examination of a (nonhuman) carcass; to evaluate the condition of a carcass by detailed examination of the organs.

Oral lesions: Circular or oval sores in the mouth (under the tongue, cheek, upper and lower palate, and inside the lips). Sores may appear canker-like and vary in size and coloration.

Organochlorine: The term organochlorine is generally used to describe organic contaminants such as various pesticides and chlorinated hydrocarbons.

Ovaries: A paired female reproductive organ which produces female sex hormones and eggs. One ovary is located at the top of each uterine horn on the reproductive tract.

Spleen: A thin, liver-colored organ near the intestine and stomach which stores blood and produces white blood cells (lymphocytes).

Sacrum: A part of the spinal column that directly connects with and forms part of the pelvis or hip structure.

Subcutaneous: Anything which occurs under the skin. Subcutaneous fat refers to the fat located between the skin and muscle layer of the carcass.

Tare: The subtraction of the weight of the container from which a tissue sample is weighed, and therefore obtaining only the weight of the tissue sample. Note that this is an automatic feature on many digital scales.

Testicle: The male genital gland and the associated enclosing structures (tunic and epididymis). We remove the enclosing structures when collecting weights and measures.

Trachea: A ribbed tube extending from the base of the throat to the lungs and functions in passing air to and from the lungs.

Ulceration: A break in the skin or other tissue causing loss of the surface tissue, often includes the disintegration or necrosis of the tissue; pus is often present.

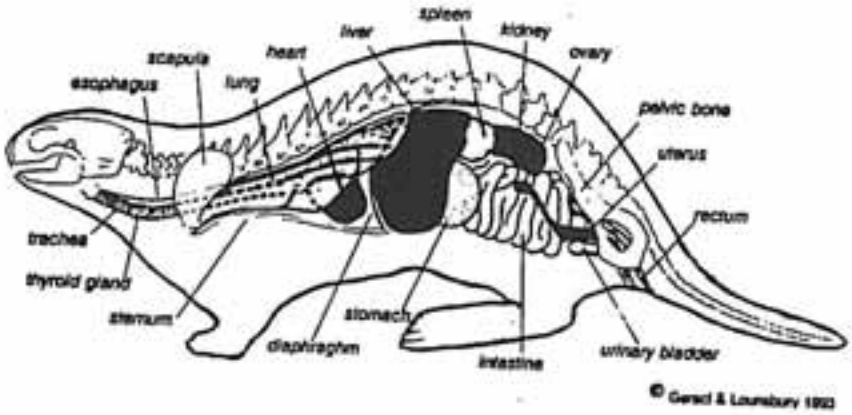
Uterus: The womb; the organ in female mammals used in the development of young prior to birth.

Ventral: Relating to the belly (abdominal) side of an animal; opposite the

backside.

Xiphoid process: The tip of the sternum (breastbone). The ribs are connected to the sternum on the ventral (belly) side of the animal.

Zygomatic arch: The cheek bone; the arch of bone that extends along the front side of the skull beneath the eye.



MEASURES AND WEIGHTS

Metric System

Length	10 millimeters	=	1 centimeter
	100 centimeters	=	1 meter
	1,000 meters	=	1 kilometer
Weight	1,000 grams	=	1 kilogram
Volume	1,000 milliliters	=	1 liter

Conversion of English units to Metric units

English units	Metric equivalent
1 inch	2.540 centimeters
1 mile	1.609 kilometers
1 pound	0.453 kilograms
1 quart	0.946 liters
1 gallon	3.785 liters

EQUIPMENT AND SUPPLIES

General Supplies

Apron
Band-aids
Binder for data sheets
Biomonitoring Log Book (for sample source information)
Calibrated spring scale (100 lbs for animal body weight)
Calipers
Camera and film (optional)
Clipboard
Cutting board
Data sheets (Tier I, II, III)
Digital scale for tissue sample weights
Disinfectant for cleaning instruments
Envelopes for teeth and whiskers
Garbage bags
Genetics vials (DMSO or similar saline solution)
Knife to skin and cut abdominal cavity
Mesh bag (nylon) for body weight
MTRP kit (for tagging hide and skull and obtaining certificate Number, where appropriate)
Nalgene bottles (250 ml, 450 ml, 1 liter)
Necropsy guide
Paper towels
Pencils, markers (X-tra fine sharpies)
Plastic tags for marking the carcasses
Pliers, to pull whiskers and remove scalpel blades
Rubber gloves
Ruler (small, plastic)
Sealable plastic bags (for frozen specimens)
Scalpel handle and blades
Sharps container (for used scalpel blades)
Sharpening stone
Strapping tape/duct tape
String
Tape measure, nylon
Toe tags
Tooth elevator, sharp
Vials (for blood and other fluid collection)

Sample preservation

Blue ice
Cooler for temporary sample storage
Fish boxes
1-2 liter nalgene bottle of 10% neutral buffered formalin
viral transport media (optional)

GUIDELINES FOR SAMPLING SEA OTTER TISSUES

1. Tissues from sea otters should be collected as soon as possible after the animal's death. Do not collect tissue samples from animals that have been dead for more than 3 days. However, tissues can be collected from animals that have been frozen within 3 days of death. In order to determine cause of death for beach-cast animals, necropsies may be conducted on animals dead more than 3 days but value of the tissues collected will be limited.

2. For subsistence hunted sea otters, it is best to collect samples the same day the sea otter is killed because studies of pathology and disease require that tissues be as fresh as possible. If it is not possible to collect samples within 2 days of the hunt, freeze the whole carcass until the sampler is able to collect all tissue samples.

   If a carcass has remained in the freezer for 2 weeks and the sampler is still unable to conduct a necropsy, ship the carcass to the Marine Mammals Management Lab in Anchorage.

   If a hunter returns with more carcasses than you can process at that time, try to conduct up to 10 necropsies with fresh animals (the Tier I or Tier II sampling procedure) and freeze the remaining carcasses to be necropsied at a later date. If freezer space is not available, ship the carcasses to the Marine Mammals Management Lab in Anchorage for necropsy.

TIERED SAMPLING SCHEME FOR SEA OTTER TISSUE COLLECTION

Tier I

The Tier I procedure is a detailed necropsy of a sea otter. Specific tissues are collected to meet a variety of objectives, including: food habits, reproductive characteristics, contaminant levels in tissues, genetics, body condition, and skeletal morphology. In addition, data on all major physiological functions, such as the respiratory system, gastrointestinal system, and the circulatory system are collected.

Tier II

The Tier II procedure is a general necropsy of a sea otter. Information gathered is limited to specific objectives including: food habits, reproductive characteristics, contaminant levels in tissues, genetics, body condition, and skeletal morphology.

Tier III

The Tier III procedure is limited to general external characteristics of the sea otter, such as sex, estimated age, and comments on the exterior body condition. The entire carcass is then shipped, either fresh or frozen, to the Marine Mammals Management Lab in Anchorage for necropsy.

Sea Otter Bio-monitoring Program Check List

NECROPSY DATE:_____ SAMPLE NUMBER:_____

TISSUE TYPE	PURPOSE	CHECK ONLY IF COLLECTED	SAMPLE PREPARATION AND STORAGE
PRE-MOLAR TOOTH	Age	☐	Any of the 4 first pre-molar teeth. Tooth root must be intact. Place the tooth in a labeled tooth envelope.
WHISKERS	Diet	☐	Pull 3-7 whiskers before skinning and place in labeled envelope.
L. KIDNEY	Histology	☐	Place thumb-nail sized piece in a labeled Nalgene bottle with formalin in about 10:1 volume of formalin to tissue. **Do not freeze.**
L. KIDNEY	Contaminants	☐	Place the rest of the **left kidney** in a Ziploc bag and freeze.
R. KIDNEY	Fat	☐	Place the entire **right kidney** in a Ziploc bag and freeze.
LIVER	Histology	☐	Place thumb-nail sized piece in a labeled Nalgene bottle with formalin in about 10:1 volume of formalin to tissue. **Do not freeze.**
LIVER	Contaminants	☐	Place the rest of the liver in a Ziploc bag and freeze.
STOMACH	Diet	☐	Tie both ends of the stomach off with string, place in Ziploc bag and freeze.
REPRODUCT--IVE TRACT	Female reproduction	☐	Include the ovaries; 2 small bean-shaped lumps at the end of each uterine horn. Follow each horn and detach the tract by cutting the tissue past the ovary. Place in labeled Nalgene bottle, 10:1 volume of formalin to tissue **(do not freeze)** or place in a Ziploc bag and freeze.
RIGHT FEMUR	Body Condition	☐	Remove the right femur and cut away attached muscle. Place in labeled Ziploc bag and freeze.
MUSCLE	Genetics	☐	Remove approximately 20 grams of muscle, place in a labeled Ziploc bag and freeze.
GENETICS VIAL	Genetics	☐	Remove a small portion of muscle and place it in the labeled vial provided and freeze.

SIGNATURE OF COLLECTOR_____