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SEA TURTLE NECROPSY MANUAL

Richard E. Wolke and Anita George

December 1981

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This manual was prepared by the authors for the National Marine Fisheries Service under contract no. NA80FAC00016. Readers are reminded that taking or possessing sea turtles is prohibited under the Endangered Species Act of 1973 (PL 93-205) unless proper permits are obtained. Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

PREFACE

INTRODUCTION

This manual is a guide for necropsies conducted on sea turtles under ideal laboratory conditions. Since most sea turtle necropsies will be conducted in the field, the manual should serve primarily as a guide. However, the manual contains information to conduct a careful and exacting necropsy, when it is possible to do so.

The value of a necropsy is dependent on the freshness of the cadaver and the number of observations. Field biologists are often pressed for time but wish as much information as possible. Unfortunately, there are no short cuts. Under these circumstances it would be best to remove the plastron and take samples for fixation from as many organs as possible.

Necropsies should always be conducted so that a minimum of logistical problems will occur. The prosector (person conducting necropsy) should dress in light, easily washable clothes and wear gloves. If possible, a shaded, well ventilated, and easily cleanable site should be used. The site should also be chosen in relation to carcass disposal. Large animals are very difficult to incinerate and must be buried. Disposal should be in compliance with authorization from proper law enforcement agents or with a valid state or federal sea turtle permit.

Descriptions are based on the anatomy of the loggerhead sea turtle, <u>Caretta caretta</u>. Left and right as used in this manual refer to the turtle's left and right. All measurements should be made in metric units.

EXTERNAL EXAMINATION

The sea turtle's weight, carapace length, carapace width and plastron length are recorded; note whether straight line or curved measurements are taken; straight line measurements are preferred. The general condition of the body is noted, because it reflects the state of decomposition and nutrition. Decomposition or postmortem autolysis is recognized by color changes of the skin, collection of blood in dependent areas (rigor mortis; Stage 1), and by the production of gas a foul odor (putrefaction; Stage 2). Within a short time after death, the ventral skin becomes pink to purple and as postmortem changes progress, frank lines of purple-green will be seen encircling the neck and limbs.

If the carcass is bloated so that gas escapes when a knife is inserted, advanced decomposition has occurred. At this point, the turtle has a foul odor. In such cases and in those instances in which the flesh is falling from the skeletal system, necropsy methods are of little value, because postmortem changes will mask antemortem events. The determination of the degree of postmortom decomposition, and therefore the value of necropsy, is subjective and learned by experience.

Nutritional state can be determined by both external and internal examination. A starving or chronically ill turtle has markedly sunken eyes and plastron. Its skin, carapace and plastron may be covered by an

abnormal number of barnacles; the muscle masses of the neck and the extremities are reduced; this may be so marked that the supraoccipital crest at the back of the head will stand out sharply and the shell appears too large for the animal. These signs are valid only if the turtle is relatively fresh and has not undergone advanced decomposition.

The skin and shell are carefully examined for color, texture and lesions. All lacerations, masses, discolorations, and scars, including rope burns, tag scars, propeller cuts, and bullet holes, are measured and described.

The head is carefully inspected for the presence of trauma and hemorrhage. The eyes are examined for abnormalities with special reference to the cornea and sclera. The oral cavity and the mucus membranes are examined for erosions, trauma and barnacles.

The extremities are then examined for lesions and palpated for fractures. The anal area is examined for protrusion, exudates, and consistency of fecal matter. A fecal sample should be placed in a small vial with 10% Formalin. Necessary scrapings or impression smears of suspected lesions are made and air dried for submission to the laboratory. Parasites are removed and placed in AFA solution for later identification (see Clinical Pathology section).

DISSECTION

Prior to dissection, the turtle is placed on its back. A short autopsy knife is idserted along the bridge between the carapace and the outermost plastron plates, and the cartilage is cut. The incisions (A-D, Fig. 1) are made through the skin along the anterior and posterior plastron edges. The plastron is removed from the underlying muscle masses. In larger turtles, this is most easily done if the plastron is pulled upward by an assistant with a hay or stevedore's hook. A mid-ventral incision (F, Fig. 2) is made between the coracoids and directed posteriorly to the pelvis. A greyish-green fat overlying the pleuroperitoneum is normal in Caretta. Incisions (G, H, Fig. 2) are then made along the lateral border of the muscle masses at the carapace edge and extending forward so that the muscles and pectoral girdle can be lifted upward and pushed toward the head, thereby exposing the underlying heart and other viscera. The muscles may then be removed by disarticulating the contained girdle at the shoulder (gleno-humoral joint). A similar procedure (I, J, Fig. 2) can be used to remove the muscle masses of the rear flippers. A mid-ventral incision (M, Fig. 2) is made separating the left and right pelvis (in larger turtles long-handled pruning shears or a saw will be necessary) followed by lifting the pelvis upward and backward with disarticulation from the femoral heads. In all of these manipulations, separate the muscle masses carefully from the underlying pericardium, peritoneum and viscera in a manner that does not disturb the relationship of organs to one another.

Check the positions of organs (Fig. 3). Frequently the pleuroperitoneal cavity will be filled with fluid. This fluid obscures the lungs and urogenital tracts and may collect in the lungs if they are

accidently cut. It is, therefore, important that this fluid (note and record color) be removed frequently by tipping the animal on its side. If water is available, the cavity may be flushed and drained.

If decomposition has progressed, the fat over the heart and on the dorso-medial side of the scapula will be black-red and gelatinous. Further, major organs lose their natural color and appear as if parboiled. The pleuroperitoneal cavity may contain large amounts of a dark-red serosanguinous fluid. If these signs are severe, the soft tissues are unlikely to be suitable for histological examination and need not be collected. However, information can still be gathered from examination of gross anatomy and such related parameters as gut contents, parasite load, toxicology screening and skeletal features.

The basic principles directing the dissection are after those of Olafson as reported by Van Kruningen (1971):

1. Each part of the carcass is examined <u>in situ</u>. Then it is removed and examined.

2. Once an organ is removed, it is dissected totally and sections are taken before continuing the gross dissection.

3. The carcass is examined from outside-in and ventral to dorsal.

I. Throat

A mid-ventral incision is made posteriorly along the neck from the mandibular symphysis (E, Fig. 1). The skin is reflected, exposing the underlying trachea and esophagus. The oral cavity is examined, as are the tongue and glottis. Two incisions (K, L, Fig. 2) are then made along the inner edges of each mandible through the muscle into the oral cavity. A finger can be inserted through the incisions encircling the tongue, and the tongue pulled upwards after its anterior attachment is severed. The tongue, larynx, trachea and esophagus are then dissected free of the neck to a point at which the trachea and esophagus disappear dorsal to the heart (Fig. 3). The thymic tissue occurs on either side of the trachea in young animals. Due to the dorsal location of the turtle's lungs, it is easiest to examine the gastrointestinal system before the respiratory system.

II. Gastrointestinal Tract

A large, bilobed liver lies over the heart and occupies the anterior abdominal cavity (Fig. 3). It is dark brown (mahogany), shiny and sharpedged under normal conditions. The right lobe is largest and lies ventral to the small intestine and pancreas. The left lobe covers the anterior portion of the stomach, which occupies the left abdominal cavity. Grasping the esophagus and pushing the liver to one side, the stomach can be dissected free along its inner curvature from the mesentery and liver. Tie with twine the anterior end of the stomach at its attachment to the esophagus to prevent the stomach contents from spilling into the body cavity (N, Fig. 4). Lift the stomach up and cut its esophageal attachment.

At the pylorus, the stomach is cut free and removed after tying both ends of the transected gut with twine (0, P, Fig. 4). The stomach is then examined internally after an incision is made along the lesser curvature. Contents are recorded and preserved in alcohol (40% isopropyl or 70% ethyl).

Before removing the liver, the duodenum is incised mid-ventrally to expose the bile-duct opening. The duct should be checked for obstructions by applying pressure to the gall bladder, which lies beneath and within the back edge of the right liver lobe (Fig. 4). The liver and gall bladder are removed and carefully examined. The gall bladder is approximately 2 x 3 cm in a 60 cm turtle and dark green. The saccular colon lies beneath the stomach. (In <u>Dermochelys coriacea</u>, the leatherback turtle, the arrangement of the anterior gut differs. The stomach lies to the left and is a U-shaped organ. The esophagus lies along the mid-line dorsally and extends posteriorly to the anterior edge of the kidneys then turns and joins the stomach. The pylorus is further forward and the duodenum, after leaving the stomach, runs backward along the edge of the carapace on the left.)

The pancreas lies along the duodenum. It is normally grey, irregular in outline and elongate. Cut the mesentery from the intestine and strip the intestine from the abdominal cavity.

One or two large mesenteric vessels to the lower intestine should be removed and fixed for blood fluke examination in the laboratory. The intestine is then tied at its entrance to the pelvic inlet, removed from the cavity and set to one side for further examination. The esophagus, duodenum, jejunum and large intestine are then opened along the entire length of the gastrointestinal tract. Lesions are noted and parasites carefully collected with their numbers and locations recorded.

The mesentery suspending the intestine is examined. The pancreas and the spleen are removed for sectioning. The spleen (Fig. 4) is antedorsal to the caecum within the mesocolon. It is slate to blue-grey, egg-shaped and smooth.

III. Lungs and Heart

The trachea is a white tube lying ventral to the esophagus and held open by a series of incomplete cartilaginous rings. It is normally unobstructed. Just below the heart, it bifurcates to form paired bronchi which enter the lungs. The paired lungs should be dark pink, partially air-filled and of a soft, expansible consistency. On cut section they are dry. Both lungs are covered by a rather thick greyish-blue sac (pleura). Their pink color is only apparent after removal of the pleura.

The trachea can be grasped and freed from underlying tissue. While holding the trachea, the lungs, which lie dorsally against the carapace, can be dissected free with the heart and great vessels. They should be removed in toto to simplify identification of the great vessels. The

heart can then be removed from the lungs and examined. Alternatively, however, if it is easier for the prosector, the heart may be removed from the lungs while still within the animal (Fig. 4). On the anterior pericardial sac, often obscured by yellow fat, are the thyroid and parathyroid glands. They are translucent and red but are difficult to see so a wide excision of the tissue is needed to assure their removal. Just lateral to these organs and the sac is the paired pinkish-grey lobular thymus. When the pericardial sac is cut, be careful to note the amount, consistency and color of the pericardial fluid. A mid-dorsal opening of the aorta will expose the aortic valves. The ventricle may be opened by an incision from its attachment to the right atrium through the apex, to the left atrium. The arterio-ventricular valves can then be examined and the incision continued into each atria.

The trachea should be opened and followed to the bronchial bifurcation. The lung parenchyma can be examined by following the respiratory tracts and by transverse incisions through the tissue. Changes in color, texture and the presence of parasites are noted and sections collected.

A possible, but not certain sign of drowning is a thick, tenacious, persistent white (or slight pink) foam which can be expressed from the nostrils by pressure on the throat or is present within the trachea, bronchi and on cut section of the lung. In addition, the lungs are greatly expanded and contain a watery fluid. When removed and placed on the autopsy table, they will collapse and fluid will run from them freely. Vomitus may be found in the trachea and bronchi. However, with current limited knowledge, a diagnosis of drowning as the cause of death, based on gross signs from necropsy, cannot be made.

IV. Urogenital System and Adrenals

The ovaries lie on each side of the posterior pleuroperitoneal cavity and can be removed with their unattached oviducts by incising the mesovarium. Note should be taken of the presence and exact numbers of eggs and degree of calcification. As the oviducts are followed posteriorly, the urinary bladder is found just anterior to the cloaca. There are no accessory cloacal bursae in sea turtles. The bladder, its cloacal orifice and the oviductal openings into the cloaca are examined. At this point, the ureters are observed and followed forward to the paired dorsal kidneys (Fig. 5).

By careful dissection, the cloacal opening and rectum can be freed, pulled upward and forward. Then the attachments of the urogenital system and the kidney can be severed and the complete system removed from the abdominal cavity. The kidneys and bladder should be removed in toto with the ovary and oviducts, incising just posterior to their cloacal attachment. The kidneys are dark red to black, covered by a grey-blue peritoneum and multi-lobed. All organs should be examined for masses, changes in color or consistency, exudates and parasites.

In the male, the cloaca can be opened laterally to expose the penis; (this organ may be difficult to find in juvenile turtles; sex determination in younger animals usually depends on histological examination of gonadal tissue). In either sex, such an incision will expose the orifices of the bladder and cloaca anteriorly. The testes and epididymides occupy the same area as the ovaries and their ducts.

The interrenal and chromaffin tissues (adrenal cortex and medulla) are not discrete and are on the ventral surface of the kidney. It is wise therefore to use wide excision when removing the kidneys and to save renal peritoneum for location of these structures.

V. Connective Tissue, Skeletal Muscle, and Osseous System

The major pectoral and pelvic muscle masses should be carefully palpated for evidence of mass lesions, and if necessary, incised and examined. The shoulder and hip joint should be opened and articular cartilaginous surfaces and synovial fluid examined, noting the color and consistency of both cartilage and fluid. At least one front flipper and one rear flipper should be disarticulated at the shoulder and hip joint and removed in toto. Whenever possible, roentgenographs of the extremities should be obtained for delineation of skeletal and joint trauma or the presence of lesions. Disarticulated limbs should either be frozen or preserved in 10% buffered Formalin, and then submitted for age determination studies. When possible, the cervical spine, consisting of eight mobile vertebrae should be disarticulated from the head and from the first thoracic vertebra, which is firmly fixed to the anterior portion of the carapace.

VI. Central Nervous System and Organs of Special Sense

The head is then disarticulated. Using a Stryker or hack saw, the dorsal calvarium of the head can be removed. This requires a transverse cut through the bone just posterior to the orbits followed by two cuts at right angles to the first cut through the dorso-lateral calvarium extending posteriorly to its ending (see Fig. 1). The only structure then holding the roof of the skull will be the underlying supraoccipital process. This can be reached with a pair of shears just above the attachments of the vertebral column to the posterior skull.

When all cuts have been made, the calvarium may be lifted away revealing three structures: two large lobulated salt glands postorbitally, to each side, and the cut dorsal surface of the supraoccipital process. If two incisions are made through the lateral portions of this process, it too may be lifted away revealing the small, elongate brain. The pineal gland is just under the roof of the skull dorsal to the pituitary and located in the middle of a line drawn behind the eyes.

Turning the head upside down, the brain will be held only by cranial nerves, olfactory tracts and optic nerves which may be carefully cut with scissors. Examine the brain and fix in 25% Formalin. Section after fixation. The pituitary, lying below the brain in a bony depression, is removed with the brain.

The eyes should be removed with curved scissors, cutting within the orbits and severing the optic nerves, then fixed in 10% Formalin. A

transverse cut made just posterior to the nostrils and joined by a frontal cut below the nostrils allows removal of the nares and examination of the nasal canals. Incise the tympanic membrane over the ear cavity and remove the slender columella bone which is attached to the skin and extends medially to the inner ear. It may not be possible to remove the bone <u>in</u> toto, but even a partial specimen can be valuable for age determination.

A portion of the cervical spinal cord should be removed and preserved. It is indeed difficult to obtain the whole cord and/or thoracic lumbar sections which require a ventral approach, hence this tissue is not obtained routinely.

In general, cause of death of a sea turtle carcass will not be discernible from gross necropsy observations. Apparent exceptions to this are obvious signs of trauma, such as bullet wounds, severe lacerations, massive hemorrhage, and decapitation; but evidence such as this might have been produced postmortem. The determination of cause of death will require histopathological examination and analysis in some cases. In other cases, the determination may be impossible because causes of death have not been adequately researched.

EQUIPMENT AND FIXATIVES

Equipment will vary from laboratory to laboratory but the following list is as suggested by Van Kruningen (1971) and modified (instrument measurements are in the English system for ease in purchasing):

- 1. Rubber gloves
- 2. Tissue forceps (6 inch 2 x 3 teeth)
- 3. Scalpel (no. 4 with no. 22 blades)
- 4. Scissors, straight and curved (6 3/4 inch)
- 5. Postmortem shears (7 3/4 inch serrated edges)
- 6. Boning knives (5 1/2 inch blade)
- 7. Hay or stevedore's hook
- 8. Pruning shears
- 9. Small hand saw
- 10. Knife sharpener and steel
- 11. Stryker electric autopsy saw, hack-saw
- 12. Bottles, waterproof labels, waterproof markers
- 13. Glass microscope slides
- 14. Sterile petri dishes
- 15. Blood agar plates; marine agar (Zobell's agar) plates
- 16. Propane burner
- 17. Spatula and bacteriology loops
- 18. Blood vials with an anticoagulant (EDTA) (ice and styrofoam container helpful)
- 19. Photographic equipment and/or tape recorder
- 20. Twine
- 21. Tape measure and/or calipers
- 22. Scales
- 23. 10% and 25% Formalin

Tissue for fixation should be thinly sliced (2 cm x 3 cm x 6 mm) and not traumatized. Sections should, if possible, contain both normal and abnormal areas. Sections should be taken from all major organs. This tissue must <u>never</u> be frozen unless it is being used for virological or toxicological examination. The best routine fixative is 10% neutral buffered Formalin (commercial formaldehyde 37-40%, 100 ml; water, 900 ml; sodium phosphate dibasic anhydrous, 6.5 gms; sodium phosphate monobasic, 4 gms). Two liters is usually sufficient for a single necropsy. Nonbuffered Formalin is acceptable if buffering agents are not available. Alcohol can be used, but <u>only in an emergency</u>. It tends to make the tissue quite hard and distorts normal cell shape.

Sections are placed in 10 times their volume of fixative for 24-48 hours. After fixation, sections may be mailed in heat-sealed, polyethylene food bags with some Formalin-moistened cotton. Make sure that all bottles and bags are well labeled. Place a waterproof label inside as well as outside the container. All necropsies should be done as quickly as possible and certainly within 24 hours of death, if possible (48 hours under refrigeration). Animals should be removed as quickly as possible from the water and placed in a cool area.

CLINICAL PATHOLOGY

All fresh tissue and tissue fluids must be submitted to the laboratory as soon as possible. Blood for hematology and biochemical studies must be taken before death. Postmortem blood specimens can be used for microbiological examination. Tissue for toxicology and virology may be frozen. Approximately 200 gms of tissue are needed, 15 ml of blood and up to 200 ml of other fluids. The tissues necessary for toxicological studies are liver, kidney, brain or spinal cord, fat, skeletal muscle, and eggs if present. Blood samples should be refrigerated and not frozen. Tissue for microbiological examination may be placed in labeled sterile petri dishes for submission. Tissues and fluids best suited to determine exposure to environmental contaminants and toxic substances are liver, muscle, bone, and stomach contents. Materials to be examined for petroleum derived hydrocarbons, PCBs, etc. should be stored in glass rather than plastic containers.

Before culturing lesions or tissue for bacteria, the bacteriological loop must be flamed and allowed to cool for a few seconds. The spatula may be flamed simultaneously and used to sear the surface of the organ for sterilization. The loop is then passed through the seared area into the organ proper. The agar is then streaked with the flat surface of the loop. Be sure to keep the petri dish top over the agar to avoid contamination. Alternatively a piece of the organ may be removed with flamed sterile instruments and placed in a sterile petri dish for later culturing in the laboratory.

Helminths should be relaxed prior to fixation. Trematodes and cestodes are refrigerated in tap water, and nematodes are relaxed in glacial acetic acid.

For the routine preservation of parasites AFA solution is excellent (85% ethyl alcohol, 85 ml; Formalin, 10 ml; acetic acid, 5 ml) or 5%

formol-saline (Formalin, 5 ml; physiologic salt, 95 ml). Relaxation can be brought about by placing the parasites in warm 70% alcohol and storing in same.

Scrapings are made with a sharp scalpel and material placed in a small vial of 10% Formalin. Impression smears are made by cutting across an organ with a knife or scalpel and pressing (dabbing) the exposed surface against an alcohol slide. The slide is allowed to air dry and is submitted in that condition.

ANAMNESIS AND RECORDS

It is imperative that a careful history be taken and that all findings be recorded. The history should include information about the data and location of stranding, water temperature, pollutants (oil, mercury), time and date of death as well as all other pertinent facts.

The prosector must report only what he sees, not what he thinks. Complete sentences are used and lesions characterized by a number, color, size, shape, consistency and location.

Photographs are helpful to document descriptions. When the prosector is alone, it is extremely difficult to record observations and quite easy to forget those made early in the necropsy. A simple, inexpensive tape recorder is most helpful in overcoming this problem.

Two pathology report forms are included in this manual. While these two forms are only samples of the kinds of forms needed, we recommend that copies of them be used, unless a better form is available. The <u>Sea</u> <u>Turtle Necropsy Report Sheet</u> (2 pages) is to be used by the prosector. The <u>Pathology Record</u> (1 page) is to be used by the pathology laboratory personnel that report the diagnosis and the cause of death.

DETERMINATION OF CAUSE OF DEATH

A carefully conducted necropsy with complete histopathological examination may uncover the cause of death in the majority of cases, but not in all cases. In those instances in which cause of death cannot be determined, the pathologist can only report morphological changes and their severity. In all cases, however, the pathologist's final report should contain a section for morphological diagnosis, etiological diagnosis, and a statement as to cause of death. Morphological and etiological diagnoses may be incidental or not of sufficient severity to have played a role in the turtle's death. Under these circumstances the pathologist must differentiate between such findings and the actual cause of death.

The determination of cause of death is dependent, along with other factors, on the knowledge of diseases and pathological conditions occurring in that species. Information regarding diseases, their prevalence, and the clinical manifestations of disease and pathological conditions in sea turtles is minimal at present. Data are being collected rapidly, but interpretation often relies on comparisons with similar observations in higher vertebrates, which may or may not be valid. An example is the diagnosis of drowning. Certain classical signs of drowning are present in warm blooded animals that have also been observed in sea turtles. However, there is no certainty with present knowledge that they are diagnostic of drowning in sea turtles.

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SPECIMEN COLLECTION CHECKLIST

1.	Skin
2.	External Parasites
3.	Skeletal Muscle
4 .	Tongue
5.	Esophagus
6.	Stomach
7.	Liver
8.	Gall Bladder
9.	Pancreas
0.	Duodenum
11.	Jejunum
2.	lleocecal Area
13.	Colon
4.	Gastrointestinal Parasites
15.	Mesenteric Vessels
16.	Spleen
17.	Trachea
18.	Lung
19.	Ventral Pericardial Sac (Thyroid and Parathyroid)
20.	Aorta and Great Vessels
21.	Atrium
22.	Ventricle
23.	Gonads and Ducts
24.	Urinary Bladder
25.	Kidney and Renal Peritoneum (Adrenal)
26.	Rectum
27.	Cloaca
28.	Salt Glands
29.	Brain (whole, sliced lengthwise)
30.	Eye
31.	Olfactory Block (see text)
32.	Scrapings and Smears (where necessary)
33.	Front Flipper and Hind Flipper
34.	Cervical Vertebrae
35.	Columella Taviaslasy Semaning (framer shout 100 - 200 grams each)
36.	Toxicology Screening (frozen about 100 - 200 grams each)
	a. Liver
	b. Kidney
	c. Brain or Spinal Cord d. Fat
	e. Skeletal Muscle f Foos

SEA TURTLE

SUBMITTING ID

NECROPSY REPORT SHEET

Submitting Prosector			Address			Phone					
Genus	Spec	ies	Carapac	e LXW	Plastr	on LXW	Head W	Weigh	nt] :		
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Moribund De	ad	Fresh F	ixed								
Tissues											
Tissues submitt	ed:		······	*··· \$, <u>*</u> <u>*</u>							
Histopatholo	ogical -										
Bacteriologi	cal (smea	urs, tissues,	cultures)	-							
Virological	(frozen)	-									
Parasitologi Toxicologica											
GROSS FINDINGS:											

External (including skin, plastron, carapace)

Head (including mouth, eyes, nares, salt glands, brain)

Gastrointestinal Tract (including liver, spleen, pancreas, mesentery)

Lungs and Heart (including trachea, bronchi, great vessels)

Urogenital System (including kidneys, bladder, gonads, accessory tubes, cloaca)

Skeletal Muscle and Osseous System

Date Submitted		PATHOLOGY RECORD MARINE PATHOLOGY LABORATORY COLLEGE OF RESOURCE DEVELOPMENT UNIVERSITY OF RHODE ISLAND KINGSTON, R. I. 02881						Accession No.		
Veterinarian	······································		Address	·····			<u></u>	Phone		
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L	C	linical Diagnosi		I <u>, , , , , , , , , , , , , , , , , , ,</u>	I ,	L		_I		
History and Clinica	al Summary:							<u></u>		
		· .								
Specimen Submitte	ed		Preservat	ion				of Specimen		
Live Animal 🗌 Tiss	Dead A	Animal 🔲	Fresh	🗌 Fro	ozen 🗖	Fixed 🔲	When Rec	eived at Lab:		
Autopsy Data Natural Death	Mode	of Euthanasia		Time and	Date of Dea	ath	Time at of Auto	nd Date opsy		
Tissues Submitted:					<u></u>	·····				
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ALPHA-NUMERIC CODES USED IN FIGURES

- Abdominal and Inguinal Fat (green) 1.
- Pectoral Muscles 2.
- Pelvic Muscles 3.
- 4. Tongue
- 5. Trachea
- Heart, Ventricle 6.
- Heart, Auricle 7.
- 8. Stomach
- Liver 9.
- 10. Duodenum
- Pancreas
- 11.
- Intestines 12.
- 13. Spleen
- 14. Gall Bladder
- 15. Luna
- 16. Mesovarium
- 17. Kidney
- 18. Gonad
- Urinary Bladder 19.
- 20. Cloaca

A-D. Incision sites for plastron removal.

Incision site to expose trachea. Ε.

Incision site to separate abdominal and pectoral muscle masses. F.

- Incision sites to allow reflection of pectoral muscle masses. G-H.
- Incision sites to allow reflection of pelvic muscle masses. I-J-M.
- Incision sites to allow reflection of tongue. K-Ł.
- Points of gastrointestional ties to prevent loss of stomach and N-0-P. intestinal contents.

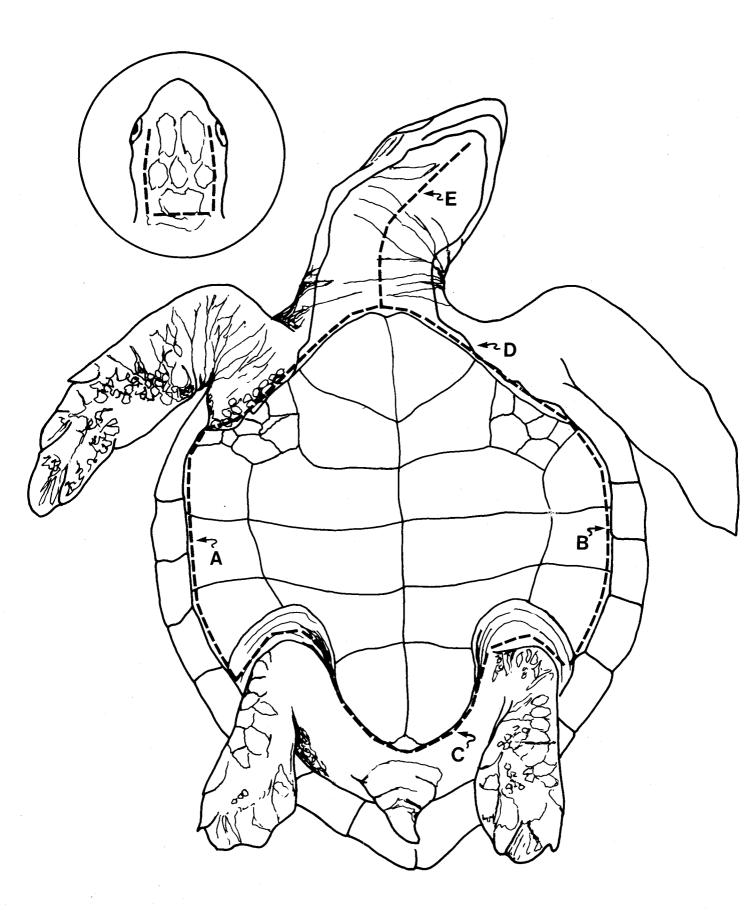


Figure 1. Turtle ready for dissection. Dotted lines indicate incision sites for removal of plastron and brain exposure.

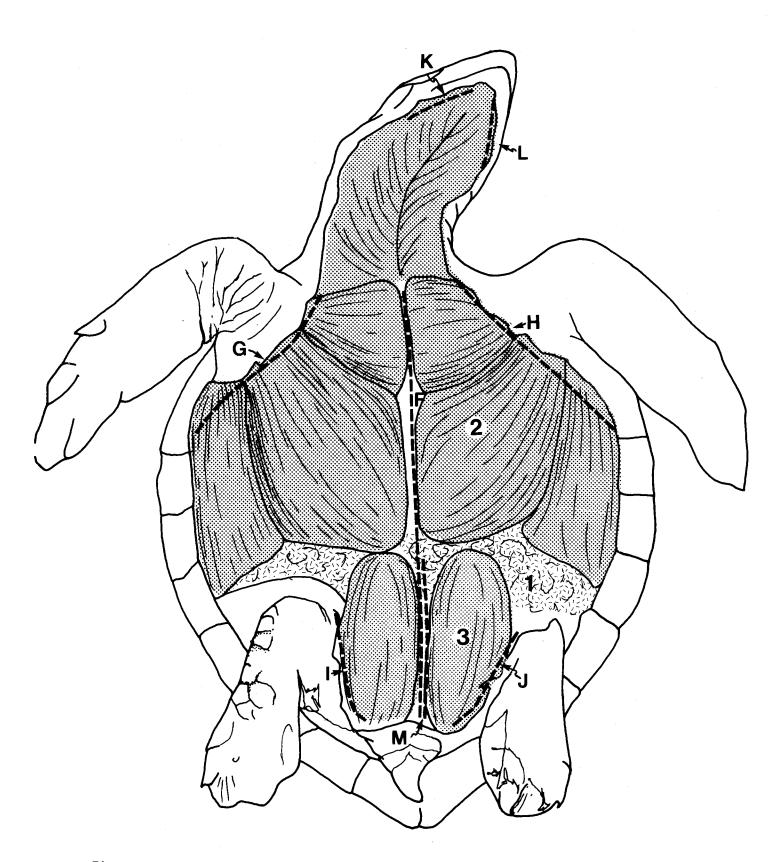


Figure 2. Muscle masses present after plastron removal. Dotted lines indicate incision sites to expose viscera.

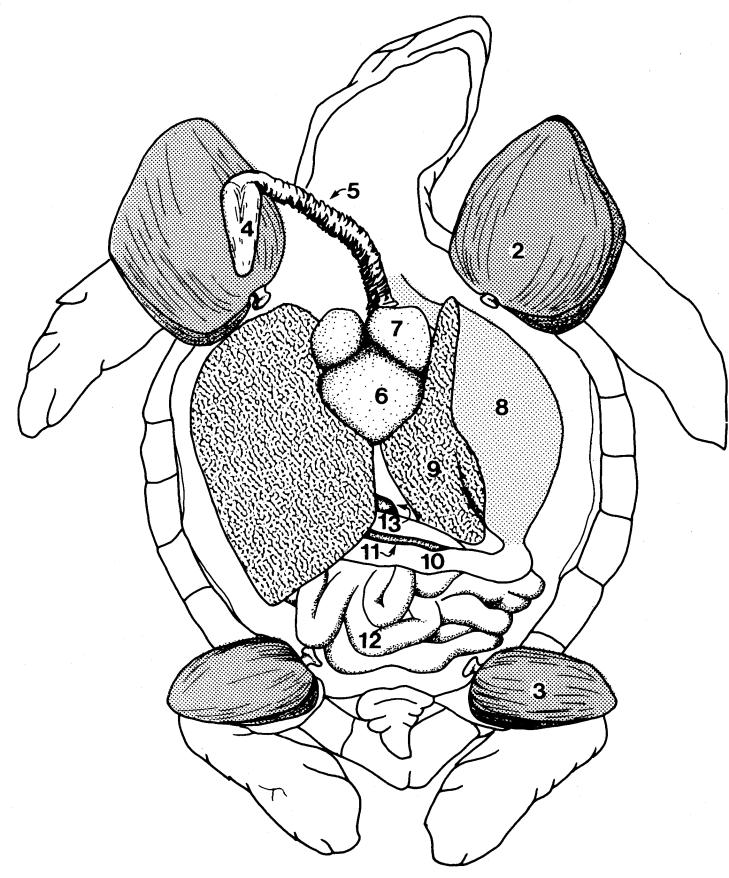


Figure 3. Muscle masses reflected to reveal viscera. Tongue and trachea have been freed and pulled ventrally.

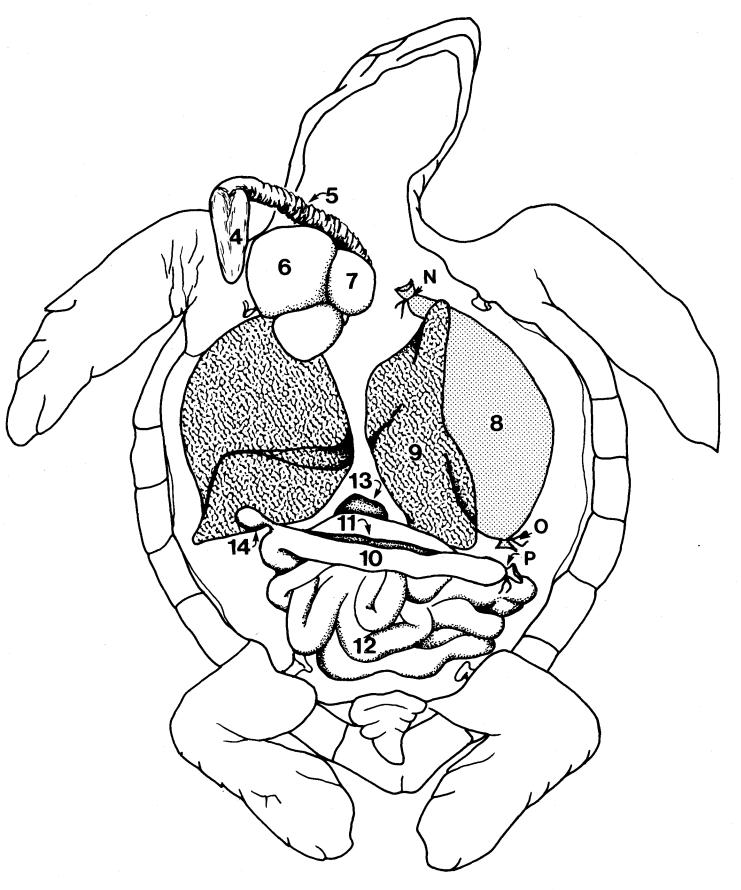


Figure 4. Viscera reflected to show location of spleen (13) and gall bladder (14), and points of gastrointestinal ties to prevent loss of stomach contents (N,0,P).

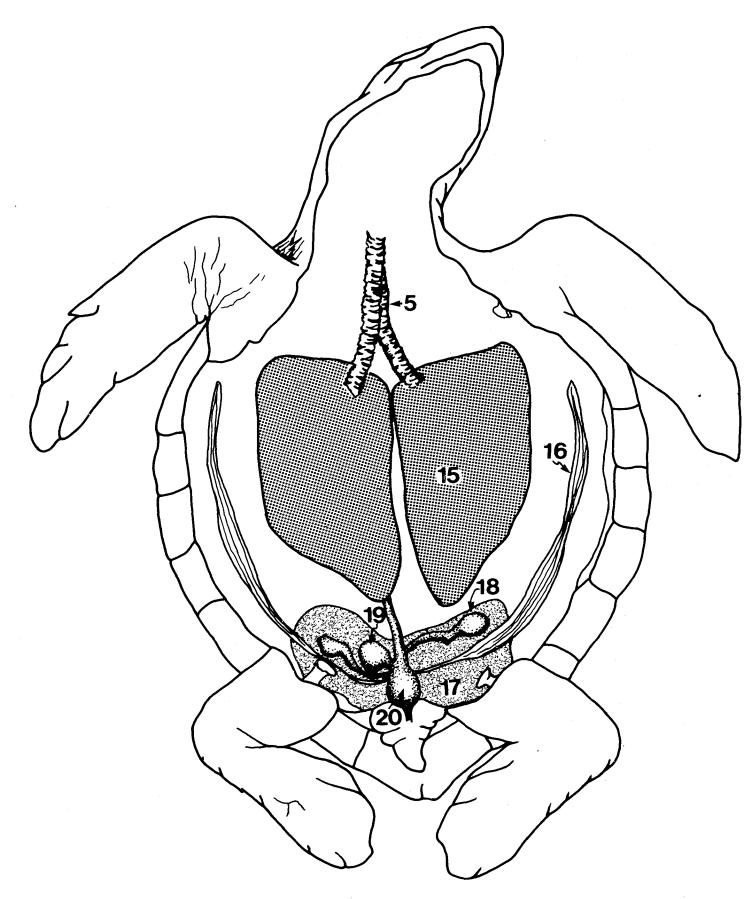


Figure 5. Liver, head, and gastrointestinal system removed revealing lungs (15) and genitourinary system (16-19).