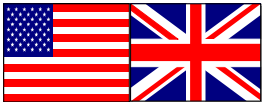


SEA TURTLE NECROPSY MANUAL FOR BIOLOGISTS IN REMOTE REFUGES

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INTRODUCTION

This manual is for biologists in remote refuges who have little to no background in necropsy techniques. It is intended to assist you in recognition of turtle organs and in procurement of appropriate samples for pathologic and other laboratory assays. The manual will probably be most useful in situations when wildlife disease specialists are unable to assist in sample collection due to remoteness or inaccessibility of the site.

WHY DO A NECROPSY?

A necropsy is one of the basic tools used to determine why an animal dies. It involves the thorough examination of a carcass externally and internally for any indications of causes of death (lesions). A good necropsy involves careful observations of lesions or abnormalities and procurement, labeling, and storage of tissue samples. Laboratory tests on properly preserved tissues allow wildlife disease specialists to systematically evaluate potential causes of wildlife mortality.

The better job you do with the field necropsy, the better the chance that wildlife disease specialists can determine what killed the animal. As such, select the freshest carcasses and, if at all possible, avoid freezing and thawing the carcass prior to necropsy as this can compromise microscopic appearance of tissues. When doing a necropsy, be observant and record your findings. If possible, take close up photos of interesting findings.

Generally, findings will deviate from normal either in shape, color, consistency, number or size. For example, a normal green turtle liver would be firm with rounded borders and be homogenous dark purple-brown. An abnormality in the liver may manifest itself in the form of abnormal coloration (spots or blotches), consistency (too soft, too hard), size (excessively large or small), or shape (lumps, bumps or scars). Obviously, many of these interpretations require knowing what a "normal" organ looks like. Although this is best learned by doing many necropsies, reference to photographs (as in this manual), will aid the novice in assessing whether or not an organ appears normal.

MATERIALS NEEDED FOR A NECROPSY

Scissors	Toothed forceps	Rubber gloves
Plastic bags	Jars	Indelible marker
Knife	Cutting board	Water
Scalpel handle	Bone saw	10% formalin
Scalpel blade	Labels	Aluminum foil
Pencil	Paper	

Additional items that would be helpful include a scale, tape measure, calipers and camera. Several types of plastic bags should be available including larger bags for carcass disposal and smaller bags (whirlpaks) to store individual organs.

The back of the manual has a recipe for making buffered formalin (a tissue preservative). It is unlikely that you will have the resources to make buffered formalin on site. An adequate substitute is mixing 15 parts of 37% formaldehyde with 85 parts seawater. **Placing organs directly in 37% formaldehyde or unbuffered formalin is unacceptable.**

SAFETY

Doing a necropsy on a turtle is hard work. Be careful with knives and sharp bones and follow proper hygiene. Wear gloves and do not eat or drink while dissecting a carcass. Remember, you don't know whether you're dealing with a disease transmissible to humans.

When working with formalin, **ALWAYS** use gloves, work in a well ventilated area and wash hands after all necropsies. All formalin containers should be clearly labeled.

LABELS

All labels should be written in indelible ink (e.g. sharpie) or pencil...**no ball point pens**. Minimum information on the label should include location of collection, date and unique specimen ID. To avoid confusion, abbreviate the month (i.e. MAR 5, 2000 not 3/5/00).

TAKING SAMPLES FOR LABORATORY ANALYSIS:

FORMALIN FIXATION (2 steps)

(Formalin fixation allows pathologists to examine tissues under the microscope and diagnose disease)

1) To ensure that enough formalin is present in the jar to allow for adequate fixation of the tissue, the ratio of formalin to tissue should be a minimum of 2 parts formalin to 1 part tissue by volume (Fig. 1). All tissues from one animal can go into one jar. **Label the jar.**

2) Ensure that tissue section is not too large to allow for adequate fixation. A piece of tissue should generally be no thicker than ~0.5 cm (1/4 in). If there is a lesion, make sure to take a portion of "normal tissue" adjacent to the lesion (Fig. 2). This is crucial as many diseases are diagnosed based on microscopic examination of the "margin" between a normal and abnormal tissue.

It is advisable to change the formalin once (say after 24 hours of fixation). This will result in better fixation and staining for microscopic analysis. Used formalin should be disposed of appropriately. **Tissues in formalin should never be frozen.**

FREEZING (1 step)

(Frozen organs can be used to isolate microorganisms or detect poisons)

1) Collect a good amount (20-30 g or 1/4 to 1/2 cup) of tissue, place in a small plastic bag, seal and label the bag using an indelible marker. In some cases, you may be asked to wrap the sample in aluminum foil prior to placement in a plastic bag. Collect tissue for freezing as early as possible during the necropsy to avoid contamination by gut contents, dirt, etc. Tissues should be placed in a freezer (-20C or colder is best) and kept frozen when shipped to the laboratory.

LID

**JAR WITH
FORMALIN AND
TISSUES
(1 part tissues
to 2 parts
formalin)**



FIGURE 1

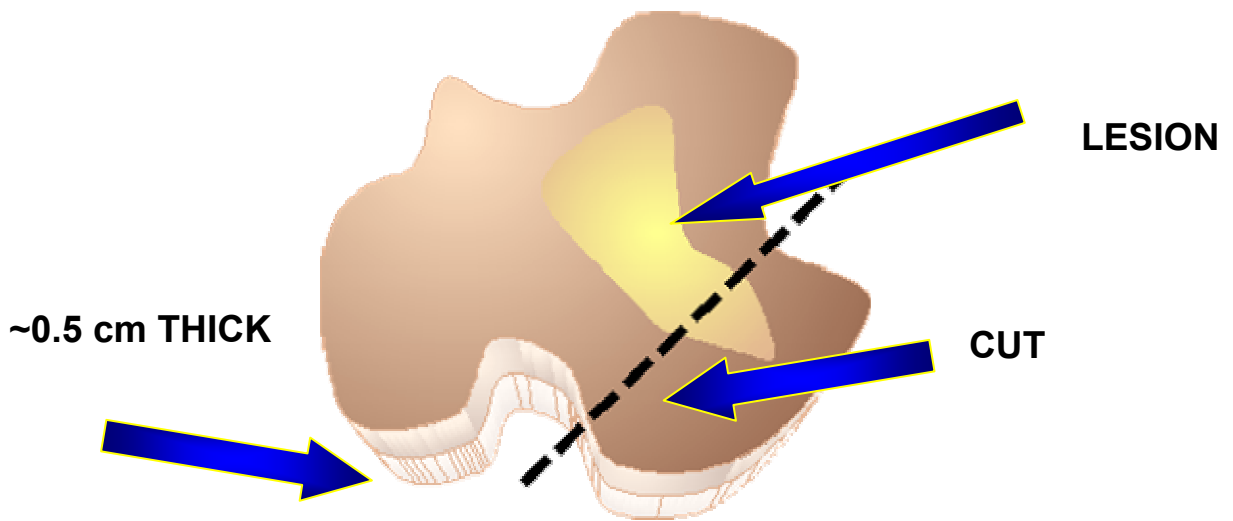


FIGURE 2

HOW THIS MANUAL IS ORGANIZED

The rest of this manual will show you, step by step, how to dissect a sea turtle carcass using a green turtle as our "model" turtle. All turtles have the organs shown here although size and shape may vary from one species to the next. The photos in this manual should give you a good general idea of what "normal" organs look like.

This manual is composed of a series of photos with a facing page of text. There are two sorts of icons throughout the text, scissors and glasses.



Sections with scissors icons are in bold type and describe the nuts and bolts of taking a carcass apart.



Sections with glasses describe organs and their appearance. Commonly encountered abnormalities appear in italics. Use these sections as a reference for taking notes on appearance of different organs. As you go through a necropsy, it would be wise to take samples of organs as you encounter them. There is a table at the end summarizing what organs you should have taken in formalin when you are done with your necropsy (P.22) and a blank necropsy form (P. 24).

NOTE: This manual assumes you are doing a necropsy on a fresh dead turtle (either you saw it die or it died within the last 12-24 hours). Appearance of some organs (and their diagnostic value) will change dramatically depending on stage of decomposition so it is best to limit your efforts to the freshest specimens available.

Finally, remember **TO NOTE AND RECORD EVERYTHING THAT YOU SEE**. There can NEVER be too much detail.

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EXTERNAL EXAM



Examine the turtle externally from head to tail for any abnormalities or damage. You may want to take photos of any abnormality or for ID confirmation. When examining the carcass, check the following :



-Plastron, carapace, and skin: Are the scutes peeling? Are there fresh or old wounds? Are there barnacles (numbers?), leeches (Numbers?), algae (Percent cover?) or other epibionts growing on the carapace? Are there abnormal growths on the skin?.

-Body condition: Turtles in good condition will usually have a nice rounded plastron. In severely emaciated turtles, the plastron is dished in and concave.

-Measurements (see page 10)

-Cloaca: Is there anything protruding out of the cloaca?

-Nostrils: Is there anything (blood or mucus) leaking out of them?

-Mouth: the mucus membranes in the mouth should be homogenous. Colors like red splotches or yellow-brown raised areas are abnormal. Note any ulcers, cuts, plaques, growths, hooks, fishing line, blood, spots or lumps in the oral cavity. Also note the presence of algae in the mouth and collect samples in a separate small vial of formalin.

-Eyes: Are the eyes collapsed, cloudy, weepy? Are there abnormal warty growths around the eyes?

-Flippers: Are there abnormal warty growths on the skin? Are the flippers intact? Is there fishing line wound around or hook embedded in the flipper?

-Any other abnormality: lumps, bumps or exudates in unusual places.

MEASURING TURTLES

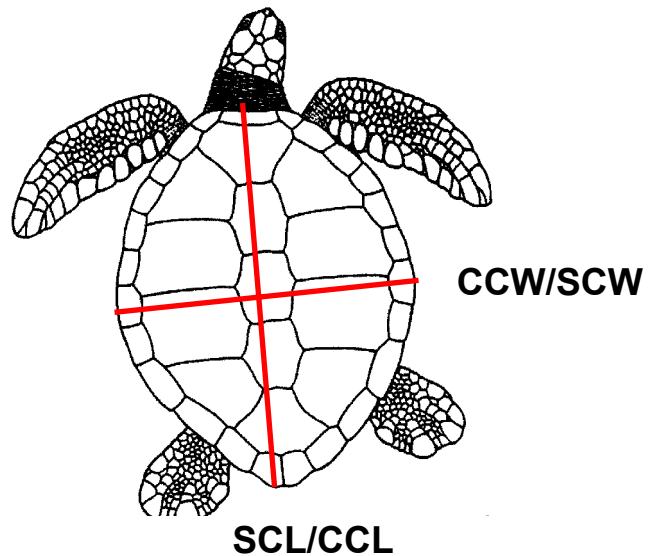
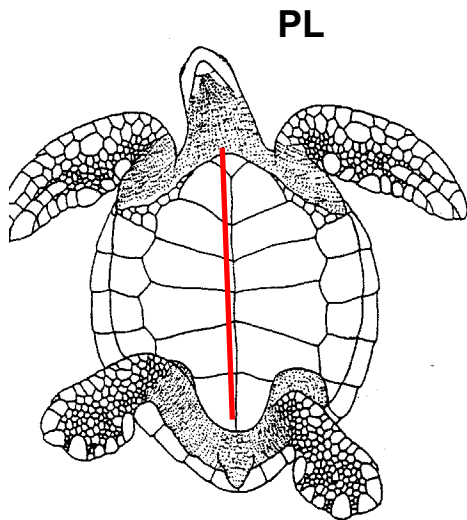
To properly measure a turtle, you need both a tape measure and calipers, and all measurements should be in centimeters. While many measurements can be taken on a turtle, a select few are critical. The diagram below illustrates how these should be taken.

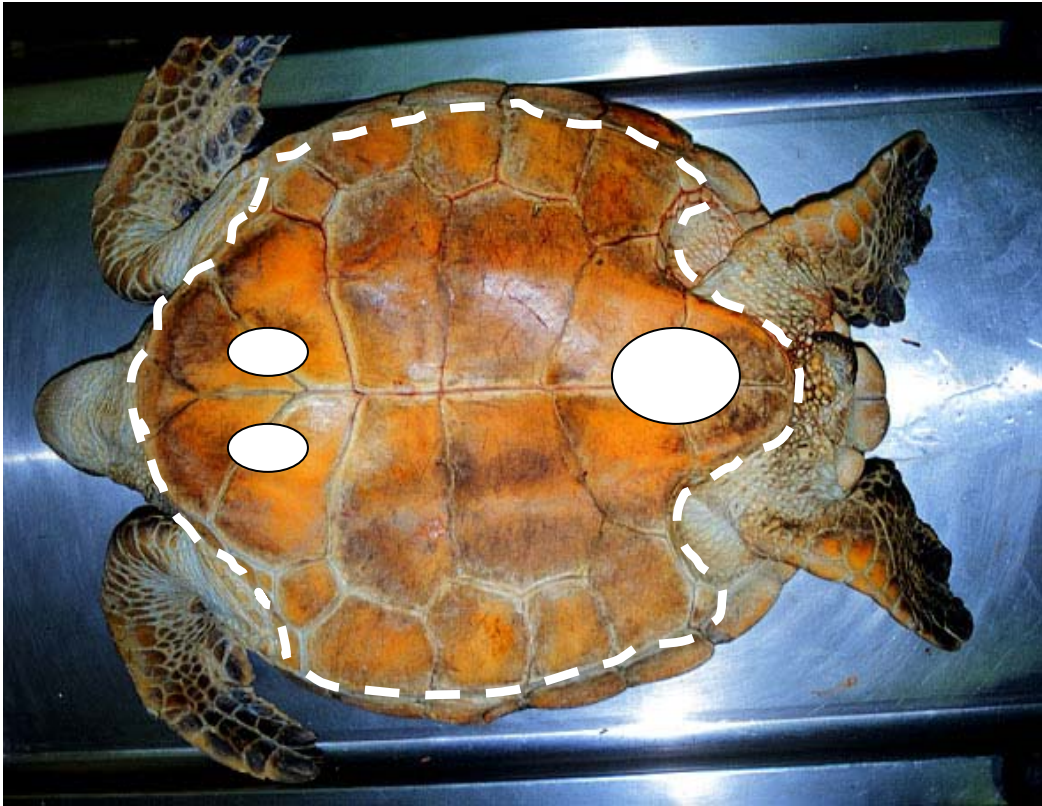
Calipers:

Straight carapace length (SCL)
 Straight carapace width (SCW)
 Plastron length (PL)

Tape measure

Curved carapace length (CCL)
 Curved carapace width (CCW)





Prior to starting the necropsy, Place the turtle on its back. Using a sharp knife or scalpel blade, cut along the dotted line (see photo). If you cut between the carapace and plastron, you should encounter only cartilage which can be cut with a knife. Pull the plastron away from the carapace as you cut skeletal muscle attachments.

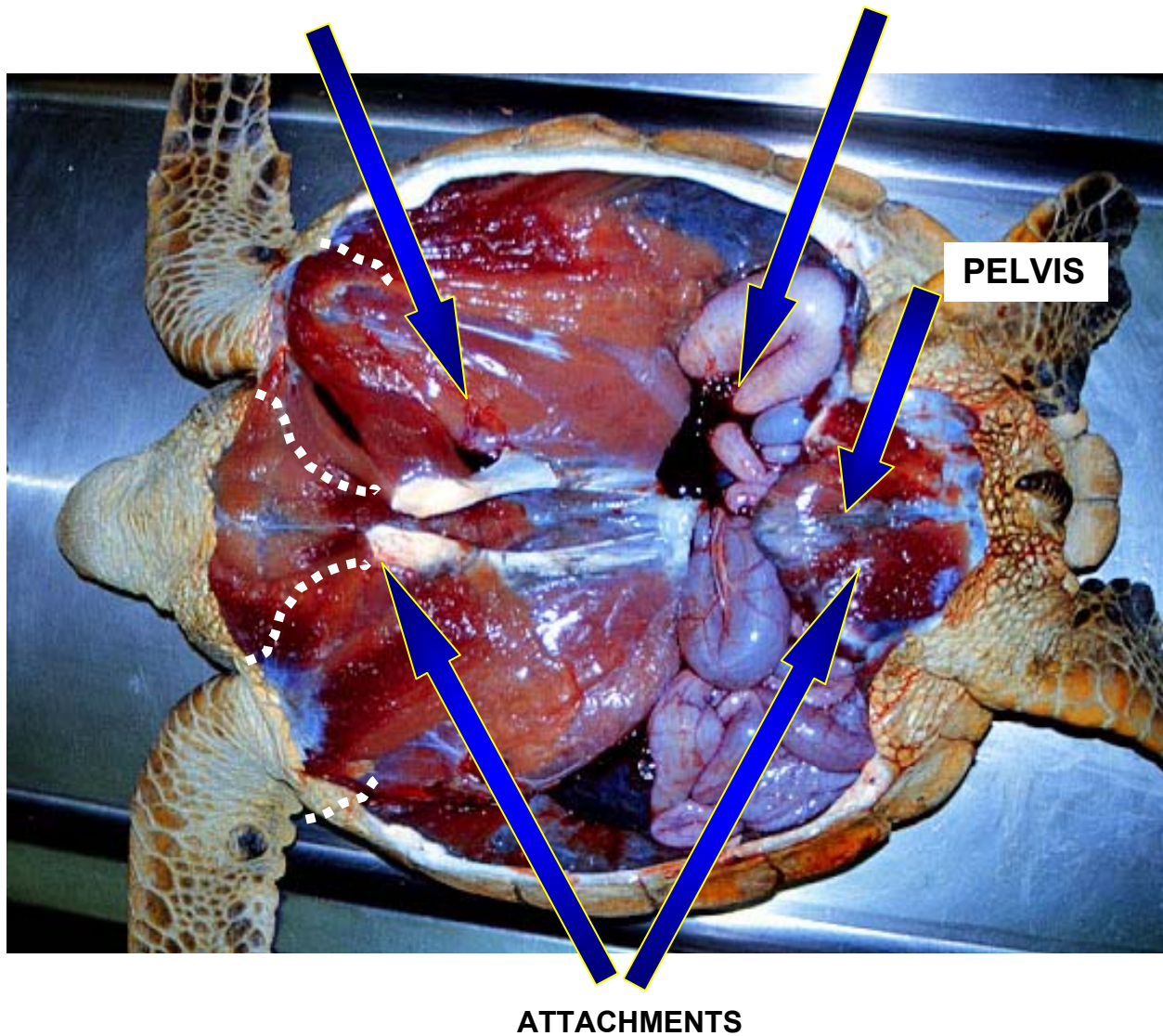
The white circles indicate areas where clavicles (forward) or pelvis (rear) attach to the plastron. These can be detached from the plastron by cutting the ligaments and cartilage close to the inside of the plastron.



Beware that in very emaciated turtles, dagger-sharp bones may protrude through the plastron.

PECTORAL MUSCLE

INTESTINE



Once the plastron is removed, you should see the pectoral muscles and intestines. Note that the pectoral muscles (the “engine” of the turtle) take up a large proportion of the body (coelomic) cavity. Note also attachments of clavicles and pelvis to plastron.

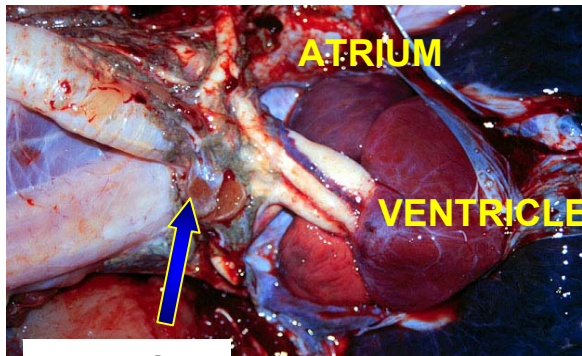


Remove the pectoral muscles and foreflippers by cutting the muscle around the flippers (dotted line, p 12) and twisting the flippers off from their attachment to the carapace. This will reveal the organs in the following page.



INTESTINES: These should be smooth and homogenous tan. In most turtles, they will be filled with algae (note it if not).

HEART: Unlike us, turtles have a three chambered heart (one ventricle, 2 atria).



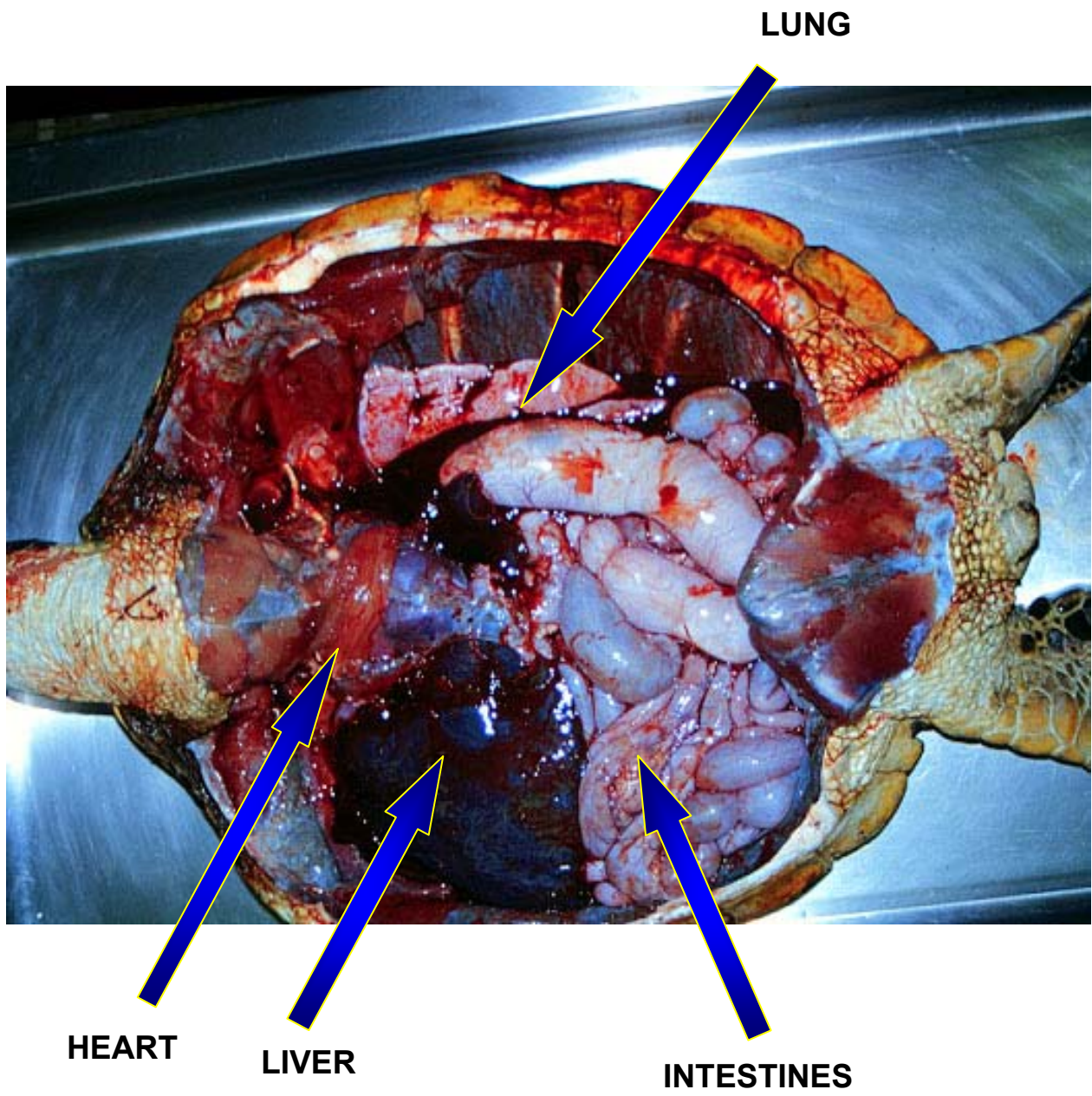
The picture below shows the heart in situ with white arteries leading out of the ventricle. If you're careful, you can see the **thyroid** gland near the heart. This is a translucent spherical organ. The heart should be firm and homogenous dark red-pink and the external and internal surface should be smooth.

Abnormalities: Tumors, pale spots on the heart muscle, a rough sandpaper like surface on the exterior or interior, semiliquid fat on the heart.



LIVER: This organ should be firm, smooth with rounded borders and homogenous dark purple brown. Like us, turtles have a bile-filled gallbladder. The consistency and texture of the liver should be homogenous on cut surface.

Abnormalities: Nodules, tumors, rough exterior, shriveled surface, discoloration in the form of spots or large pale areas.





Remove the heart and liver. You should also cut the skin on the midline of the ventral neck to expose the esophagus and trachea (windpipe). Once you have done this, your turtle should look something like the facing picture.



TRACHEA: It should be tan and have a smooth lumen on cut surface. The trachea bifurcates into two bronchi.

Abnormalities: Froth, blood, or food material in lumen, rough surface in lumen, tumors in opening of trachea (glottis).



ESOPHAGUS: The soft tubular organ next to the trachea. The lumen mucosa contains numerous large spines...this is normal in a sea turtle (Photo). This contrast with the smooth surface of the stomach mucosa. The external and internal surface should be smooth and tan.



Abnormalities: Hooks or fishing line embedded in mucosa.



CROP: Note in the facing photo how the esophagus dives between the bronchi and becomes the crop. The crop is a pouch that stores food before it goes into the stomach. Crops in green turtles are found only in individuals from Hawaii.

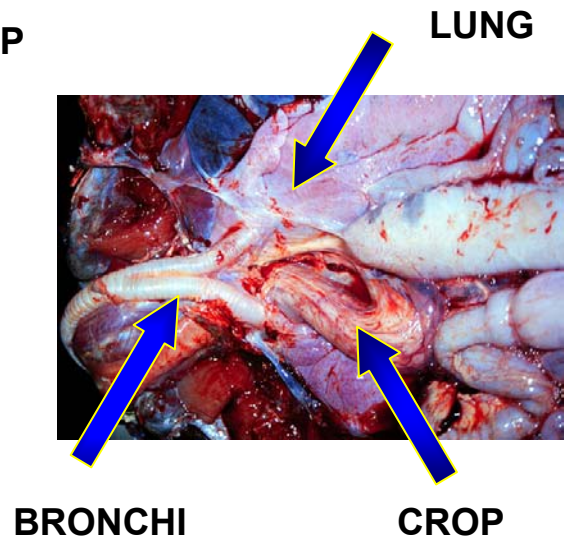
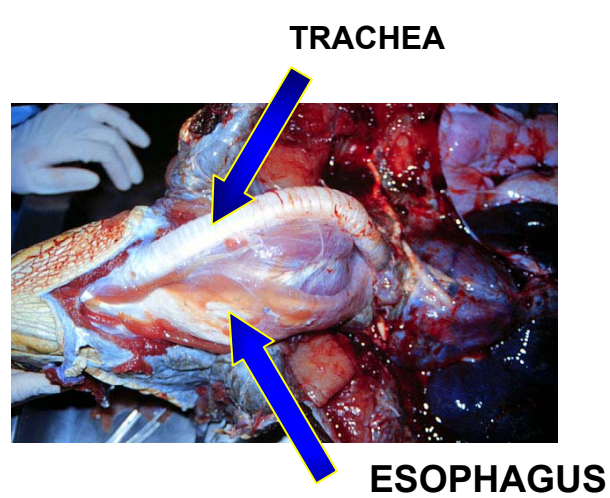
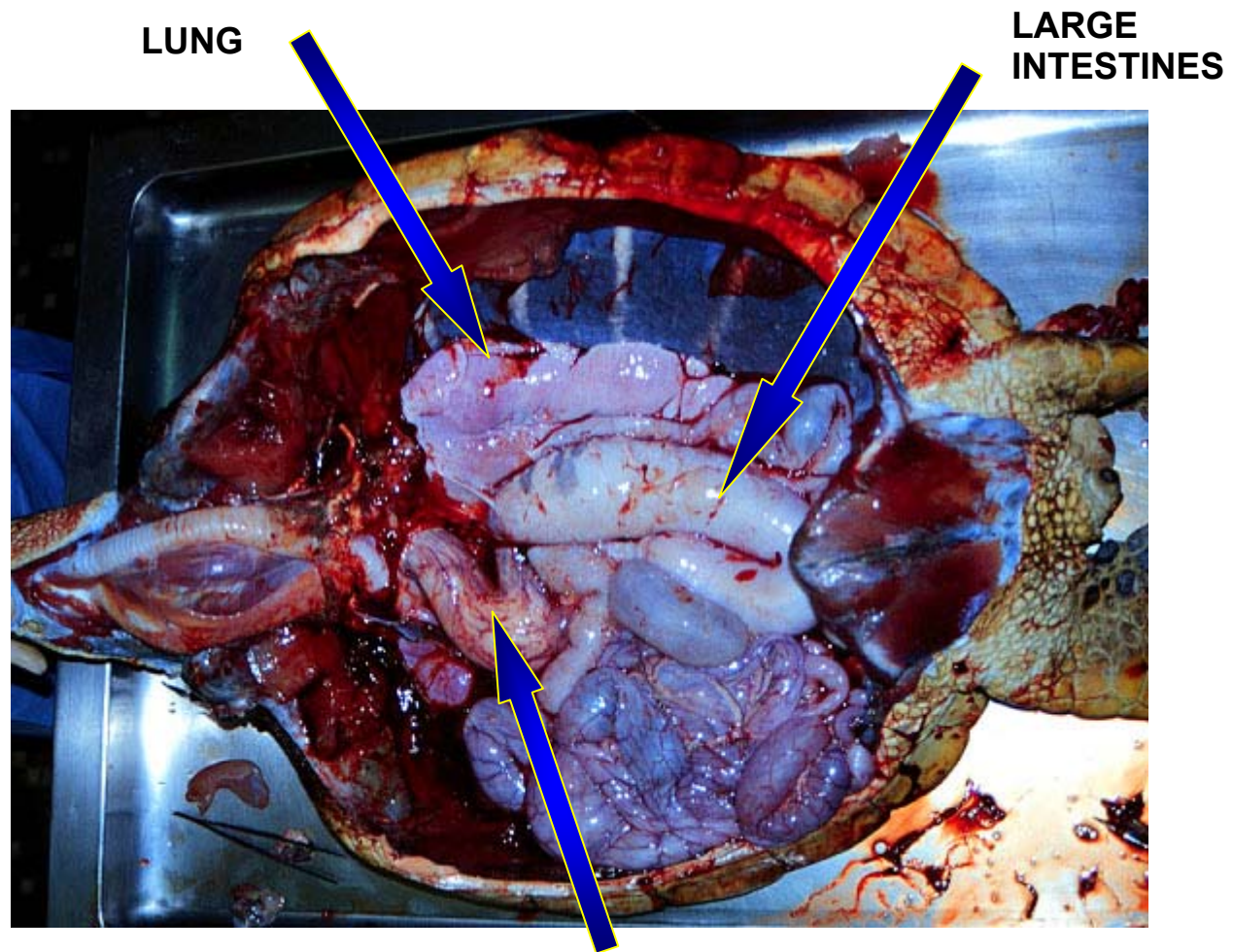
It should be full of algae and the mucosa should be tan.

Abnormalities: Rough sandpaper-like consistency of mucosa, hooks or fishing line embedded in mucosa.

This is a good time to take samples for food habit studies



LUNG and LARGE INTESTINES: Those two organs should also now be visible. Normally, the large intestines contain large amounts of macerated vegetation (for herbivorous turtles)





GASTROINTESTINAL TRACT: This is the entire gastrointestinal tract laid out from mouth to cloaca. The following organs should be visible:

HYOID APPARATUS-This is the same as your Adam's apple. Underneath (dorsally) is the glottis which is the opening to the trachea.

ESOPHAGUS: mentioned earlier

CROP: Mentioned earlier

STOMACH: The mucosa will generally have smooth ridges.



SMALL INTESTINES: Note that they have been cut and the contents emptied. Hence the reason they look smaller than they did in previous pictures. In many turtles, the mucosa will have a "honeycomb" like appearance.



LARGE INTESTINES: Note that they also have been emptied of contents. The mucosa of the large intestines is smooth and tan.

Abnormalities in GI tract: Hooks, fishing line, or other foreign matter in lumen, blood in mucosa, rough sandpaper consistency in mucosa, parasites (worms).



SPLEEN: This round organ is part of the turtles immune system. It is usually firm, smooth and tan-pink and closely associated with the pancreas. The spleen can be found near the small intestines as they exist from the stomach.

Abnormalities: Tumors, pale areas, dark spots, sandpaper-like surface.

HYOID

ESOPHAGUS

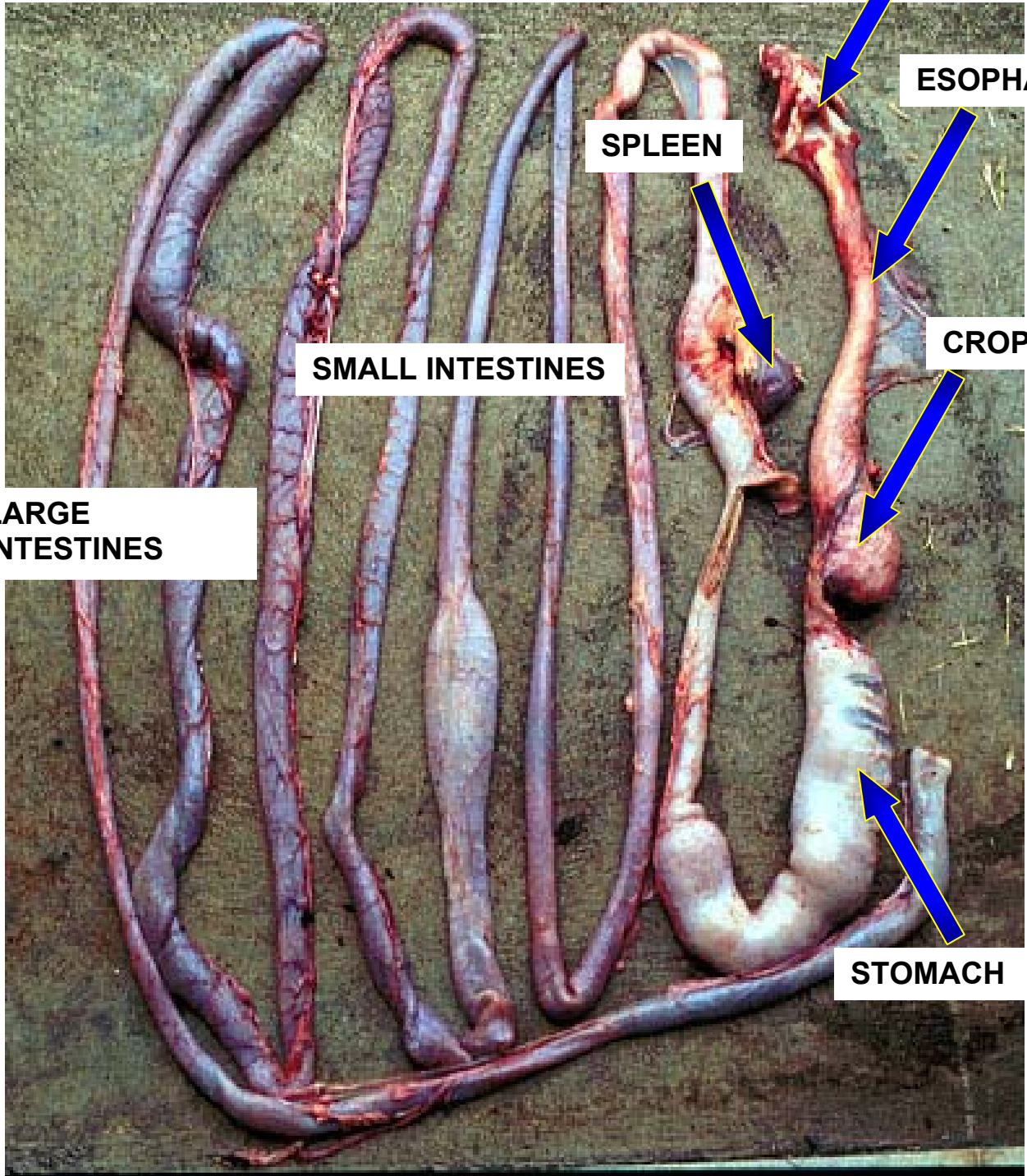
SPLEEN

CROP

SMALL INTESTINES

LARGE
INTESTINES

STOMACH





Once you have removed the gastrointestinal tract, you should be left with the something like the facing picture:



LUNGS: These should have a spongy consistency and be smooth and homogenous pink on surface and cut surface.

Abnormalities: tumors, nodules, large areas of discoloration, dense consistency, large amounts of frothy blood exiting from small airways on cut surface.



DESCENDING AORTA: this is like your aorta except that turtles have two of them. They should be smooth and homogenous tan to white.

Abnormalities: Nodules, rough sandpaper like surface on lumen.



BLADDER: This is a thick walled sack that holds urine and that is located just above the large intestines and under the pelvis. The bladder may contain clear yellow urine that may have white flecks (mucus) in it. The mucosa will appear wrinkled and may have dark pigmentation.

Abnormalities: Parasites in lumen.



KIDNEYS: These are hidden under the carapace just behind the lungs and under the pelvis (outlined in yellow in photo). They should be firm and homogenous brown with a rough nodular surface.

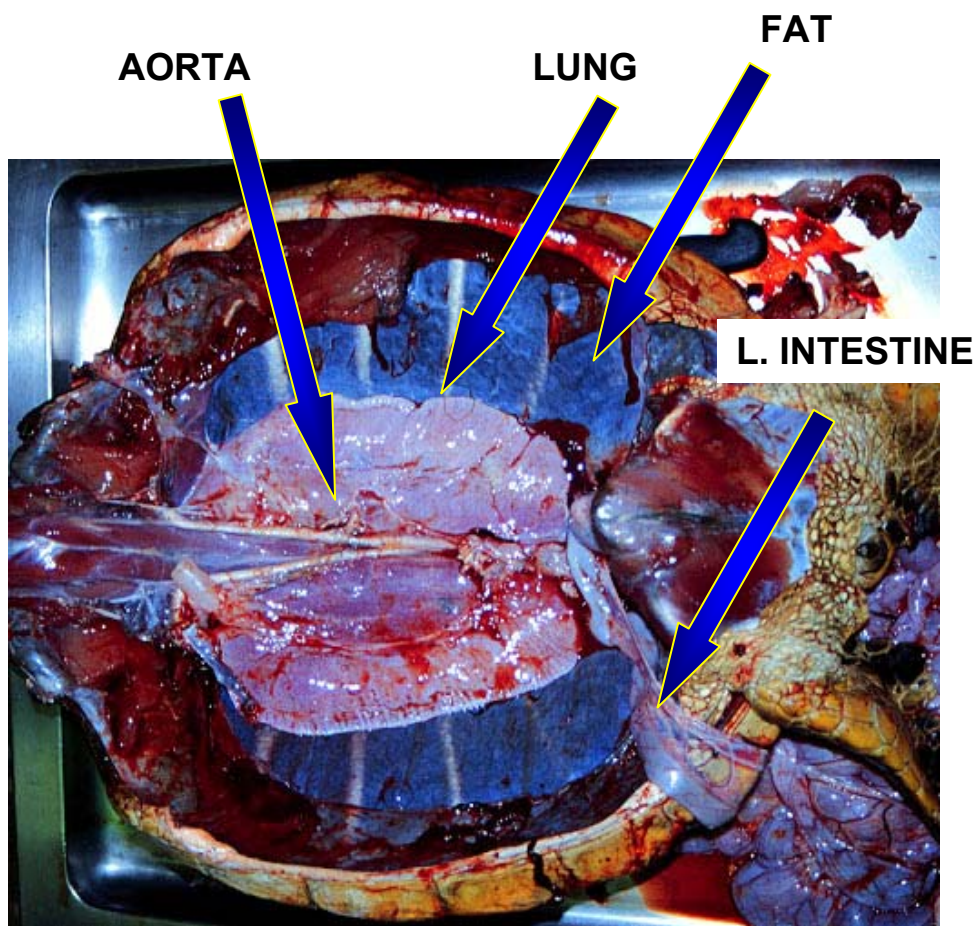
Abnormalities: Large pale round firm white tumors.



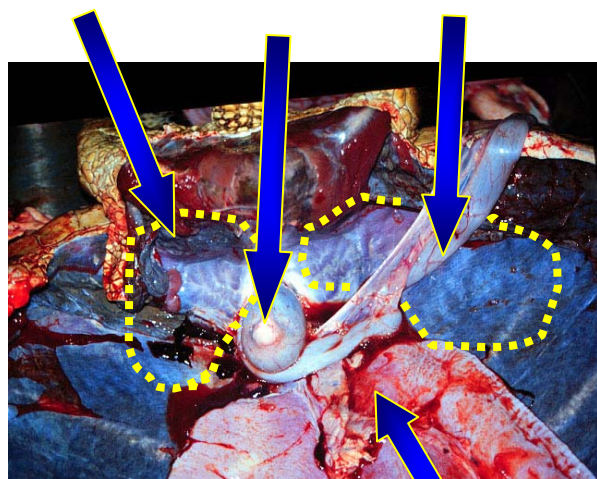
GONADS: These lie just above the kidneys. Adults are easy to differentiate, however, in immatures, this is harder. MALES have a smooth tan gonad. FEMALE gonads look like small clusters of grapes.



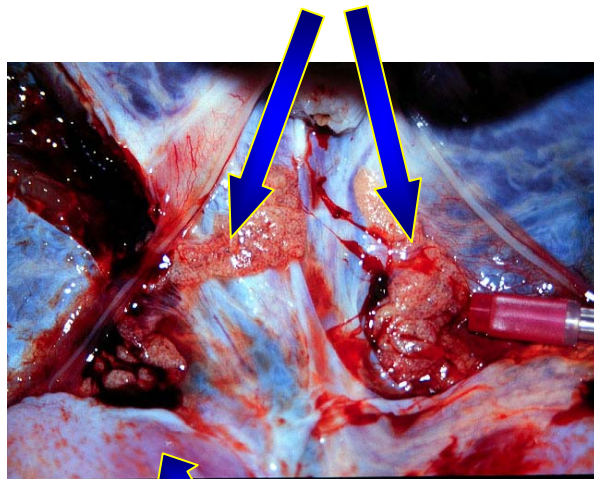
FAT: This is also a good time to check fat reserves. Turtles in good body condition have a nice layer of firm green to tan fat under the shell. This fat is jelly-like and watery in thin turtles.



KIDNEY **BLADDER** **L. INTESTINE**

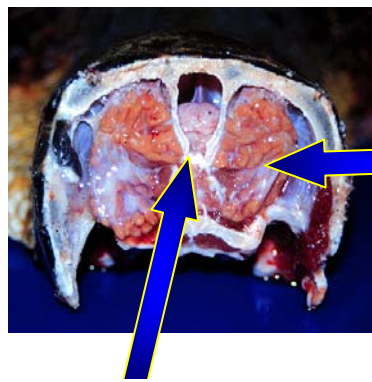


GONADS



LUNG

LUNG



SALT GLAND

BRAIN



The last part of the necropsy involves removing the brain and salt gland. To do so, simply saw the skull on the dotted line and you will see something like the picture above.



BRAIN: This organ should be firm and homogenous tan. You will note that it is rather small relative to the size of the head.



SALT GLAND: These are important for osmoregulation. They are firm, lobular and pink to light brown.

Abnormalities: Pale spots, gritty textures.

WHEN YOU ARE DONE WITH A NECROPSY, ENSURE THAT:

- 1) All samples and jars are labeled with a unique number referring to the animal along with date of collection. All organs collected (see checklist p. 23).
- 2) All information on the necropsy record sheet is complete (see form pp. 24).
- 3) All "dirty" gloves and other material are disposed of properly. Any sharp items such as scalpel blades and needles should be disposed of in a rigid, sealable puncture proof container (i.e. a plastic jug).
- 4) Any used formalin is to be stored in sealed containers labeled with the following words: "WARNING: FORMALDEHYDE: HANDLE WITH GLOVES" and disposed of appropriately.

TWO RECIPES FOR 10% FORMALIN

RECIPE 1

If you have graduated cylinders and scale mix the following:

Na ₂ HPO ₄ (Sodium phosphate dibasic)	6.5 g
NaH ₂ PO ₄ .H ₂ O (Sodium phosphate monobasic)	4.0 g
Fresh water	900 ml
37% formaldehyde	100 ml

RECIPE 2

If you have no scales or measuring apparatus

37% formaldehyde	150 ml or 15 parts
Seawater	850 ml or 85 parts

WHEN PREPARING FORMALIN, USE GLOVES AND WORK IN A WELL VENTILATED AREA

CHECKLIST OF ORGANS YOU SHOULD HAVE TAKEN IN FORMALIN

Plain numbers indicate where organ is mentioned in text.

Bold numbers indicate figures in which organs are labeled.

ORGAN	PAGE(S)
TRACHEA	15, 16
ESOPHAGUS	15, 16, 17, 18
MUSCLE	12
LIVER	13, 14
HEART	13, 14
THYROID	13
CROP	15, 16, 17, 18
SPLEEN	17, 18
STOMACH	17, 18
SMALL INTESTINE	17, 18
LARGE INTESTINES	15, 16, 17, 18, 20
LUNGS	15, 16, 17, 18, 20
KIDNEYS/BLADDER	19, 20
GONADS	19, 20
BRAIN/SALT GLAND	21

NECROPSY DATA SHEET
(all measurements are metric)

Species _____ ID# _____ Date Collected _____ Date _____
 Necropsied _____
mmddy mmddy

Collection site _____

History: _____ SEX (M/F/U) AGE: _____

Weight _____ (kg) SCL ____ CCL _____ PL ____ SCW ____ CCW _____

(Circle most appropriate term(s)). Add notes as you see fit.

BODY CONDITION: (Good, fair, poor)

POST-MORTEM CONDITION: (Fresh dead, ~1 day old, >2 days old)

EXTERNAL EXAM (Skin, carapace, eyes, nostrils, cloaca)

MUSCULOSKELETAL: (*Pectoral muscle atrophy*-None, moderate, severe; *Fat*: firm, soft, jelly-like; *body cavity*-Lots of fluid, small amounts of fluid, no fluid)

LIVER: (*Surface*: smooth, rough, granular, wrinkled; *Consistency*: firm, friable; *Color*: homogenous/mottled, red, black, brown, purple, tan, yellow.)

HEART: (*Surface*: smooth, rough, granular, wrinkled; *Consistency*: firm, friable; *Color*: homogenous/mottled, red, black, brown, purple, tan, yellow.)

LUNGS: (*Surface*: smooth, rough, granular, wrinkled; *Consistency*: firm, friable, spongy; *Color*: homogenous/mottled, pink, tan, yellow, grey, red, brown; **TRACHEA-Lumen**: smooth, rough; *Color*: homogenous/Mottled, tan, white, red, brown, green, pink.)

SPLEEN: (*Surface*: smooth, rough, granular, wrinkled; *Consistency*: firm, soft; *Color*: homogenous/mottled, brown, tan, red, black, brown, yellow.)

KIDNEY: (*Surface*: smooth, rough; *Consistency*: firm, soft; *Color*: homogenous/mottled, brown, tan, red, black, brown, yellow.)

GONAD: (*Surface*: smooth, rough; *Consistency*: firm, friable; *Color*: homogenous/mottled, red, black, brown, purple, tan, yellow.)

THYROID: (*Surface:* smooth, rough; *Consistency:* firm, friable; *Color:* Translucent/mottled, orange, red, tan, yellow.)

ORAL: (*Mucosa:* smooth, rough, granular, pitted; *Color:* homogenous/mottled, pink, tan, yellow, grey, red, brown); Contents?

ESOPHAGUS-*Mucosa:* smooth, rough; *Color:* homogenous/Mottled, tan, white, red, pink.) Contents?

CROP: (*Mucosa:* smooth, rough; *Color:* homogenous/mottled, tan, red, yellow, black, brown,) Contents?

STOMACH: (*Mucosa:* smooth, rough; *Color:* homogenous/mottled, tan, red, yellow, black, brown) Contents?

SMALL INTESTINES: (*Mucosa:* smooth, rough; *Color:* homogenous/mottled, tan, red, yellow, black, brown) Contents?

LARGE INTESTINES: (*Mucosa:* smooth, rough; *Color:* homogenous/mottled, tan, red, yellow, black, brown) Contents

BLADDER: (*Mucosa:* smooth, rough; *Color:* homogenous/mottled, tan, red, yellow, black, brown) Contents

BRAIN: (*Surface:* smooth, rough; *Consistency:* firm, friable; *Color:* Homogenous/mottled, tan, red)

SALT GLAND: (*Surface:* smooth, rough; *Consistency:* firm, friable; *Color:* Homogenous/mottled, brown, pink, tan, orange)

SAMPLES:

Formalin: _____

Frozen: _____

Other: _____