STANDARD PROTOCOL FOR POST-MORTEM EXAMINATION ON SEA TURTLES

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PREFACE

Sea turtles are marine reptiles with peculiar anatomy, physiology and habits. Considered "threatened" by the IUCN, a lot is still unknown about these animals. Fortunately, in the last few years an increasing number of research facilities have developed growing interest in these animals, but still very little bibliography is available for a systematic data collection.

Some protocols have been developed for the dissection of sea turtles, but clear schemes for sample collection are lacking even now.

Drawing inspiration from similar protocols in other endangered wild species, this protocol is a mix of previous procedures and our experience, providing a practical approach for the conduction of routine data and sample collection. Furthermore, this protocol is designed to improve knowledge about diseases of sea turtles in the wild by providing guidelines to complete more comprehensive necropsies and disease testing.

Using standardized necropsy protocols is important to facilitate comparison of data among stocks or population. Screening for specific pathogens (i.e., Herpesvirus and *Mycobacterium chelonii*) is of increasing importance for assessing population health and the presence of potential zoonoses. In some cases, negative results are as meaningful as positive ones.

It is our hope that once people on the Adriatic Sea realize the need to learn more about diseases of sea turtles, this protocol will also increase available knowledge through the execution of complete postmortem necropsies.

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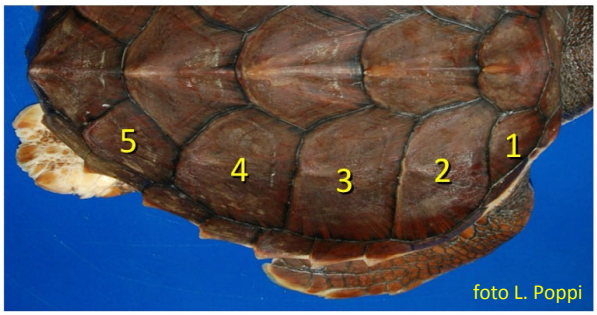
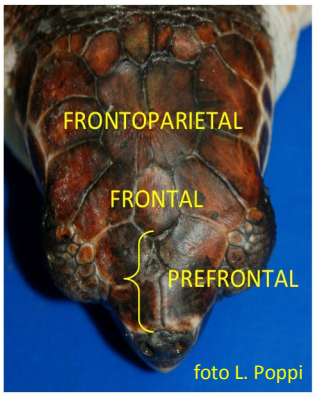
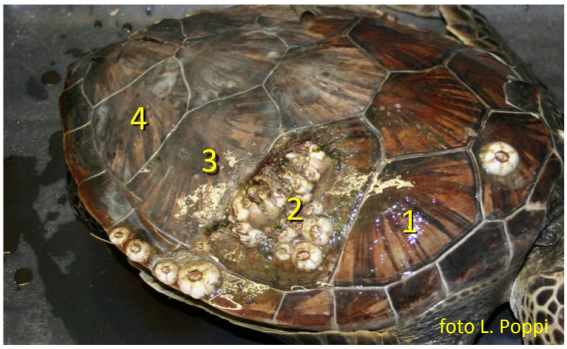
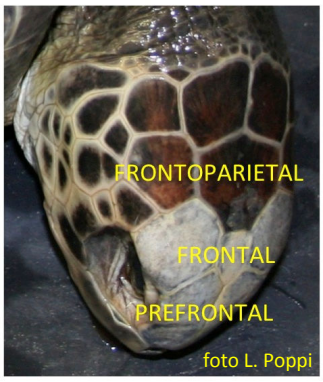
1. EXTERNAL EXAMINATION

1.1 Signalment

1.1a - **Species Identification:** there are several elements that identify the species of a sea turtle, but the most efficient are the number and disposition of costal scutes and prefrontal scales. Subjective variations in these patterns are possible.
Caretta caretta: 5 lateral scutes, 2 pairs of prefrontal scales,
Chelonia mydas: 4 lateral scutes, 1 pair of prefrontal scales
Dermochelys coriacea: no scutes - 5 dorsal ridges, no scales

Caretta caretta		
Chelonia mydas		
Dermochelys coriacea		

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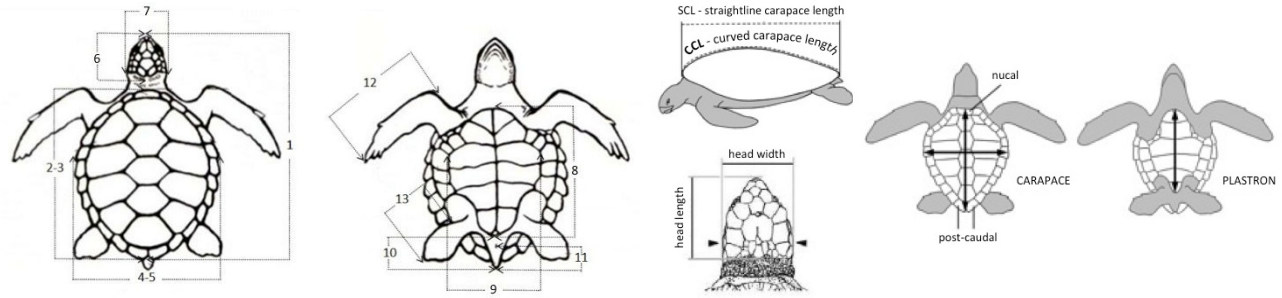
1.1b - **Collection of Morphometric Data:** different scheme are proposed from different scientific groups. The most important are: weight, curved carapace length (CCL), head length and width (HL and HW), total tail length (TTL), and vent-tip length (VTL). For measurements, use scales, measuring tape and/or callipers. See Annex I.



1.1c - Sex Determination: to determine the sex of a sea turtle is not easy, because adult females differ little in external morphology from large, immature males.
Typically, females have a short tail and the cloacal opening (vent) is located roughly half way between the tip of the tail and the plastron's anal scute. Within the cloaca, the genital papilla remains small as the clitoris on the floor of the cloaca. Adult males are characterized by a long tail with the cloacal opening near the tip, and strongly curved claws on the second digit.
In younger animals (< 60 cm CCL), the sex of the animal may be known for certain only during the necropsy trough accurate examination of the gonads. However, in hatchling and very young subjects (< 30 cm CCL) it may be very difficult to establish if gonads are ovaries or testicles due to the underdevelopment of the reproductive system and the temperature-dependent sex determination. In hatchlings, histologic analysis will help.
Regarding sex determination, species differences are not noteworthy.

1.2 Condition code.

Before initiating the necropsy, carcass condition must be determined. Preferably, necropsies are performed on fresher carcass (within 48 hours of death); however, environmental conditions can greatly impact condition code.
If human interaction is suspected or forensic data are of value, necropsies should be performed irrespective of tissue quality. Carcasses are classified in one of five code categories depending on the level of decomposition.

Code 0: Alive or just died (< 2 hours post mortem).
Code 1: Fresh carcass (< 24 hours post mortem). Normal appearance, usually with little scavenger damage, fresh smell, minimal eyes drying, eyes clear, carcass not bloated.
Code 2: Moderate decomposition. Bloated carcass with characteristic mild odour. Head: integral or with partial loss of skin; eye: sunken or liquefied; tail: present or absent; limbs: integral; carcase and plastron: integral.

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Code 3: Advanced decomposition. Collapsed carcass with strong odour. Head: complete loss of skin; eye: liquefied tail absent; limbs: partially exposed skeleton; carapace and plastron: partial or total loss of skin.
Subgroup "a": distinguishable internal organs.
Subgroup "b": liquefied internal organs.

Code 4: Mummified carcass or partial carcass. Incomplete carcass; skull: visible; carapace: broken with separation of parts.

Code 1

Code 2

Code 3

Code 4

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1.3 Nutritional condition (1).

The body condition of a sea turtle is not easy to asses. A good idea of the body condition can be obtained by looking at the roundness of the neck, the depression of the eyes and of the axillary and inguinal regions. Nevertheless, in a fresh carcass (condition code 0 and 1), sunken eyes and neck are also signs of a dehydrated animal, and flaccid axillary area may be considered muscle cachexia. Furthermore, in condition code 2 axillary and inguinal areas up to the tail are severely bloated, so can be impossible to establish if there are fat o muscles in these positions.
Then, the correct nutritional code is assignable only by looking the ventral fat deposition after plastron removal. Also in this case, very important to keep in mind that fat deposition is strongly

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dependent on sex, size, season and reproductive stage.

1.4 Integument.

The first note to take from an external examination is the presence of injuries, scars, deep parallel cuts or absence of scutes on the carapace or plastron. Equally important is to note the number, type, size and disposition of (if present) ectoparasites (barnacles, leeches) and algae on the carapace, plastron, visible skin and mucosae. Loss of some scutes is normal in condition code 3 and 4. The examination should include the investigation and description of the eyes, mouth, vent, and skin.
When examining the eyes, look for discolouration, injuries, or discharge, and for ectoparasites on or near the eyelids.
Document any lesions in the mouth or of the tongue: fracture of the jaw, cuts (especially if linear and deep), and foreign bodies. Pay attention to the normal detachment of the rhamphotheca in condition code 4 and sometimes 3.
Look for parasites and discharge around the vent. Obtain samples of abnormalities for histology, microbiology and molecular investigations. Sample parasites for parassitology diagnosis.
Thoroughly examine and document any scars, ulcerations, erosions, wounds on the carapace and plastron, especially if regular in shape and in disposition (linear, parallel..). Make note of the size (length x width x depth/height), shape, colour, texture, location and distribution of all abnormalities.
Remove about 30 g of scutal cheratin from the carapace for genetic analysis (frozen and DMSO) and small pieces of skin with lesions for histology. Be sure that all morphometric measurements have been completed before collecting these samples.

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2. INTERNAL EXAMINATION

2.1 Plastron removal.

To perform a sea turtle necropsy, plastron removal is the first step.
With the carcass placed on its back (if necessary, stabilize the carcass with paper, cloth, beach towel or make a hole in the sand), cut with a scalpel all around the plastron. Pay attention to the direction and the depth of the cut, particularly between marginal and inframarginal scutes. In this position the coelomic cavity is particularly close (especially in summer, when there is less fat), whilst under the cranial and caudal part of the plastron, the subcutis and muscles are present between the plastron and body cavity. Pulling the plastron upwards cut the underlying muscle (anterior part of the ventral muscles - *pectoralis major*) with cuts parallel to the plastron in a cranio-caudal direction. At approximately a quarter of the plastron length (more or less central where the gular scutes meet the humeral scutes) note the white connective strong connection of the plastron with the acromion processes of the scapula. Cut it as close as possible to the plastron to avoid damaging the pericardial sac and/or the heart. Regarding the second half of the plastron, a large portion of the underlying surface is covered by muscle (caudal part of the ventral muscles - *rectus abdominis*). As before, continue cutting close to the plastron: in the central part of this area, usually fat deposition is found between the plastron and underlying peritoneum, but for cachectic animals or during the summer this could be very thin.


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Once the plastron has been removed a surface covered by muscles and fat is observable. The muscles are the large pinnate (feather-shaped) “chest” muscles used for swimming, and the fan-shaped pelvic muscles that were attached to the plastron. Fat is usually partially liquefied, shiny, perfuse and with holes; the colour is olive-green or brownish-orange, depending on the type and percentage of minerals inside, resultant from feed. Fat covers entire ventral area.
During the execution of all these procedures, development, thickness, texture and colour of muscles and thickness and colour of fat deposition must be noted, as well as presence of haemorrhages, haematomas, oedema or other focal/multifocal lesions over the entire field.
Obtain muscle samples for histology and contaminants and fat samples for toxicology and fat composition analyses.

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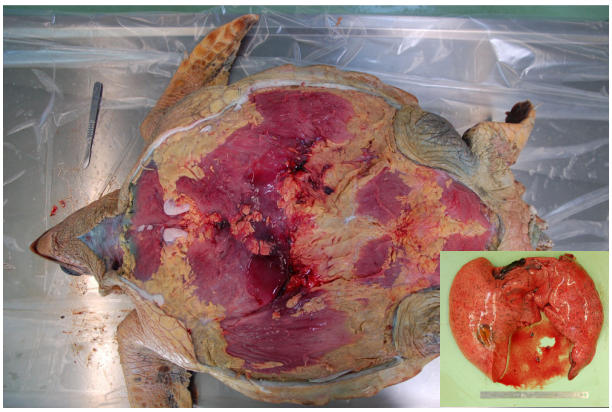
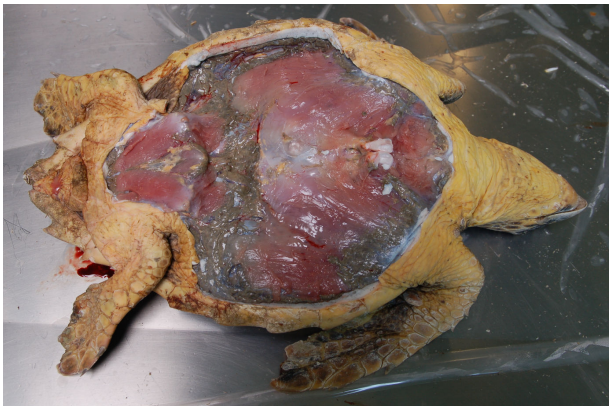
2.2 Nutritional condition score (2).

Once the plastron is removed, it is possible to assign the correct nutritional condition score. It is very important to keep in mind that fat deposition (localization, thickness and colour) is strongly dependent on sex, size, season (feed, water temperature, time spent in feed activities) and reproductive stage.

Score 1 - Excellent: Integral or partially liquefied adipose tissue covers entire ventral area. Hepatic lipidosis is also present.
Score 2 - Very good: Integral or partially liquefied adipose tissue covers entire ventral area.
Score 3 - Fair: Integral or partially liquefied adipose tissue covers peripheral parts of ventral area.
Score 4 - Scarce: Integral or partially liquefied adipose tissue is present only in limited peripheral parts of ventral area.
Score 5 - Not valuable: Mummified carcasses.

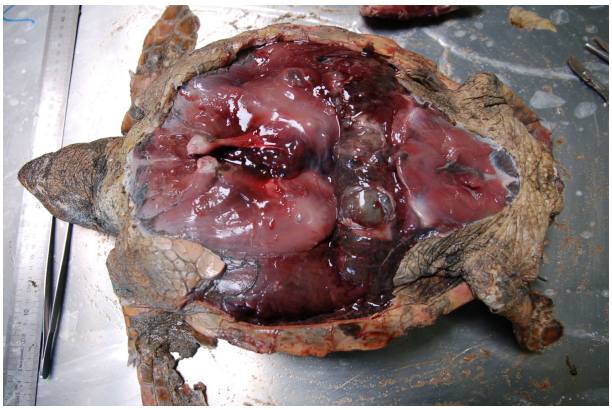


Score 1 (plus hepatic lipidosis)



Score 2

Score 3



Score 4

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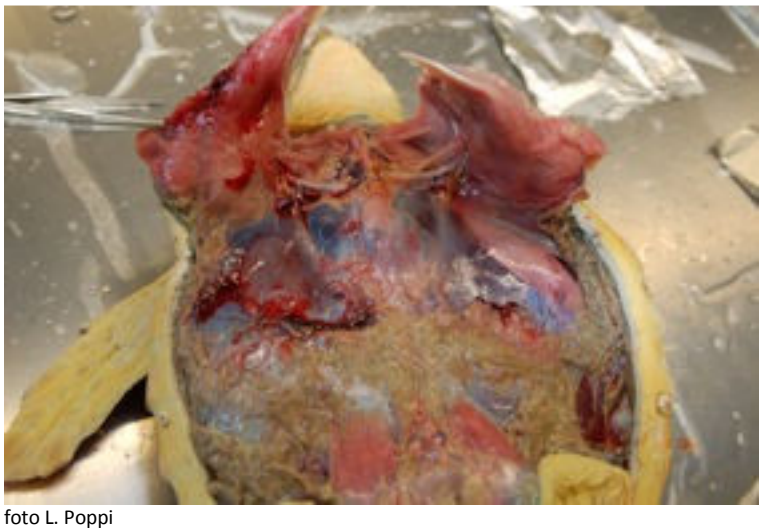
2.3 Removal of the Scapula and approaches to the viscera.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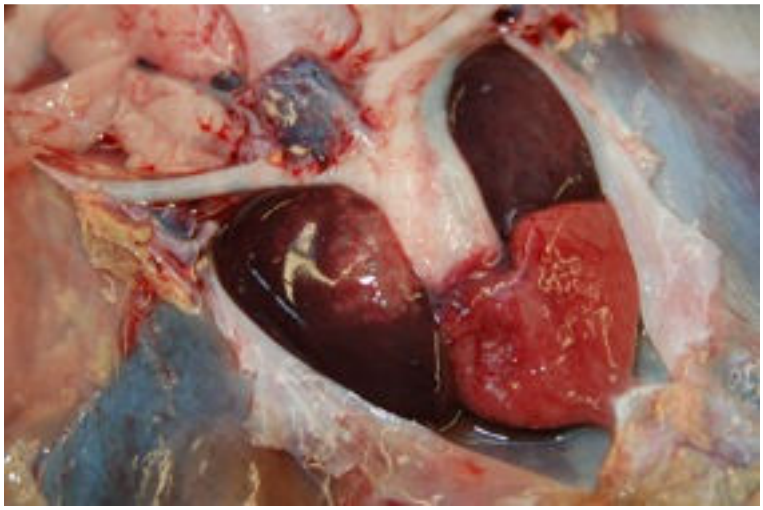
To uncase the viscera, perform a sagittal cut through the pectoral muscles, gently separating all the muscular masses including the scapula of both sides from beneath the pericardium and peritoneum, rotating the entire flippers anteriorly outwards. In this way, the brachial artery, axillary vein and brachial plexus are observable and by reversing the flippers under the carcass, it is definitively stabilized. Cut the peritoneum sagittally (evaluate the fat thickness if possible) and observe location, size and colour of the liver and gastrointestinal tract respectively and if there are exudates or fibrin depositions. Cut the pericardium and observe the heart and great vessels. Look for lesions and exudates and describe them (localisation, appearance and quantity). In any case, if fluids are present, measure them and make cytological smears from them.
For an overall view of the main organs, gently move the gastrointestinal tract to see the lungs, gonads, bladder and adrenal glands above it and identify retroperitoneal renal masses. The pancreas and spleen are observable along the intestine.

2.4 Heart and Great Vessels.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The heart, great vessels, thyroid gland and thymus are found in the space beneath the muscles located between the two acromion and the coracoid processes. Particularly the pericardium is very thin and near the muscular masses, so it will usually be damaged during the scapula removal.
Before handling the heart, observe and describe the pericardium. Note if there is excessive fluid and describe the characteristics. Also, note the thickness of the tissue. Trim away the pericardium and observe the epicardium (external surface of heart) *in situ*. Note

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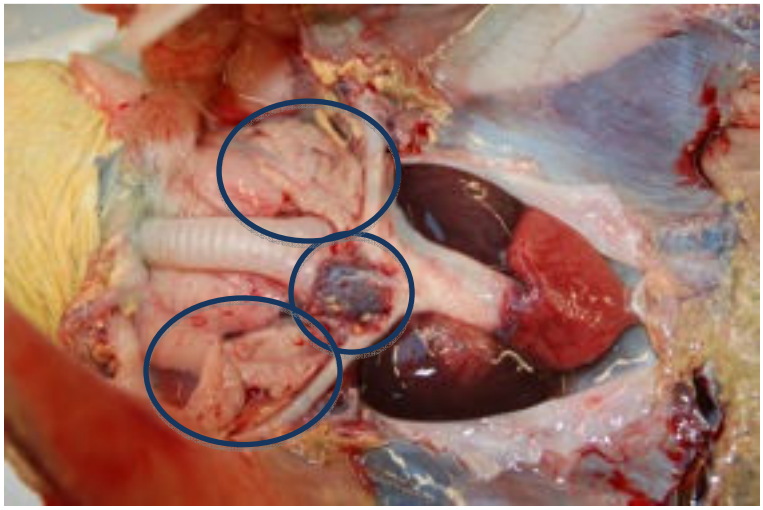
size, colour, and texture of each heart structure: *sinus venosus*, two large (right and left) atria and a ventricle, and the great vessels: two aortas (right and left) and a pulmonary trunk. The ventricle is attached to the pericardium via a fibrous connective tissue cord called the *gubernaculum cordis*. To remove the heart, it is necessary to cut the sinus venosus and the gubernaculum cordis. Whilst opening the heart chambers and the great vessels, examine them closely to look for parasites or lesions caused by them. Sample the ventricle, the atria and the aorta for histology.

2.5 Thyroid, Thymus, Parathyroid and Ultimobranchial Body.

Thyroid - The thyroid sits in the ventral neck, often coated with a thin layer of yellow fat, which masks it very well. In fresh carcasses is gelatinous, liquefied in other carcass conditions.



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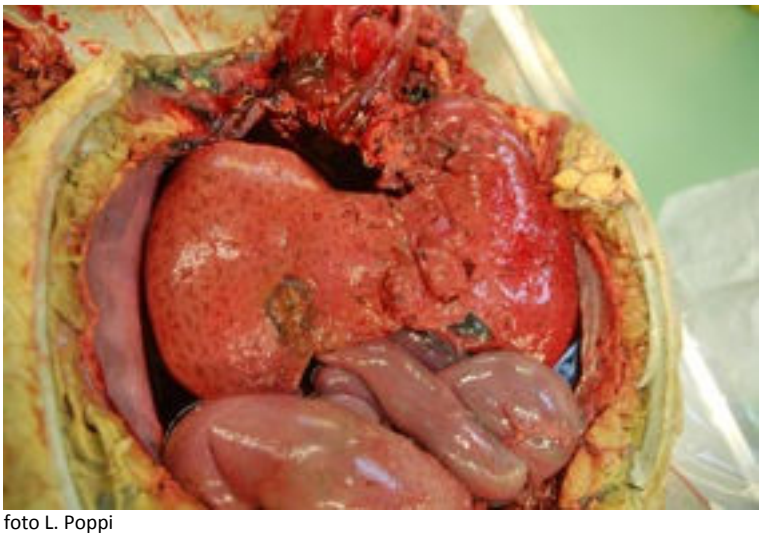
Note the size, shape, colour and texture. Sample for histology, microbiology and molecular investigations.

Thymus - The thymus is a large, lymphoid organ formed by two structures. In decomposed carcasses, it become more similar to the surrounding fat.

Note the size, shape, colour and texture. Sample for histology, microbiology and molecular investigations.

Parathyroid and Ultimobrachial body - These two glands are distinguishable only histologically. They are very difficult to identify even in a really fresh carcass. They are located along the carotid and ventral cervical arteries. If found, sample for histology.



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2.6 Liver and Gallbladder.

The liver is the largest visceral organ and surrounds the heart. In fresh carcasses and in some periods of the year it is not unusual to find the liver pale or orange and soft (severe lipidosis).

Examine the surfaces of the liver and note colour pattern, texture and size of the lobes. Examine the parenchyma of the liver and the gallbladder by performing several

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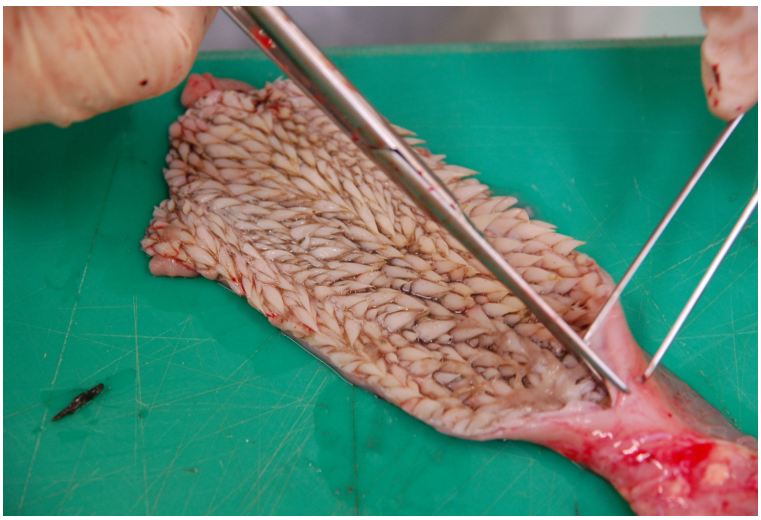
parallel cuts through the tissue. Again, note the internal colour and texture and look for parasites. Sample for contaminants, histology, microbiology and molecular investigations.

2.7 Oesophagus.

The oesophagus is located left of the trachea, then it passes through the tracheal bifurcation and it takes position above the lungs and under the heart and liver (with the carcass on its back).
To expose the oesophagus, cut the skin of the ventral neck from the position of the intergular scute (previously removed) to the jaw, separate the underlying muscles and move aside the trachea. Cut the oesophagus and let it slide through the tracheal bifurcation; then, draw it away with the entire gastro-intestinal tract (after tying the rectum). Using scissors, cut through the entire length of the oesophagus from the cranial part to the stomach; observe the serosal and mucosal surfaces of the oesophagus and the presence, quantity and type of feed and parasites.
Note, colour, texture and contents. Sample for histology.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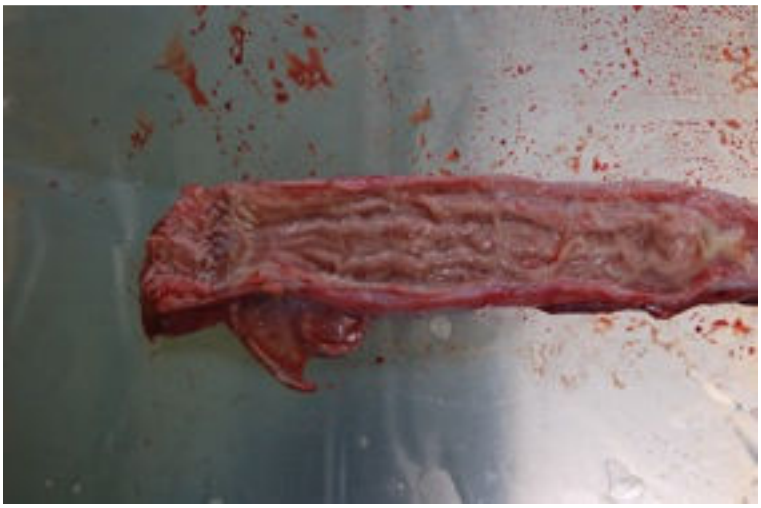
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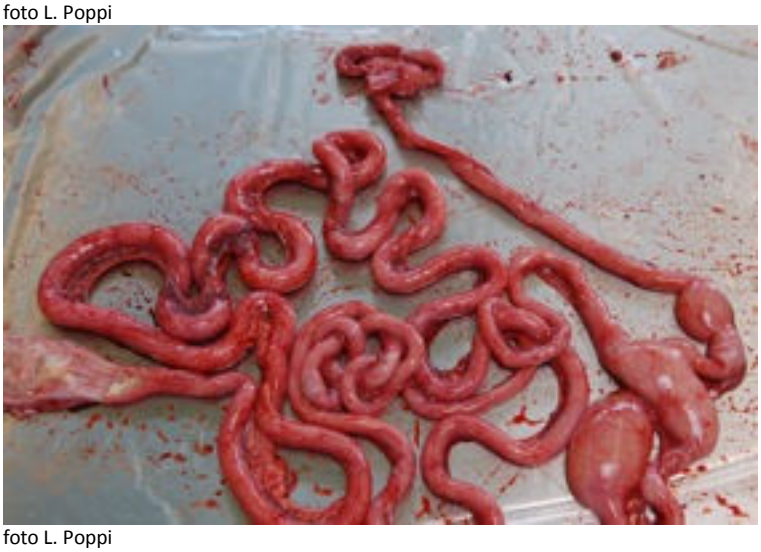
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2.8 Gastrointestinal tract.

After the oesophagus, the digestive apparatus is formed by stomach and intestine.
The stomach forms a moderate dilation in the digestive tube. To avoid contaminating the remaining tissues or losing contents, it is

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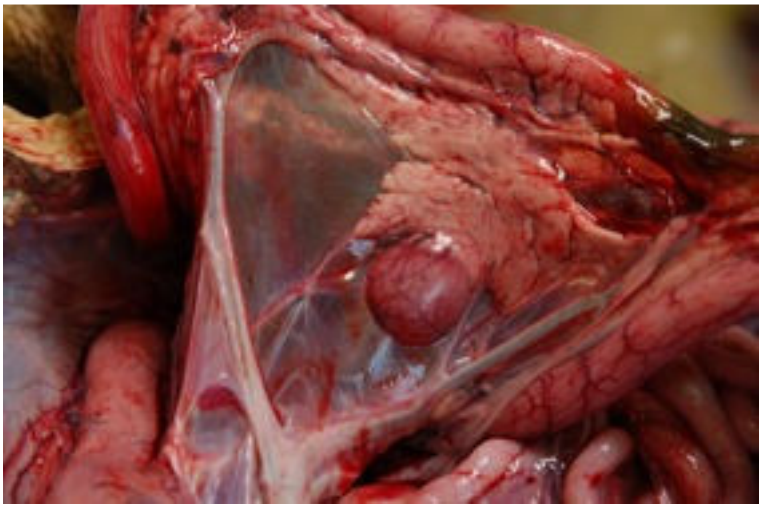
necessary to tie off both ends of every part of the digestive tract (stomach, small and large intestine) prior to cutting. With some twine, tie a tight, secure knot at the location of the attachment of the esophagus to the stomach. A second piece of twine can be tied just below the base of the pylori where the small intestine begins. Unlike in mammals, the intestine is poorly differentiated between small and large intestine, so a third knot may be tied at approximately half the length of the tube. The last knot has to be tied at the rectum just before the bladder. Examine the external surface of the gastrointestinal tract for discolouration and lesions.

Separate each section and empty the contents into individual identified containers through a sieve. Thoroughly examine to look for fluid, mucus, whole or partially digested fish, fish bones, parasites, and foreign objects; in particular note the presence of marine litter and/or fishing devices (hooks, fishing lines, fishing nets). Save all foreign objects for human interaction documentation. Once empty, cut the wall to examine the mucosa; note the colour and texture, look for ulcers, parasites, areas of discolouration and other abnormalities. Sample for histology and for toxicological analysis.



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2.9 Pancreas.

The pancreas is attached to the mesentery along the duodenum, past the stomach. and half surrounds spleen with its caudal pole. Remove the pancreas from the cavity by detaching it from the connective tissue and duodenum.

Note the size, shape, colour, and texture of the surface. Cut into the parenchyma and note changes in colour or texture, or look for very small discoloured nodules, referable to multifocal hyperplasia or eventually parasitic egg infestation. Take samples for histology investigation.

2.10 Spleen.

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The spleen is located in the mesentery at the end of the pancreas.

Note size, shape, colour and texture of both the surface and the parenchyma of the spleen. Sample for histology, microbiology and molecular investigations.

2.11 Urinary Bladder.

The bladder is found centrally, just under the pelvis and above the rectum (with the carcass on its

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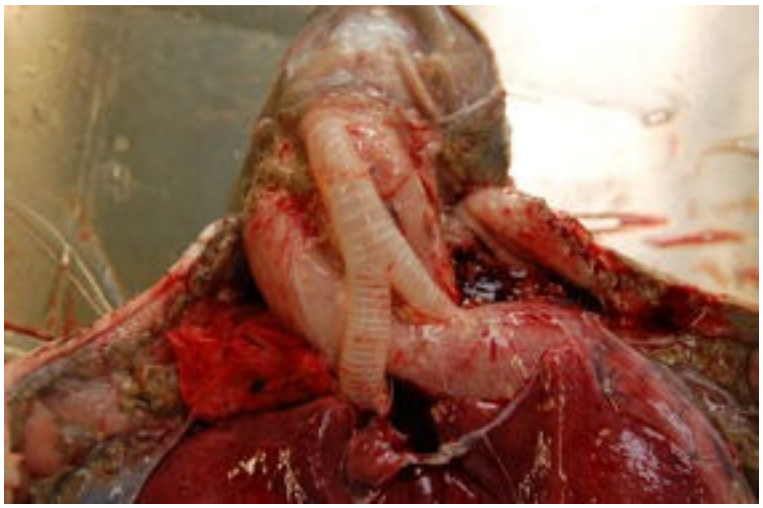
back).

Before removing the bladder from the body, be sure to clamp the bladder using a haemostat in order to retain urine. The anatomical position of the bladder sometimes allows the entrance of foreign materials: faecal materials, sand, eggs in females and also parasites. Open the bladder in a small container, collecting all the content: very often there are parasites inside. Note colour, consistency, amount of urine and presence of parasites; refer the content to the parassitologist.

Examine the bladder internally by cutting along the length of the organ to expose the mucosal surface. Note colour and texture of the mucosa.

Sample the cranial tip of the bladder for histology.



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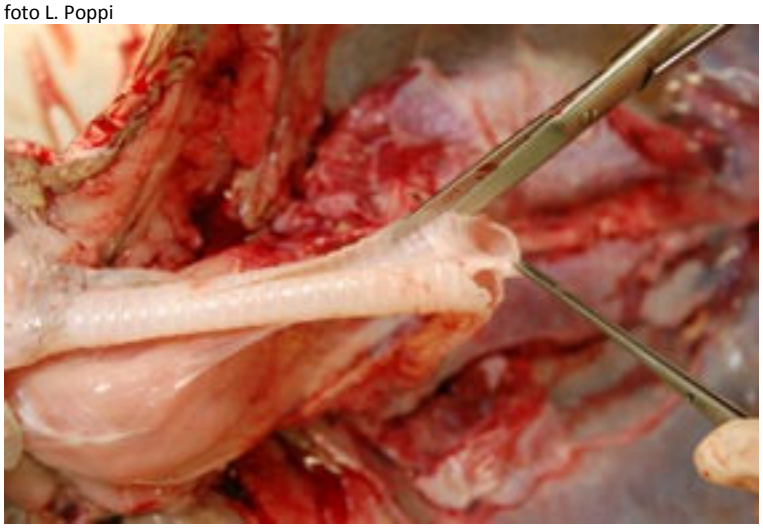

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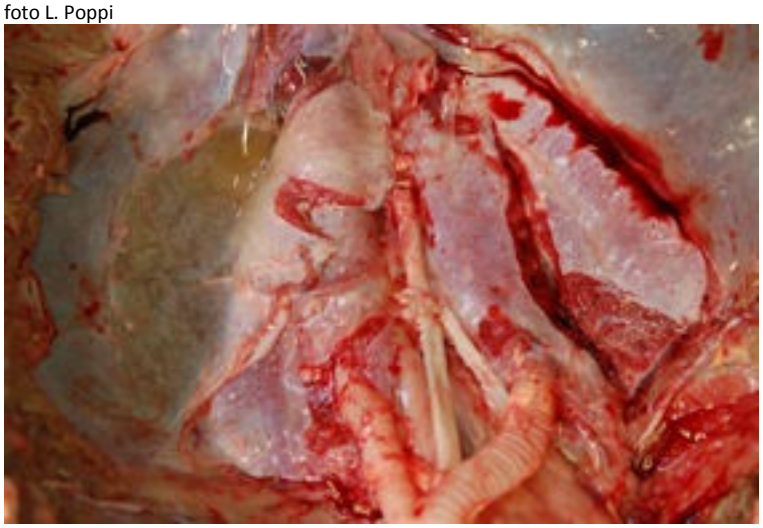

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2.12 Trachea.

To expose the trachea, cut the skin of the ventral neck from the position of the intergular scute (previously removed) to the jaw and separate the underlying muscles. Using scissors, cut through the entire length of the trachea from the bifurcation up to the apex of the throat.

Examine the mucosa and identify and describe contents (froth, fluid, blood, colour, etc.) and look for parasites. Sample for histology.

2.13 Lungs.

The lungs are attached to the carapace and vertebral column. They are easier to investigate directly in the body. Removal the lungs may be difficult and time consuming, versus the possibility to touch and cut lungs and bronchi and observe texture and colour and look for parasites directly *in situ*.

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Cut the trachea opening where it bifurcates into two bronchi, and then follow the bronchus that enters the lung and continues. Next, in the lung, make serial cuts parallel to the long axis of the body to examine the parenchyma. This is best done with a long knife using a single sweeping cut in order to avoid tearing or serrating the lung tissue. During the cut, note whether fluid, froth and/or parasites are present and describe amount, colour, *etc*. Examine the parenchyma and pleural surface: note colour pattern and texture.
Sample for histology, microbiology and molecular investigations.

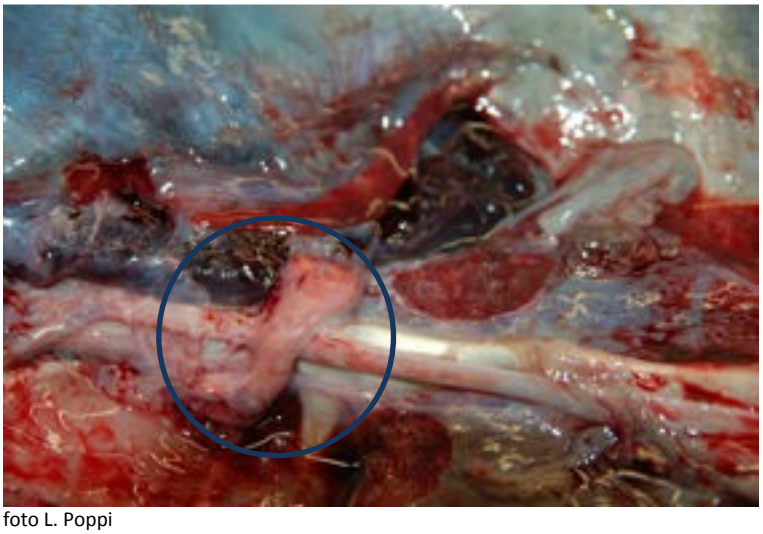


foto L. Poppi

2.14 Adrenal Gland.

The *Caretta caretta* has a single adrenal gland, located centrally, close to the dorsal aorta, between the kidneys
To remove the adrenal, grasp and pull the tissue away from the body wall and cut the surrounding connective tissue. Before sectioning, measure (LxWxH) and weigh the adrenal. Note size, shape, colour and texture of the tissue. Sample adrenal for histology investigations.

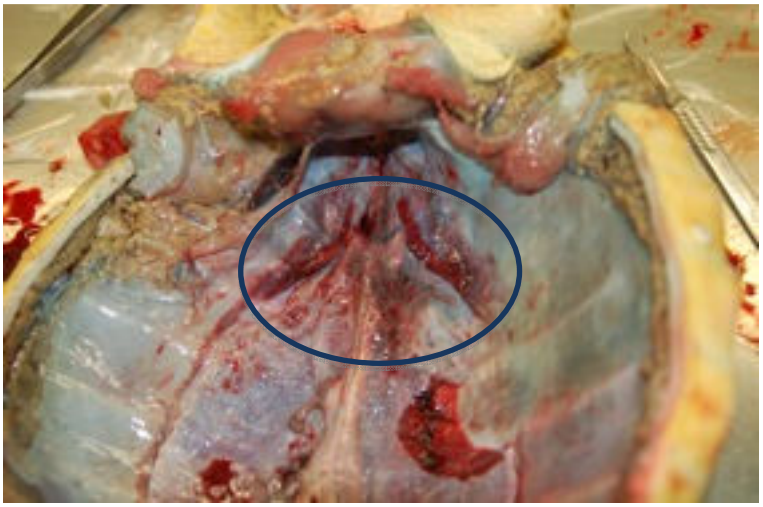
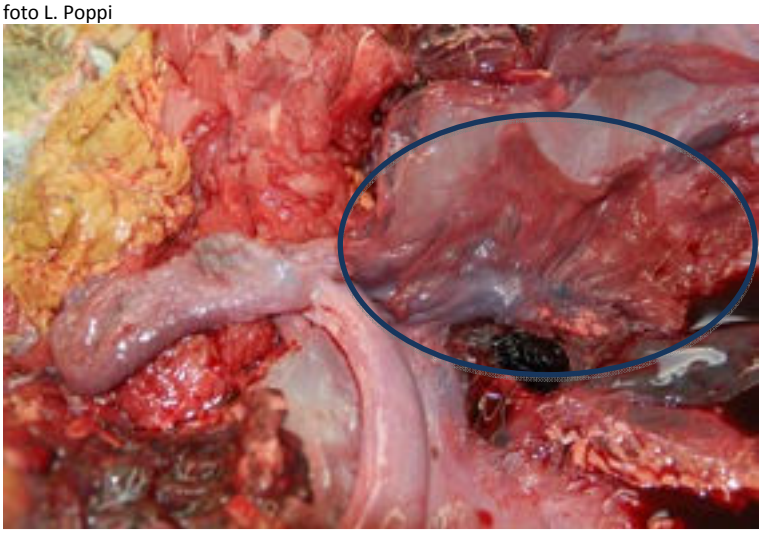


foto L. Poppi



2.15 Urogenital system.

The urinary and genital systems form a unique system with the terminal of digestive tract (cloaca). During the necropsy, the gonads are easy to examine.
<u>Ovaries and oviducts</u> - The ovaries and oviducts change a lot in size and composition with age and between breeding and nonbreeding seasons. Obtain measurements (LxWxH) and

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weight of each ovary. Examine the size, and the developmental stage. Sample each ovary for histology

Testes - Testes are attached by the mesorchium to the peritoneum.

Obtain measurements (LxWxH) and weight of each testis. Examine the size, shape, colour and texture externally and internally. Sample each testis for histology.

2.17 Kidneys.

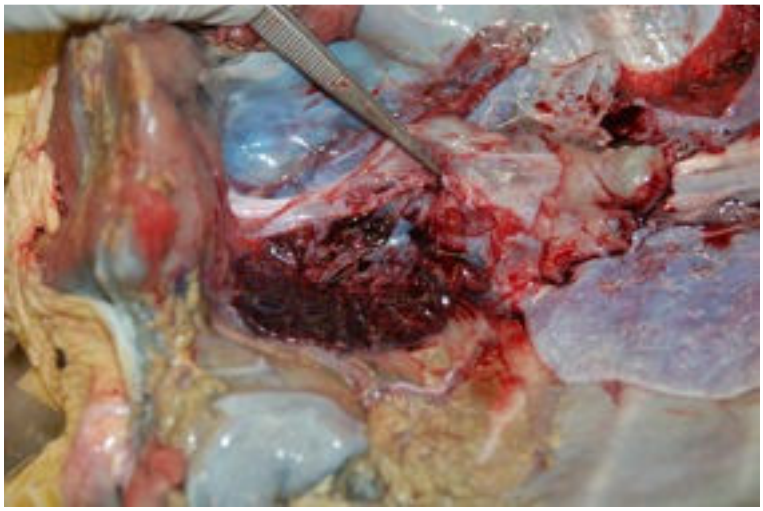

foto L. Poppi

The left and right kidneys are retroperitoneal and attached to the caudal roof of the carapace.

To detach kidneys from the carapace may be difficult and time consuming. The easiest way to examine them is to perform a deep single sweeping cut and examine the internal surface and the capsule. Look for eventual oedema, other lesions or fluid; note colour, thickness and opacity. Note the size, shape of both kidneys.

Sample for contaminants, histology, microbiology and molecular investigations.

SKULL

2.18 Removal of the Brain.

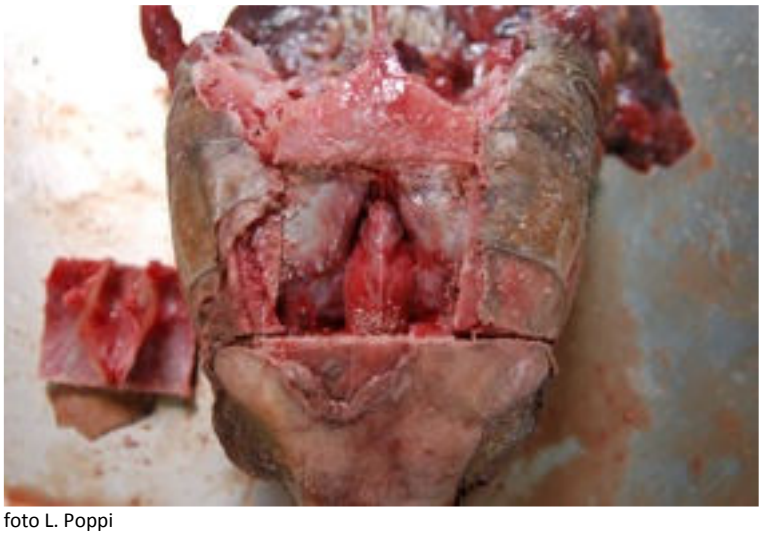

foto L. Poppi

The brain is the most fragile and easily disrupted tissue in the entire body, thus extreme care must be taken when removing the brain from the skull.

First the head must be detached from the body to safely remove the brain. Do so by cutting behind the supraoccipital crest (at the start of the neck) down to the joint between the skull and cervical vertebrae, and then completing the cut ventrally. Once separated, firmly stabilize the head, then, using a Stryker saw or a hacksaw, make a parasagittal cut through the head from one of the nostril backwards along the major axis

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of the head and uncase the brain and the large subdural space containing cerebral spinal fluid, the third ventricle and cerebral aqueduct ventral. Alternatively, cut away the top of the head with a minimally inclinated cut from the dorsal margin of the eyes towards the middle of the supraoccipital crest. In this way, the tubular brain appears white and elongated in the braincase, covered by the two-layer meninges and immersed in the clear cerebral spinal fluid. Olfactory tracts to the nose, optic lobes, cerebral hemispheres and cerebellum may be seen. The two round, lobed structures visible laterally to the brain and dorsally and posteriorly to the eyes are the salt glands.

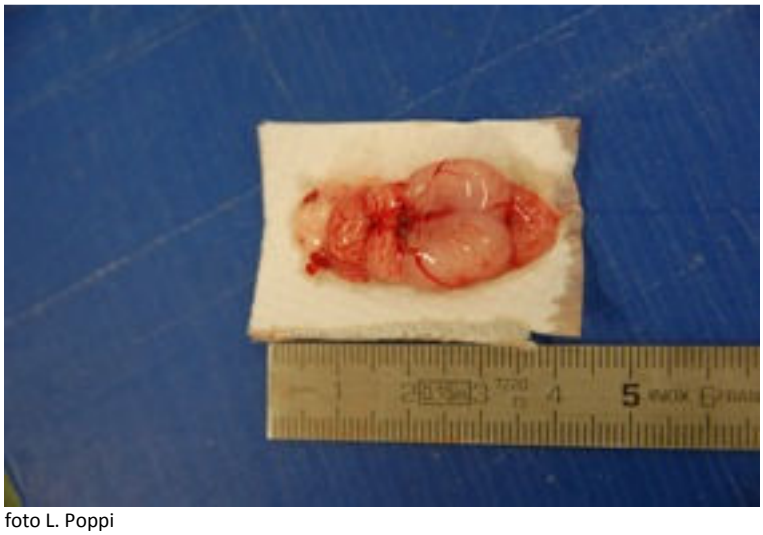

foto L. Poppi

It will take some practice to successfully cut the head without penetrating the brain, also because different sea turtles differ slightly in brain morphology and location, even between cheloniids, and even more when compared to Dermochelys.

Using a scalpel, gently cut the meninges, sample cerebral spinal fluid with a sterile needle and syringe for cytology and culture, work under the brain to sever each cranial nerve. Inversion of the head often allows the brain to gently descend in to the palm of your hand.

2.19 Examination of the Brain.

As stated before, the brain is the most delicate tissue in the body and will fall apart if handled excessively.

Observe the external surface of the brain and note symmetry of each distinct structure (right and left cerebral hemispheres, cerebellum, and brain stem) while noting the colour, texture and presence of exudate or lesions. Vascular congestion can be a result of positioning or post mortem lividity.

The best way to preserve the brain is to cut it sagittally and put an entire half brain in formalin and sample the other half brain for microbiology and molecular investigations.

During the cut, note symmetry, colour, texture and the presence of lesions.

2.20 Pituitary Gland.

Once the brain has been removed, the pituitary gland can be seen immediately under the crossover of the optic nerve The organ is within a bony recess and is usually small. It can be extracted after incision through the overlying dura by lifting it out using small forceps and a scalpel blade.

Sample for histology and other priority testing.

2.21 Salt Glands.

The salt (lacrimal) glands are dorsal and medial to the eyes, and are responsible for removal of excess salt from the body.

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3. SAMPLING PROTOCOL

3.1 Life History

Code 1, 2 and 3, ideal; 4 and 5 limited.

- Age determination –
  - Humerus, should be kept frozen.
- Reproductive Status –
  - Gonads and Uterine samples fixed in 10% NBF.
- Feeding Habits –
  - Stomach contents can be collected into a sealable plastic bag or jar, freeze.
  - Carapace keratin, for stable isotopes should be kept frozen. What the animal has been eating recently.

3.2 Genetics

Codes 1; 2-4 are suitable. Only one of the following needs to be collected, unless there are specific requests.

- Internal organs, Muscle, Bone - Better to freeze tissue samples, in case the tissue is used for something other than genetics. Genetic tissue samples can be fixed in DMSO saturated with NaCL.
- Blood – can only be collected from Code 1 and 2 animals. Minimum amount is ~10 ml; 50-100 ml is optimal for DNA studies.

3.3 Microbiology

Code 1 and 2 are ideal; 3 limited; 4 and 5 useless. Take separate samples for bacteriology and virology. Lesions should be sampled from several distinct locations, include normal tissue with the infected tissue sample.

- Bacteriology – Avoid freezing samples for bacteriology if avoidable. Refrigerate samples at 4 C. Freezing at –70°C is preferable to decomposition.
  - External samples can be taken with a swab from the eye, and vent. Culture swab in a bacterial transport medium.
  - Internal samples can be taken from the intestine, heart, kidneys, lungs, liver, spleen, bone with marrow, and tissues showing pathological changes. Culture swab in a bacterial transport medium or 6 x 6 cm sample placed in a sterile container.
  - Fluid samples can be taken from the pleural fluid, peritoneal fluid, urine, blood, fluid from abscesses. Store in appropriate aerobic or anaerobic vial.
- Virology – Refrigerate samples at 4° C.
  - External samples use a sterile swab dipped in viral transport medium. Take samples from the eye and vent. Place swabs in the vial that **contains** the viral transport medium.
  - Internal samples can be taken from the CNS tissues, lungs, liver, spleen, kidneys, tissues with pathological changes, intestinal contents. 6 x 6 cm sample placed in a

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sterile container.
- Fluid samples from pleural fluid, peritoneal fluid, pericardial fluid, urine, blood from heart. Store in sterile container.

3.4 Parasites

Samples taken from Code 1-4 animals are suitable for examination.

- Barnacles – first fix in 10% NBF, for no more than 24 hrs, then transfer to 70 % EtOH.
- Copepods & Amphipods – place directly into 70% EtOH.
- Nematodes (roundworms) – Fix in GAA for 5-10 minutes first if possible. Otherwise use 70% EtOH or 10 % NBF. If formalin is used, fix only for a few hrs. Then transfer to 70% glycerin alcohol.
- Trematodes (flukes/flatworms) – Dead or alive, fix in AFA for up to 3 days, transfer to 70% EtOH. Do Not use glycerin alcohol.
- Cestodes (tapeworms) – Fix for 5-10 min. in AFA solution and water, 4:1 ratio. Transfer to 70% EtOH. Include cestode head when removing from the host, if necessary cut host tissue.
- Acanthocephalans – Fix in AFA for up to 24 hrs. Then transfer to 70% glycerin alcohol.

3.5 Histopathology

Code 1, 2 and 3 are ideal. Rare / endangered species should be thoroughly sampled. Lesions, fractures, lacerations, and gunshot wounds of any code should be sampled in this manner.

- Tissues should be preserved in 10% NBF.
- Tissue samples should be no larger than 3 x 3 cm and approximately 1 cm in thickness.
- Ideally, histo samples should be cassetted and placed into a labeled jar for the appropriate Institution / researcher. Individual requests should be noted and tracked.
- Samples of gross lesions should include abnormal and normal tissue.

3.6 Contaminants / Biotoxins:

(organochlorines, heavy metals)

Code 2 is ideal, 1 and 3 is limited; 4 and 5 questionable to useless.

- Biotoxin Analysis – Code 2 animals only. Collect stomach contents, liver and/or kidney tissue. Freeze.
- Contaminant Analysis – All of the samples should be frozen in plastic zip lock bags and/or wrapped in aluminum foil.
  - Skin and subcutaneous tissue, muscle, liver, kidney, and brain (if possible include cerebrum and cerebellum).

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4. BIBLIOGRAPHY

WYNEKEN, J. 2001. The Anatomy of Sea Turtles. NOAA Technical Memorandum NMFS-SEFSC-470. http://csi.whoi.edu/biblio/anatomy-sea-turtles

LUTZ, P.L., MUSICK, J.A. 1997. The Biology of Sea Turtles. CRC Press, USA.

LUTZ, P.L., MUSICK, J.A., WYNEKEN, J. 2003. The Biology of Sea Turtles, Vol II. CRC Press, USA.

WYNEKEN, J., LOHMANN, K.J., MUSICK, J.A. 2007. The Biology of Sea Turtles, Vol III. CRC Press, USA.

MADER, D.R. 2006. Reptile Medicine and Surgery, second edition. Chapter 7. Saunders Elsevier, St. Louis (MO, USA)

WORK, T.M. 2000. Sea turtles necropsy manual for biologists in remote refuges. US Geological survey, National wildlife health center, Hawaii field station; 2000, 25 pp.

MC ARTHUR, S., WILKINSON, R., MEYER, J. 2004. Medicine and Surgery of Tortoises and Turtles. Blackwell publishing Ltd, Oxford (UK)

Frye, F.L. 1991. Reptile care: an atlas of diseases and treatments. Tsh Publications, Neptune City (NJ, USA)

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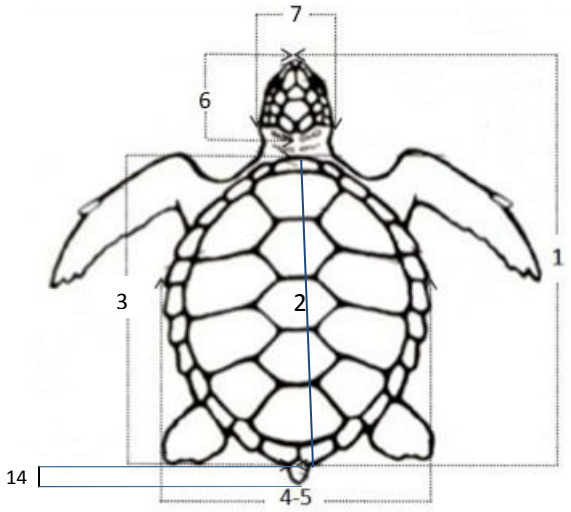
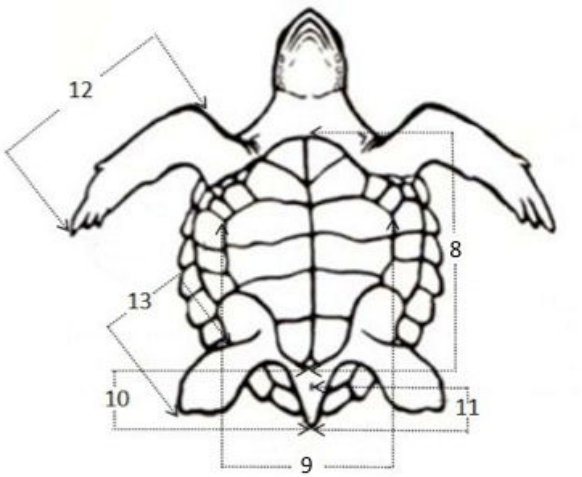
5. ANNEX I

MORPHOMETRICAL DATA

DATE	
ANIMAL N°	
STRANDED AT	
WEIGHT	





BASIC MEASURES	
1 = TL	
2 = CCL	
3 = SCL	
4 = CCW	
5 = SCW	
6 = HL	
7 = HW	

COMPLEMENTARY MEASURES	
8 = PL	
9 = PW	
10 = TTL	
11 = VTL	
12 = FoL	
13 = HiL	
14 = CaTT	

TL: total length
CCL: curved carapace length notch to tip
SCL: straightline carapace length notch to notch
CCW: curved carapace width
SCW: straightline carapace width
HL: head length
HW: head width

PL: plastron length
PW: plastron width
TTL: total tail length
VTL: vent-tip length
FoL: foreleg length
HiL: hindleg length
CaTT: tip of carapace-tip of tail

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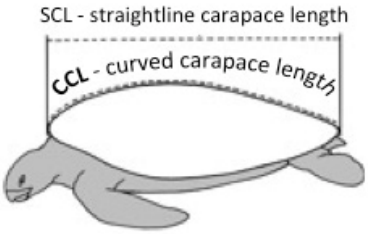
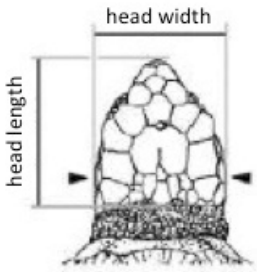
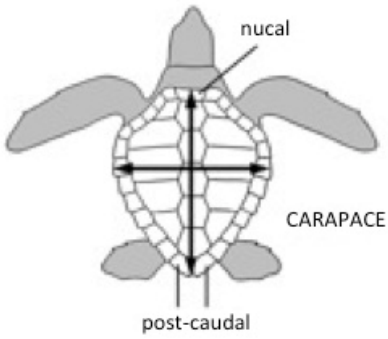
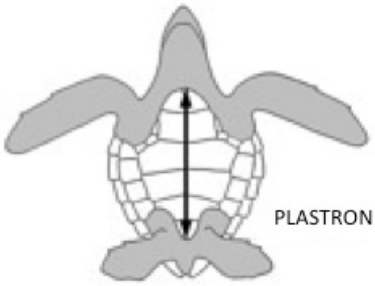
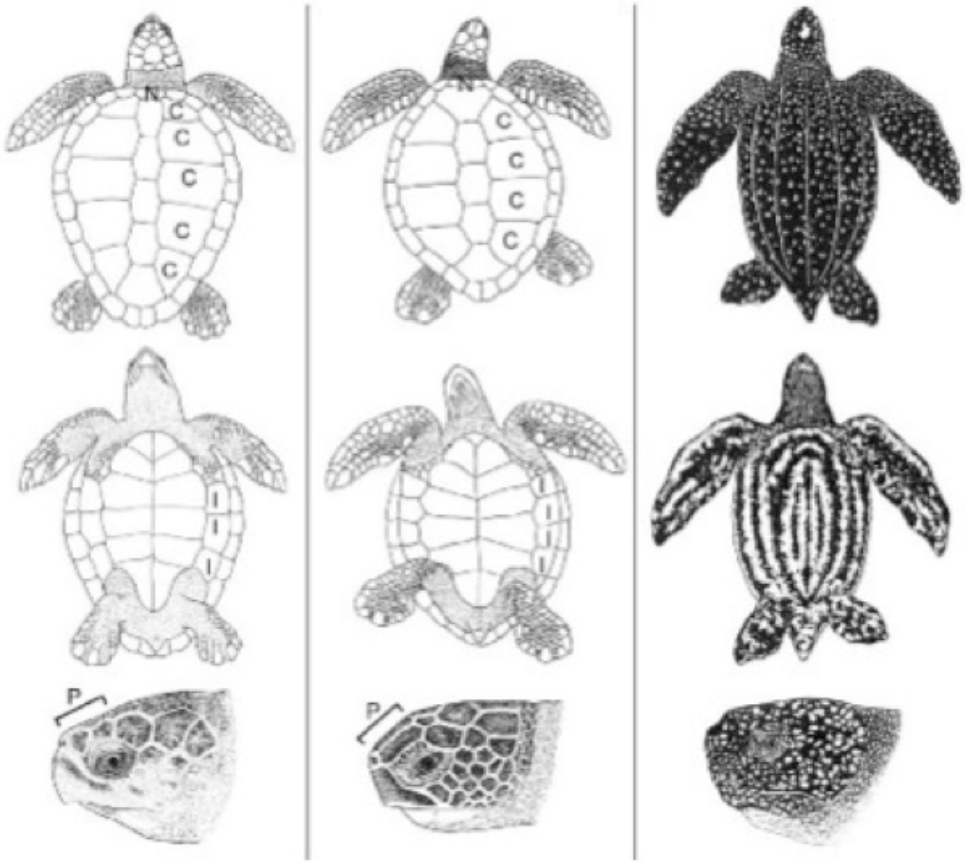








Caretta caretta *Chelonia mydas* *Dermochelys coriacea*



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6. ANNEX II

GUIDELINES FOR SEA TURTLES NECROPSIES

1 ANAMNESIS AND SIGNALMENT (WHERE? SPECIES?)

2 EVALUATION OF PRESERVATION STATUS (*SEE* PRESERVATION SCORES TABLE)

3 COLLECTION OF MORPHOMETRICAL DATA

4 DISSECTION:

4.1 external examination of the animal (EXTERNAL WOUNDS? SKIN ABNORMALITIES? EXTERNAL BLEEDING?)

→ ***PARASITOLOGY****: external parasites research by simply observation (how many barnacles? Leeches?)*

4.2 removal of plastron

4.3 evaluation of nutritional score (*SEE* NUTRITIONAL SCORES TABLE)

4.4 removal of scapula and associated muscles (HAEMORRHAGES? PALENESS?)

4.5 medial cut on neck skin: observation of thyroid gland and thymus (SIZE? COLOUR? SHAPE?)

4.6 opening of pericardium; observation of great vessels, removal and dissection of the heart (PERICARDIAL EFFUSIONS? EPICARDIAL OR MIOCARDIAL LESIONS?) – weight

→***PARASITOLOGY****: blood flukes research in the heart and great vessels or related lesions on vessels wall*

4.7 opening of celomic wall and general observation of the cavity (CELOMIC EFFUSIONS? TOPOGRAPHY? COLOUR ABNORMALITIES? SPREADING LESIONS?)

4.8 liver evaluation and removal (COLOUR? CONSISTENCE? SIZE? GROSS LESIONS?) - weight

→ ***PARASITOLOGY****: research of liver parasites by sedimentation exam*

4.9 removal of whole gastrointestinal tube (EXTERNAL WALL LESIONS? INTUSSUSCEPTIONS?) - length ; opening of the entire tube (GASTRIC ULCERAE? INTESTINAL WALL LESIONS-PETECHIAE? CONTENT-ANY FOREIGN BODY? WHICH AND HOW MUCH FOOD?)

→ ***PARASITOLOGY****: research of adult parasites by simply observation of content + sedimentation exam; research of eggs by qualitative coprologic exam from rectum content; research of Cryptosporidium oocysts from smears of rectum content by modified Ziehl-Neelsen stain*

4.10 pancreas and spleen evaluation (COLOUR? CONSISTENCE? SIZE? GROSS LESIONS?) - spleen weight

4.11 external observation, removal and opening of urinary bladder (INTERNAL WALL LESIONS?)

→ ***PARASITOLOGY****: research for adult parasites in bladder content (usually Trematoda) by simply observation and/or sedimentation exam.*

4.12 opening of the remaining part of esophagus (FOOD? FOREIGN BODIES? WALL LESIONS?)

→ ***PARASITOLOGY****: research of parasites in the esophagus (usually Nematoda at junction with stomach)*

4.13 observation of lungs (CONSISTENCE? COLOUR? GROSS LESIONS?); opening of the trachea (WALL LESIONS? ANY FLUID INSIDE?)

→ ***PARASITOLOGY****: research for adult parasites in trachea and bronchi by simply observation after longitudinal opening of their cartilage*

4.14 genital organs observation and removal (WHICH GONADS?? OVARIC FOLLICLES PRESENT? EGGS?)

4.15 kidneys observation (GROSS LESIONS?) - weight

4.16 eyes observation and removal (GROSS LESIONS?)

4.17 brain and salt glands removal (cut away the top of the head)

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5 SAMPLE COLLECTION

histology: all lesions

bacteriology: pericardium, lungs, intestine, kidneys, lesions

virology: lung, liver, kidney, brain and/or periferical nerves, intracardiac blood clot

toxicology: liver, muscle, kidney, skin, gonads

genetics: visceral organs, skin, scutes keratin, muscle, bone

PRESERVATION CONDITION SCORE

Score	Preservation status	Description	Practicable exams
1	Fresh carcass	Death occurred in the previous 24 hours	histology, cytology, virology, microbiology, parasitology, pollutants, biotoxins, genetics
2	Moderate decomposition	Head: integral or with partial loss of skin; Eye: sunken or liquefied; Tail: present or absent; Limbs: integral; Carapace and plastron: integral; Bloated carcass	histology (limited), virology, microbiology, parasitology, pollutants, biotoxins, genetics
3	Advanced decomposition	Head: complete loss of skin; Eye: liquefied; Tail: absent; Limbs: partially exposed skeleton; Carapace and plastron: partial or total loss of skin; Collapsed carcass	histology (limited), parasitology, pollutants (limited), genetics
		a) internal organs: still distinguishable	
		b) internal organs: liquefied	
4	Mummified carcass or partial carcass	Incomplete carcass; Skull: visible; Carapace: broken with separation of parts	genetics

NUTRITIONAL CONDITION SCORES

Nutritional status	Description
Excellent	Integral or partially liquefied adipose tissue covers entire ventral area. Hepatic lipidosis is also present.
Very good	Integral or partially liquefied adipose tissue covers entire ventral area.
Fair	Integral or partially liquefied adipose tissue covers peripheral parts of ventral area.
Scarce	Integral or partially liquefied adipose tissue is present only in limited peripheral parts of ventral area.
Not valuable	Mummified carcasses.

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7. ANNEX III

NECRO REPORT

Event Info	Animal Info
Strand Date: _____	Species: *C. caretta* *C. mydas* *D. coriacea*
Recovery Date: _____	Sex: M F (after necropsy)
Euthanized / Died	CCL: _____cm
Date & TOD: _____	Weight: _____ Kg
Necro Date & Time: _____	Condition at Stranding: 1 2 3 4
Storage Prior to Necropsy: _____	Condition at Necropsy: 1 2 3 4
Stranding Location: _____	Human Interaction: Yes / No
_____	
Lat/Long: _____N/_____W	# Animals: _____

CARCASS DISPOSITION:

HISTORY:

COMMENTS:

Necropsy Observations: Please note general observations of color, condition, textures, etc. even when utilizing NA= not applicable, NE= not examined, NSF= no significant findings, NVL= no visible lesions. List weights (g) next to each organ examined.

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EXTERNAL EXAM

Carapace/Plastron/Skin (color, condition):

Wounds/Scars:
mark the figure

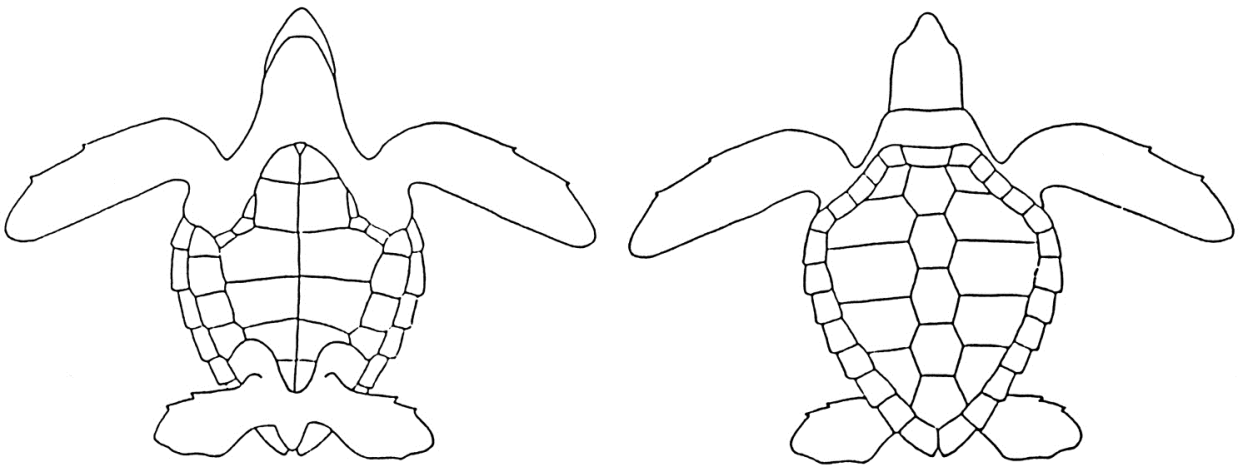
Lesions:
mark the figure

Parasites (what, where):

Vent:

Mouth (tongue, ulcers)/ Mucous membranes (color):

Eyes (discharge, color, ruptures):



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NUTRITIONAL CONDITION

after plastron removal
Fat deposition: Excellent [1] - Very good [2] - Fair [3] - Scarce [4] - Not valuable [5]

Coelomic cavity (fluid content, colour, quantity):

MUSCOLO/SKELETAL SYSTEM

Muscle (haemorrhages/haematomas/discoloration/atrophy):

Ventral muscles:

Posterior muscles:

CIRCULATORY SYSTEM

Pericardium:

Heart:

Vessels (parasites):

LIVER and GASTROINTESTINAL SYSTEM

Liver (color, congestion, lesions, size) and gallbladder:

Esophagus (parasites):

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Stomach (content, ulcers, mucosa, parasites):
weight full _____ weight empty_____

Intestine (contents, ulcers, parasites):

Pancreas:

Spleen:

RESPIRATORY SYSTEM

Trachea:

Bronchi (parasites):

Lungs (color, condition, edema, congestion, consolidation, granulomas, emphysema, lesions, parasites):
(R)

(L)

URINARY/REPRODUCTIVE SYSTEMS/ADRENAL

Bladder (parasites):

Testes / Ovaries: Immature / Mature with eggs / follicles / corpora albicans

(R) **Lx W x H cm:**

(L) **Lx W x H cm:**

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Kidneys (color, condition):
(R)

(L)

Adrenal:

Lx W x H cm

OTHER GLANDS

Thyroid:

Thymus:

SKULL

Brain:

Salt glands:

Differential Diagnosis from Gross Exam

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8. ANNEX IV

PROTOCOL FOR EXAMINING SEA TURTLES FOR SINGS OF HUMAN INTERACTION

1. GENERAL INFORMATION			
N. ID		Species	
Weight	CCL	Examiner	
Cause of death		Date of death	
Location of necropsy examination		Date of exam	
Video YES NO		Photo YES NO	
Condition Code 1 2 3 4		Fresh o frozen	
Note **ND**: Not Determined – **NE**: Not Evaluable			

2. EXTERNAL EXAM			
a. Body condition			
Emaciated	Not emaciated	ND	NE
b. Sings of fishing net or lines (indicate if YES, NO, ND, NV for each area and in the positive case describe the lesion)			
Head	Mouth		
Flipper sx	Flipper dx dx		

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2. EXTERNAL EXAM			
c. Presence of fishing nets on the animal		YES	NO
Fishing nets have been preserved?		YES	NO
d. Penetrating wounds			
YES	NO	ND	NE
Describe gunshot wounds, puncture wounds, from harpoon, parallel deep wounds, etc.			
e. Mutilations			
YES	NO	ND	NE
Describe ctus, tears, cracks in the shell, missing appendages, etc.			
f. Suspected haemorrhages and haematomas (ventral neck, limbs)			
YES	NO	ND	NE
Describe extension and area.			
h. Post-mortem damage from scavengers and opportunists			
YES	NO	ND	NE
Describe extension and area.			

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3. INTERNAL EXAM				
a. Muscular hemorrhages				
YES	NO	ND	NE	
Describe extension and area.				
b. Fractures				
YES	NO	ND	NE	
Describe.				
c. Content of airway and lung				
AIR	FLUID	FOAM	ND	NE
Describe lungs' appearance (heavy, consolidated areas, color variations, etc.) and airway's content.				
d. Stomach content				
Describe stomach content, amount, presence of parasites and foreign bodies.				
Stored in frozen		YES	NO	
e. Histopathology		YES	NO	

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3. INTERNAL EXAM			
f. Presence of macroscopically visible lesions			
YES	NO	ND	NE
Describe.			
g. DIAGNOSTIC HYPOTHESIS			

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9. ANNEX V

SAMPLES SCHEMA

	Standard Samples							
	Life History	**Genetics**	**Parasites**	**Histo.**	**Contam.**	**EnteroBT**	**HerpesVR**	**Biotox**
Tissue	(Frozen or fixed)	(Frozen &/or DMSO)	(70% EtOH)	(10% Formalin)	(Foil wrapped and frozen)	(Culture swab)	(Frozen9	(Frozen)
Adrenal				■				
Blood/Serum								■
Brain				■	■		■	
Carapace	■		■					
Esophagus			■	■				
Fat deposition	■							
Feces			■					■
Heart			■	■		■		
Humerus	■							
Intestine			■	■		■		
Kidney (R)				■	■	■		■
Kidney (L)				■	■	■		■
Liver			■	■	■	■		■
Lung (R)			■	■			■	
Lung (L)			■	■			■	
Muscle		■		■				
Oral Mucosa				■				
Ovary	■			■	■	■		
Pancreas				■		■		
Skin		■	■	■	■		■	
Spleen				■			■	
Stomach			■	■		■		
Stomach Contents	■							■
Testis	■			■	■			
Thyroid				■				
Trachea			■	■				
Urine					■			■

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