A veterinarian’s guide for sea turtle post mortem examination and histological investigation

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Preface

These instructions have been composed to aid training pathologists in the field of marine turtle post mortems. They have been written for the expressed use within The University of Queensland but may be of use in other institutions.

The authors emphasise that marine turtles are protected species, and the procuring of specimens or any part thereof without a permit is prohibited, for example, under the Environment Protection and Biodiversity Conservation Act 1999 in Australia and its specific state or territory acts, and the Endangered Species Act of 1973 in the United States of America. Post mortem examination of turtles requires permits issued by local governing authorities.
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Introduction

There are seven species of marine turtle in the world: the Green (*Chelonia mydas*) including the Black subspecies (*Chelonia mydas agassizii*), the Loggerhead (*Caretta caretta*), the Leatherback (*Dermochelys coriacea*), the Hawksbill (*Eretmochelys imbricata*), the Flatback (*Natator depressus*), the Olive Ridley (*Lepidochelys olivacea*), and the Kemp’s Ridley (*Lepidochelys kempii*). All of these species, except for the Flatback, are listed as vulnerable, endangered or critically endangered on the IUCN Red List of Threatened Species. The flatback is listed as ‘data deficient’; it is classified as such due to limited available population information to determine its status.

In coastal waters, Green and Black turtles feed primarily on sea grasses and algae; Loggerheads on invertebrates; Olive Ridley and Kemp’s Ridley turtles on crabs, molluscs, shrimps, jellyfish and vegetation; Leatherback turtles on jellyfish, tunicates and other soft-bodied animals; Hawksbill turtles on sponges, tunicates, shrimps, jellyfish and vegetation; and Flatback turtles on soft bodied invertebrates (seapens, soft corals, and sea cucumbers).

In threatened species such as marine turtles, detailed post mortem examinations combined with concurrent monitoring of in water and nesting beach populations may prove invaluable to the understanding of morbidity and mortality factors of demographic importance, especially in populations where the loss of a few individuals can have significant effects. For the optimal execution of a post-mortem investigation, a standardised approach to documenting the collection of samples, and submission of collected samples to appropriate diagnostic laboratories is required.

There are four freely available comprehensive instructional guides for the post mortem of marine turtles with view to collecting samples for disease diagnosis:


This manual is designed to complement the above resources and provide the post mortem examiner with an illustrated guide to normal and abnormal morphology at the gross and microscopic level. It is hoped that by providing standardised collection techniques as well as visual aids, these instructions
will be a reference source that maximises the possibility of determining cause of death.

The green sea turtle (*Chelonia mydas*) has been selected as the model for post mortem examination. Significant anatomical variations found in the other species will be noted where appropriate. The instructions are presented in order of dissection.

Common terminology used in this guide is presented in Appendix I.
Morphometrics

All post mortem examinations should start with collection of morphometric data including species, tag numbers and measurements.

Species
Identify and record the species using anatomical cues (Figures 1 and 2).

Tags
Examine the carcass for identification. Record all tag numbers in their entirety and report to relevant authority (Appendix II). Marine turtles previously caught during population surveys are tagged in one of four ways.

1. **Applied tag** (most common): self-piercing and self-locking metal (titanium, inconel or monel) tags or plastic tags. Multiple tags may be applied to a turtle depending on the number of times it has been caught. These tags may be applied to the left or right fore or hind limb, but are most often found on the fore limbs only. Depending on the tagging project, tags may have been placed anywhere along the caudal aspect of the flipper. Tag numbers are comprised of letter prefixes or suffixes and up to six numbers. Relevant authority contact details are seen on the reverse of all tag types.

2. **PIT tag** microchips are approximately 12 x 3 mm in size. They are applied in the interdigital space of the proximal tarsals or carpals of the left or right limbs, the cranial aspect of the shoulder, the soft tissue areas associated with the cranial aspect of the carapace, or in the subcutaneous tissue of the tail. PIT tags may be identified by most microchip scanners. All turtles should be scanned for PIT tags.

3. **Notches**: This practice has ceased in marine turtles in Australia but individuals are frequently recaptured that underwent this practice. Shaped notches were placed on the marginal scutes by excising the germal layer of the scute. Number of notches and scute number were used to identify the animal. Notches may be difficult to discern from trauma. Dorsoventral photographs should be taken, focused to enable scale counts to aid in species identification and forwarded to relevant authorities who can distinguish notches from trauma and allow for confirmation of identification. To aid in identification, the carapace should be cleaned so that injuries, deformities and notches can be assessed.

4. **Living tag** (US and Caribbean): This is defined as the transplantation of a patch of carapace to plastron and vice versa, creating a dorsally un-pigmented and ventrally pigmented transplant of living tissue. The scales each transplant is present on should be noted and photographs taken to confirm location and distinguish from natural scar tissue by experts. Photographs should be forwarded to relevant authorities for confirmation.

5. It is common for turtles to have no identification, as only subsamples of populations are surveyed. Under these circumstances it is important to
determine sex and estimate breeding status during the internal examination.

Figure 1. Photographic anatomical cues of marine turtles of the Indo-Pacific region.
Figure 2. Illustrated identification key for marine turtles of the Indo-Pacific region.

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Listed below are several key turtle morphometric measurements. It is advised measurements are taken that are the standard measurements used within that region. For example, carapace length measure used in Australia is midline curved carapace length, whereas in Hawaii midline straight carapace length is used. All linear measurements are made to the nearest 0.1 cm.

**Weight:**
Weigh the animal using appropriate-range scales to the nearest 0.5 kg. Green turtles may weigh over 220 kg and Leatherback turtles over 500 kg. Block and tackle pulley systems with tripods may be necessary to weigh larger specimens with animals secured using large cargo nets, as is the case for nesting females in Hawaii, or soft leg ropes as is used in Queensland. Ensure safety to the operators prior to use and that all OH&S requirements are being followed. All of the turtle must be clear of contact with fixed objects.

**Midline curved carapace length (CCL)**
Measure and record the CCL using a fibreglass measuring tape; plastic tapes stretch and give false readings. CCL is a measurement used to approximate the age class of a turtle. CCL is measured from the cranial skin/carapace junction to the caudal margin of the suture of the post-central scutes (Figure 3).

**Curved carapace width (CCW)**
Measure and record curved carapace width from the lateral aspect of the marginal scutes, at right angles to the midline at the widest section of the carapace (Figure 3).

**Figure 3. Carapace measurements. Midline curved carapace length (blue line) and curved carapace width (red line).**
**Plastron length (PL)**
Measure mid line plastron length from the cranial skin/plastron junction of the intergular scute to the caudal skin/plastron junction of the suture of the anal scutes (Figure 4).

*Figure 4. Plastron length measurement (red line).*

**Straight carapace length (SCL)**
Measure mid line straight carapace length from the cranial skin/carapace junction to the caudal margin of the suture of the post-central scutes as for the curved length, but using callipers.

**Carr's straight carapace length (Carr's SCL)**
Measure Carr’s straight carapace length from cranial most aspect of the carapace to the caudal margin of the suture of the post-central scutes using callipers. Carr’s SCL may be used to measure a turtle while in dorsal recumbency.

**Straight carapace width (SCW)**
Measure straight carapace width from the lateral aspect of the marginal scutes at right angles to the midline at the widest section of the carapace as for the curved width, but using callipers.
**Standard depth (SD)**

SD is determined by placing the turtle on its side and measuring the maximum depth of the carapace with callipers by selecting the midline apex of the carapace (dorsal) and measuring at 90° to the corresponding midline of the plastron (ventral) (Figure 6). It is measured from the callipers using a steel or fibreglass tape. SD is a measurement that may be used in conjunction with CCL and weight to determine the body condition score of a turtle.

**Figure 5. Measurement of standard depth.**
**Tail length (TL)**
Measure and record the tail length using a fibreglass measuring tape. TL is a measurement used to denote maturity, particularly in males. For example, in a green turtle, a TL of > 20.0 cm is an indicator (only) that the specimen is a mature male. TL is measured from the caudal aspect of the suture of the post-central scutes to the distal caudal aspect of the extended tail (Figure 5a).

If the tail extends past the caudal aspect of the suture of the post-central scutes, it is denoted by a positive measurement. If the tail does not extend past the caudal aspect of the suture of the post-central scutes, it is denoted by a negative measurement.

**Head Width (HW)**
Determine the head width using callipers. Measure and record HW from the callipers using a steel or fibreglass tape. HW is a measurement used to confirm the species of the specimen, when examined as a ratio of CCL. HW is measured transversely at the widest part at the quadrate bones (Figure 5b).

Figure 6. Measurements of (a) Tail length; (b) Head width.
**Body condition (BC)**

Estimate body condition subjectively by visual inspection of the carcass. Body condition is classified as good, average, poor or emaciated (Figure 7 a-d).

**Figure 7. Body condition.** (a) Good; (b) Average; (c) Poor; (d) Emaciated.
Turtles in good condition have a rounded plastron and good muscle mass particularly around the forelimb axilla and neck ± a fat tail.

Turtles in average condition have a less rounded plastron and reduced muscle mass.

Turtles in poor condition have a flat to slightly concave plastron and notable reduction in muscle mass, both around the fore and hind limbs.

Emaciated turtles have a sunken, concave plastron which is easily depressed by pressing on it, with significant loss of muscle mass around the fore and hind limbs and loss of fat seen in areas of soft tissue (fore and hind limbs and neck) and eye sockets. Emaciation also often results in softening of plastron cartilage which will manifest as softness or pliability of plastron. In extreme cases, plastron detaches from carapace revealing coelomic cavity or the heads of the humerii erode the plastron over the pectoral girdle resulting in penetration of bone through the plastron (Figure 8).

Figure 8. Bilateral erosion of humeral bone through the plastron of an emaciated turtle.
Body Condition Index (BCI)
Calculate the body condition index using Equation 1 derived by Bjorndal et al. (2000). BCI quantifies body condition based on a ratio of weight and SCL (Table 1).

\[
BCI = \left( \frac{\text{Weight (kg)}}{\text{SCL(cm)}^3} \right) \times 10000
\]

Table 1. Body condition index corresponding to subjective visual observations in Green turtles (C. mydas).

<table>
<thead>
<tr>
<th>Condition Index Code</th>
<th>Body Condition Index</th>
<th>Subjective visual interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;1.20</td>
<td>Very Good</td>
</tr>
<tr>
<td>1</td>
<td>1.11-1.20</td>
<td>Good</td>
</tr>
<tr>
<td>2</td>
<td>1.00-1.10</td>
<td>Average</td>
</tr>
<tr>
<td>3</td>
<td>&lt;1.00</td>
<td>Poor</td>
</tr>
</tbody>
</table>


External examination
Conduct a detailed external examination of the carcass prior to commencing the internal examination. Note any abnormalities as outlined below. All findings should be entered on a post mortem examination datasheet. An example of such a sheet is provided in Appendix III.

Carcass condition
Estimate the elapsed time between death and presentation for post mortem examination to determine the stage of decomposition, if any.

The following codes which have been adapted from standard decomposition codes may be used to classify the level of decomposition (Table 2).
Table 2. Carcass condition and code as assigned in Australia.

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Live but subsequently died; unsuccessful rescue.</td>
</tr>
<tr>
<td>D2</td>
<td>Dead, carcass in good condition.</td>
</tr>
<tr>
<td></td>
<td>• fresh; suitable for pathology or resembling a carcass fresh enough for eating.</td>
</tr>
<tr>
<td>D3</td>
<td>Dead, carcass fair.</td>
</tr>
<tr>
<td></td>
<td>• decomposed but organs intact; autolysis noted on gross examination.</td>
</tr>
<tr>
<td>D4</td>
<td>Dead, carcass poor</td>
</tr>
<tr>
<td></td>
<td>• advanced decomposition with internal organs falling apart.</td>
</tr>
<tr>
<td>D5</td>
<td>Dead, mummified carcass with skin holding bones together.</td>
</tr>
<tr>
<td>D6</td>
<td>Dead, disarticulated bones with no soft tissue remaining.</td>
</tr>
</tbody>
</table>


Carcass condition is a subjective measure. The exact time of death, as for any species, is usually difficult to discern, with predictive algorithms in canine and human models showing that time of death is usually over-estimated. As such, state of decomposition offers a more accurate terminology to describe changes post mortem. The more decomposed the animal, the less diagnostic value, so care must be taken with interpretation of decomposed samples.

Photographs
It is essential to take photographs of the carcass, with a reference scale included (e.g. tape measure but more preferably a scale that the identification number of the animal may be written on). Standard photographs to be collected are ventral (plastron) and dorsal (carapace) surfaces, head (dorsal-ventral and rostral-caudal), forelimbs and hindlimbs, and any identifying marks (trauma or tags). All photographs should allow for the confirmation of species identification via scale counts and, if present, notches. In addition to these photographs, take detailed images of any external abnormalities (e.g. tumours, epibionts, wounds, missing limb or eyes, deformities, skin lesion, etc.).

Oral
Assess the mouth for mandibular or maxillary fractures, haemorrhage, lacerations, calcium deposits, ulceration, deformities, tumours, oral leeches (Ozobranchus margo) (Figure 9a), fish or fish spines and foreign bodies (eg. fishing hooks and line). Record the size (length x depth x width) of any noted abnormality.
Ocular
Examine the eyes for degree of retraction (sunken), trauma, opacity, presence of eye, and tumours. Sunken eyes in a D1 or D2 carcass may be indicative of dehydration.

Carapace
Place the turtle on its plastron. Examine the carapace for evidence of lifting scutes (e.g. caused by natural sloughing, usually in conjunction with leeches colonising the scale bed, nutritional deficiency or fungal infection), trauma such as boat strike (generally exemplified by fracture of carapace, multiple parallel linear lacerations [propeller], single linear laceration [skegs]), and epibiotic (barnacles, leeches and algae) load. Classify and count the absolute number of barnacles (Figure 9 b,c) and leeches and estimate the percentage of algal cover. In some species such as greens, high epibiont loads may be indicative of a health problem, that has resulted in the inability to dive to and stay at cleaning stations while cleaner fish remove epibionts. Care must be taken in interpreting epibiotic loads depending on the species involved; for example high loads are normal for loggerheads and hawksbills.

For boat strike cases, measure the length and depth of each laceration as well as the distance between lacerations if more than one strike is present. This information allows experts to determine the size of propeller responsible.

Plastron
Place the turtle in dorsal recumbency (on its carapace). Examine the plastron for evidence of lifting scutes, trauma, and classify and estimate epibiotic load.

Figure 9. Common parasites and epibionts. (a) Ozobranchus margoii (oral leeches); (b) Tubicinella cheloniae (burrowing barnacle); (c) Chelonibia testudinaria (barnacle).
Neck
Examine both the dorsal and ventral aspect of the neck. Assess for tumours, lacerations, leeches, masses and other abnormalities.

Cloaca and Tail
Examine the cloaca for prolapse, haemorrhage, lacerations, leeches, deformities, tumours, and foreign bodies (e.g. fishing hooks and line). Record the size and length of any foreign bodies. Examine the tail for condition, fractures and deformities.

Appendages
Examine both the axial and abaxial surfaces of the fore and hind limbs, including the associated axillary and inguinal soft tissue areas. Note and record any tumours, evidence of trauma or fracture and presence of foreign bodies. Record the size and length of any foreign bodies.

Fibropapillomas
Fibropapilloma tumours are neoplastic masses that may occur internally or externally in turtles (Figure 10). They are strongly associated with presence of a herpes virus, but their cause is unknown. In appearance, fibropapilloma tumours may be smooth or papillary, flat or rounded. They are usually grey to black to white depending on location (darker dorsal, lighter ventral). They range in size from <1 cm to >30 cm in diameter. If present, measure the width, length and depth of each tumour mass using a fibreglass tape. The exact location of each mass should also be recorded. From this information, determine the fibropapilloma score (Table 3). It may be necessary to adapt this table to accurately reflect a disease state for specific areas as disease manifestation may vary with location.

Table 3. Fibropapilloma tumour score as defined by tumour size and number.

<table>
<thead>
<tr>
<th>Tumour size</th>
<th>Number of tumours</th>
<th>Tumour Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A : &lt;1cm</td>
<td>0</td>
<td>1-5 &gt;5 -</td>
</tr>
<tr>
<td>B : 1-4cm</td>
<td>0</td>
<td>1-5 &gt;5 -</td>
</tr>
<tr>
<td>C : &gt;4-10cm</td>
<td>0</td>
<td>- 1-3 &gt;3</td>
</tr>
<tr>
<td>D : &gt;10cm</td>
<td>0</td>
<td>- - 1 or more</td>
</tr>
</tbody>
</table>


Photographs of individual tumours should be taken; the site should be indicated in the image. Resolved tumours leave areas of scar tissue, the locations and sizes (length) of which should be recorded.
Figure 10. A green turtle covered in large numbers of fibropapilloma tumours.

Histologically, fibropapilloma tumours have a hyperplastic epidermis up to 30 cells thick, often with orthokeratotic hyperkeratosis and sometimes with cornified inclusion cysts, overlying a fibrous tissue core (Figure 11 a,b). Hyperkeratosis is not noted if tumours arise from non-cornified epidermal sites including the cloaca and conjunctiva. The epidermis may be thrown into papillary projections, with anastomosing rete ridges extending into the dermis. The basal cells are often vacuolated with individual cell necrosis and there can be dermal-epidermal clefting with epidermal necrosis and ulceration. Single-cell or more extensive vacuolation in the stratum spinosum may be associated with acantholysis and epidermal pustule formation. Areas of epidermal ballooning degeneration with eosinophilic intranuclear (herpesviral) inclusions have been identified in some early cases.

Figure 11. Photomicrographs of fibropapillomas. (a) The thickened (hyperplastic) epidermis (arrow) overlying a projecting, fibrous core (asterisk). Magnification x 40; (b) Epidermal inclusion cysts (arrow). Magnification x 100. Haematoxylin and eosin.
**Blood samples**

If the turtle is to be euthanased, blood should be collected ante-mortem to aid in a definitive diagnosis. Ten millilitres of blood is obtained by standard dorsal cervical fossa venipuncture using 10 mL heparinised vacutainer tubes fitted with sterile 18–21 G needles (Figure 12) as outlined by Owens and Ruiz (1980). This should be conducted prior to euthanasia. A blood smear is immediately made and air dried. At the same time, duplicated heparin coated micro-haematocrit capillary tubes may be prepared to determine pack cell volume (PCV) and total protein percentage (TPP), although this may be done later in the laboratory. The vacutainers and haematocrit tubes are labelled and placed in coolers until separating, which must occur within 6 hours of collection. To separate plasma from blood cells, samples should be centrifuged at 400 g for 3 minutes. Once separated, plasma is decanted and frozen or immediately run for plasma biochemical analysis.

**Figure 12.** Collection of blood from the dorsal cervical fossa. The fossa is located deep to the biventer cervical muscle when a needle is inserted on the medial aspect of muscles. Due to low circulatory pressure, inverting the turtle achieves increased sinus pressure.
Internal examination

Once morphometric and external examinations are completed, an internal examination may be conducted. Diagrams to assist are included in the following text. An equipment list of required tools to perform a post mortem examination is provided in Appendix IV.

The recommended sequence of dissection for an internal post mortem examination of a marine turtle is to remove and examine the plastron, forelimbs, coelomic mesentery, heart, thyroid, liver, trachea, tongue, oesophagus, coelomic cavity, lungs, urogenital system, kidneys, adrenal glands, distal aortas, central nervous system, brain, salt glands, and gastrointestinal tract. To avoid faecal contamination, it is best to remove the gut whole and to proceed with the internal examination of this system after all other tissues and organs have been examined. In addition to a standard post mortem examination, samples of the eyes, muscle, skeleton, blood and faeces may be collected for histological, toxicology, microbiology, ageing, serology and parasitology studies. It is critical that all organs be systematically examined in the same order every time.

Histology

It is critical that specimens be taken for histological examination from all organs, including those in which no gross lesions are noted. Multiple samples should be taken from larger organs, from representative sites. Many diagnoses cannot be confidently made without this comprehensive histological examination. All samples should be stored in 10% neutral buffered formalin at a ratio of ten parts formalin to one part tissue (10:1). Samples should not be crushed with forceps, and the mucosa of the gastrointestinal tract or the urinary bladder should not be handled or washed with water during collection. When encountering a lesion, ensure that both normal tissue and lesion are collected in one piece. Tissue collected should not exceed 5 mm in thickness. In general, a sample of tissue should be 20 x 20 x 3 mm in size. The exception to the above sample size is spinal cord and brain, which are collected and fixed whole for later sectioning by the pathologist.

If 10% neutral buffered formalin is not available, pure formalin (formaldehyde solution at 37-40%) may be mixed with filtered seawater at a ratio of 15 parts pure formalin to 85 parts seawater (15:85) to create a temporary buffered solution.

It is advisable that the formalin in stored samples is changed 24 hours post collection. Samples may then be stored for extended periods of time.

DO NOT freeze any samples intended for histological examination.

Toxicology

Specimens from skin, adipose, and skeletal muscle tissues and internal organs including the liver may be taken for toxicological assessment including levels of toxins, heavy metals, polychlorinated biphenyls and organochlorine compounds (e.g. dichlorodiphenyltrichloroethane (DDT). As screening can be
expensive, it is important to consult with a toxicologist prior to obtaining and storing specimens.

In general, samples taken for heavy metal analysis should be frozen and placed in separate plastic bags; whereas samples taken for pesticide analysis should be frozen and wrapped in inert packaging such as aluminium foil.

**Microbiology**

Specimens from skin, internal organs, and fluids may be taken for microbiological assessment including isolation and identification of bacteria, viruses, fungal elements and pathogenic protozoa.

Samples may be stored in medium such as agar, an aspirate or impression smear, a swab or a serum sample. All samples should be stored in a cool, dry environment. In the case of serum, blood should be collected into a clot tube so serum and red blood cells can be separated. Serum samples may be frozen long term if required at -20 °C. Assessment should be conducted as soon as possible after collection to prevent sample degradation and contamination.

Caution should be aired when sampling the gastrointestinal tract for microbiological assessment as numerous commensal bacterial species mask meaningful findings; however specific pathogens (e.g. Salmonella sp.) may be identified.

**Genetic sampling**

Blood, muscle or skin samples may be taken for genetic sequencing to aid in population and migration studies.

Samples may be collected in a variety of methods. Skin biopsies may be collected by using a biopsy punch or scalpel to remove a 5 - 10 mm section of epidermis to be stored in a NaCl-saturated solution of 20% DMSO. Five to 10 mL of blood may be collected via cardiac puncture or the dorsal cervical sinus to be either frozen or suspended in a long term storage buffer (100 mmol Tris, 100 mmol NaCl, 10 mmol EDTA.2Na, 0.5% SDS)(Dethmers et al. 2006). Muscle may be collected and stored as per skin.
Dissection for an internal post mortem examination of a marine turtle

Place the turtle in dorsal recumbency. If required, place blocks around the carapace to stabilise the carcass.

**Plastron**
Remove the plastron completely by cutting through the soft tissue at the plastron-carapace junction (junction of the marginal and inframarginal scutes) on the lateral aspects of the plastron and through the soft tissue of the ventral aspects of the limbs, neck and cloaca (Figure 13a). At this stage, note any significant gas release that may indicate putrefaction. Retract the plastron using a hook and carefully excise all soft tissue (skeletal muscle and connective tissue) as close to the plastron as possible (Figure 13 b,c). The plastron is attached to the carcass by cartilage, muscle and thick connective tissue at three points; two ‘clavicles’ (acromion processes of the pectoral apparatus) and the pelvis (lateral pubic processes of the pelvic girdle). These may be disarticulated using a knife.

Examine the plastron for evidence of trauma. Note and record the density and colour of the adipose tissue, if any. In a normal turtle in good body condition, coelomic fat should be firm, tan to grey to green to yellow, and have a thick buttery consistency. As turtles become emaciated, fat cells replace lipid with water and fat adopts a more watery consistency (serous atrophy of fat). Turtles that are losing condition undergo serous atrophy of fat; as the fat is replaced by water, pigmentation can become more apparent i.e. a darker (black) colour may be noted. The presence and severity of serous atrophy of fat should be noted.
Forelimbs
Examine the forelimbs to assess muscle mass and identify any foreign bodies. Make a circumferential incision through the skin around each forelimb (Figure 14a). Elevate the caudal aspect of the scapula (coracoid process) and excise the connective tissue of the dorsal surface of the pectoralis major muscle group for each forelimb (Figure 14b). In a healthy turtle, pectoralis major muscles should be red to pink, firm and ample. As animals become emaciated, muscles atrophy and become pale. Rotate the flipper medially. Several complete rotations should remove the flipper by tearing the connective tissues (Figure 14c). All underlying structures should be left intact (Figure 14d). Preserve the forelimbs if skeletal chronology tests or toxicology screening is to be conducted.
For skeletal chronology, strip the forelimb of muscle and connective tissue to expose the humerus. Cross-sectional samples of the mid-shaft humerus should be collected for measurement.
For toxicology screening, remove a 50 x 50 x 25 mm (if possible) piece of representative skeletal muscle. A common site for muscle harvesting is the forelimbs. In addition, collect a representative sample of cardiac muscle by excising a suitable sized full thickness piece of the ventricle. Consult
respective analytical laboratories for proper sample handling and shipping methods as these vary greatly depending on chemical being analysed.

**Figure 14. Forelimb excision.** (a) Incising through skin and muscle around the limb (arrow); (b) Elevating the caudal aspect of the scapula; (c) Rotating the limb to remove it; (d) Ventral view of a carcass with the plastron and forelimbs removed.

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**Coelomic (gastrointestinal) mesentery**

Examine the coelomic mesentery. It contains lymphatic and blood vessels. Much of the functional lymphatic system of a marine turtle is within the mesentery. Note the colour and width of the vessels; normal mesentery is nominally vascular and appears tan to light pink and smooth. Abnormal mesentery shows evidence of reaction and congestion, i.e. a red appearance with deposition of fibrin on surfaces in more severe cases. In abnormal mesentery, the vessels may be engorged and dark red to black; and the lymphatics may be distended and range form clear to dark yellow (Figure 15). Neoplastic masses may be present. If required, remove the mesentery to gain access to the internal organs.
Heart, thyroid and thymus

Examine the heart and thyroid gland in situ. Turtles have a four chambered heart comprised of the sinus venosus, one ventricle and two large atria. The thyroid gland is a translucent pink to orange spherical organ adjacent to the heart. The thymus is a grey to pink organ lying cranial and to either side of the thyroid gland.

Open the pericardial sac on the ventral aspect. The heart of a healthy turtle is bathed in small amounts (1-3 ml) of colourless to yellow clear fluid. Frank blood, large amounts of fluid, or fluid that is cloudy are all abnormal (Figure 16). There may be red discolouration of the pericardial fluid in autolysed carcasses.

Remove the heart by cutting transversely through the left and right aortae, pulmonary artery and the brachiocephalic trunk of the right aorta, if the latter is located proximally to the heart. Examine all surfaces for signs of abnormalities (parasites, tumours, pale colouration of cardiac muscle and calcium deposits/ fibrin tags). Examine the interior of each chamber and vessel via longitudinal incisions. In some cases, vascular flukes can be visualised adhering to endocardium or within clotted blood.

If there is question as to the origin of the vessels, leave the heart in situ to dissect. Record any evidence of parasite, tumours, abnormal colour, or fibrin tags.
The most common pathological findings in the heart and other regions of the cardio-vascular-respiratory systems are Spiorchiid flukes and associated lesions. The adults infect blood vessels of various organs and grossly can often be found in the heart chambers and distal aorta (Figure 17 a). Gross and histological lesions associated with these infections can include mural endocarditis, arteritis and thrombosis (Figure 17 b) that is frequently accompanied by aneurysm formation, nodules containing parasites, papillary proliferations in blood vessels (Figure 17 c), luminal calcium deposits, perivascular oedema, and infarcts. Walls of muscular arteries may be thickened in certain organs including the lungs, intestine and spleen (Figure 17 d).

There is a granulomatous inflammatory response to the eggs that exteriorises them through the vessel wall, resulting in nodules on the adventitial surfaces. Microscopically the brown-shelled eggs, that can be identified in or adjacent to blood vessels in virtually any organ, are surrounded by variably sized granulomas (i.e. infiltrates of macrophages and histiocytic multinucleate giant cells) that may be observable grossly (up to several mm in diameter) (Figure 18 a,b). As lesions associated with spiorchiid trematode parasites and eggs will be seen in most turtles, it is a matter of experience in terms of estimating
whether they are more numerous, associated with more severe inflammatory lesions or altered in distribution versus “normal background pathology” for that particular population or subpopulation.

Next, incise the thyroid gland and thymus. Note any masses or abnormalities in consistency or colour of this structure and record.

Figure 17. Lesions associated with spirorchiid trematode infection (a) Adult parasites within clotted blood (arrow) that is distending both aortae; (b) Photomicrograph of a large artery containing adult parasites, with erosion of the inner surfaces of the wall (on the right). The remaining (narrowed) lumen of the artery, containing blood, is on the left. Marked inflammatory cell infiltrates surround large numbers of dark brown eggs (arrows). Magnification x 20. Haematoxylin and eosin; (c) Photomicrograph of arteritis. Papillary projections containing mononuclear inflammatory cells (arrows) are extending into the lumen. Magnification x 100. Haematoxylin and eosin; (d) Photomicrograph of a splenic artery with a markedly thickened and inflamed muscular wall. Magnification x 40. Haematoxylin and eosin.
Figure 18. Granulomas associated with spirochid trematode eggs (a) Grossly they are observed as black, often coalescing nodules, here in large numbers on the intestinal serosal surfaces (arrows); (b) Photomicrograph of intestinal serosal granulomas showing brown eggs surrounded by inflammatory cells. Magnification x 20. Haematoxylin and eosin.

Liver
Examine the liver in situ. The liver is a large bilobed organ lying in the cranial coelomic cavity. The left lobe is attached to the stomach by the gastrohepatic ligament. The hilus attaches to the gall bladder and the duodenum. The liver should be firm, smooth, have rounded borders, and be a homogenous dark purple to brown colour. Hepatic lipidosis associated with emaciation (i.e. mobilisation of fat to the liver) and granulomatous inflammation associated with parasitism, are common histological findings. Make a small incision in the duodenum and squeeze the gall bladder to assess for patency of the common bile duct. Remove the liver including the gall bladder. Assess the liver for internal abnormalities by serially incising at 10 mm intervals. Open the gall bladder and examine the mucosal surface. Trematode parasites may be present, although this finding is rarely considered to be pathologically significant. Assess the bile for colour, density and solids. Normal bile should be dark green and clear.

Trachea (tongue and oesophagus)
Examine the tongue for plaques, ulcers, trauma, or other abnormalities. To examine the trachea, incise the skin and muscle on the ventral midline of the neck. The incision should extend from the coelomic inlet to the midline of the ramus of the mandible. At this stage, the incision should deviate to bisect both mandibular rami and follow a full thickness cut into the oral cavity along the medial ventral aspects of the left and right mandible to the symphysis. It should be noted the leatherback turtle is the only species to have a true mandibular symphysis. Retract the tongue ventrally through the incision (Figure 19 a). To advance the tongue, oesophagus and trachea past the cranium, the hyoid apparatus will need to be disarticulated. In larger turtles, this may require cutting the hyoid apparatus through the distal aspect using secateurs (Figure 19 b). Remove the oesophagus and trachea down to the
level of the thoracic cavity by freeing the soft and connective tissues (Figure 19 c).

Transversely cut the trachea at the bronchial bifurcation and open. The trachea should be white to tan with a smooth lumen. Examine and record any evidence of haemorrhage, neoplastic change, foreign bodies, or foam that may be indicative of pathology. Haemorrhage of the trachea and surrounding soft tissue may be indicative of injury sustained when on the deck of fishing trawlers or being caught in netting, where the head extends through the space of the net.

Figure 19. Removal of the tongue, oesophagus and trachea (a) Retracting the tongue through the incision; (b) Cutting the hyoid apparatus on one side using secateurs; (c) Removing the tongue, oesophagus and trachea down to the level of the thoracic cavity.

Alimentary tract
Separate the oesophagus from the trachea. Marine turtle alimentary tracts consist of oesophagus, crop (green turtles from certain geographic areas only), stomach, duodenum with adjacent spleen and pancreas, jejunum, ileum, caecum (proximal colon), colon and rectum (Figure 20).

Remove the entire digestive tract from its points of attachment by use of blunt dissection. Place surgical forceps as caudally as practical along the tract. Make a transverse incision through the colon immediately caudal to the forceps. The gastrointestinal tract should be removed and laid out ready for examination. Estimate and record gut fill (as a percentage of an entirely filled gut). Assess for evidence of intussusceptions, strictures, blockages, plications, fishing line ingestion torsion and parasites.

Sections should be taken from all gastrointestinal segments for histological examination. It is important to avoid washing those specimens with water, and to avoid touching the mucosa with hands or instruments as it is easily destroyed. For intestinal specimens, cut out an intact segment approximately 0.5 mm in length from each region prior to opening the entire gastrointestinal
tract from the oesophagus to the colon using gut scissors. Ensure the stomach is opened along the lesser curvature. Estimate the volume, collect and record digesta samples from each section of the gastrointestinal tract. Collect tissue samples from each section of the gastrointestinal tract including associated organs (spleen and pancreas).

Figure 20. The alimentary tract of a green sea turtle.
Parasite (spirorchiid trematode) eggs are often identified as dark raised masses 2-3 mm in diameter as noted above (Figure 18). Spirorchiid trematodes and coccidia (protozoan) are the two most commonly reported internal parasites, however nematodes have previously been reported in the gastrointestinal tracts of loggerhead turtles; diagnosis of such infections may require parasitological examination of gut content samples and histological examination of tissues (Figure 21), therefore where possible, collect faecal samples in addition to the standard tissue samples.

**Figure 21.** Coccidial (Caryospora cheloniae) elements in the thickened crypt epithelium of the intestinal tract of a green turtle. Very large numbers of various stages are noted within the epithelial cells (arrows). Magnification x 200. Haematoxylin and eosin.

The presence of Coccidia and Trematode (Sporocheilidae) parasite burdens can be determined by use of faecal floatation and Baermann funnel techniques, respectively. Following examination of organs and collection of samples for histology, each organ should be serially dissected at approximately 10 mm intervals and irrigated with saline solution to flush eggs, larvae and adults into a separate collection vessel for each organ. Similarly, the coelomic cavity should be irrigated and collected to catch parasites seeping from vessels when organs are removed. The contents of the saline are allowed to sediment over 15 - 30 minutes. Supernatant is decanted to leave the sediment and placed in a shallow clear dish for scanning under a dissecting microscope. By use of a pipette, any adult, larval or egg parasites can be extracted and placed into preservative. It is not recommended sieves are used to extract specimens as this method may cause damage to the microstructure of the parasite.
It is recommended 70-98% ethanol is used as a preservative, as ethanol provides superior storage if genetic sequencing (PCR) is being undertaken when compared with storage in formalin, as well as optimising the likelihood of morphological structures remaining undisturbed. If ethanol is unavailable, formalin may be used only if the parasites are gently pressed during fixing; otherwise this preservative may cause contraction of morphological features. Preservation in formalin decreases the success of genetic analysis. When properly preserved, samples may be stored indefinitely.

**Oesophagus**
Examine the oesophagus. The oesophagus extends from the tongue to the crop in some green turtles, or the tongue to the stomach in most marine turtles. Both external and internal surfaces should be white to tan. The lumen mucosa has numerous sharp, keratinised papillae (spines) (Figure 22). These spines should be directed caudally towards the stomach, and rigid. In turtles that are in poor condition, spines may be flaccid. Inspect for foreign bodies such as fishing line and hooks that may be embedded in the mucosa.

**Crop (some green turtles only)**
Examine the crop (Figure 22). The crop is a pouch of the oesophagus proximal to the stomach. Unlike a bird’s crop, which in some species serves to secrete nutritional material for squab, a turtle crop functions solely for retention of food prior to entry into the stomach. Both external and internal surfaces should be tan. Inspect for foreign bodies such as fishing line and hooks embedded into the mucosa or retained marine debris.

*Figure 22. Oesophagus of a green sea turtle with numerous mucosal spines (arrow), leading into the crop.*
Stomach
Examine the stomach, described by the cardiac sphincter proximally and the pyloric sphincter distally, as in other species. It is the first part of the gastrointestinal tract responsible for the digestion of food. Both internal and external surfaces should be tan. The internal surface should have smooth transverse ridges (rugae) (Figure 23). Inspect for foreign bodies such as fishing line and hooks embedded into the mucosa or retained marine debris, and areas of ulceration or inflammation.

Duodenum with spleen and pancreas
Examine the duodenum. The duodenal mucosa is a “honeycomb” complex that is white to tan (Figure 23) and produces mucous. At the proximal end, the ampulla of Vater (bile duct opening) should be detected and the duct assessed for patency (by squeezing the gall bladder) if not done previously. Discolouration (by bile i.e. green) occurs at this site in normal healthy marine turtles. Inspect for any abnormalities.

Figure 23. The mucosal surface of the stomach, showing rugae. Note the honeycomb appearance of the duodenal mucosa (asterisk), following the pyloric sphincter (arrow).
Serially incise the spleen (attached to the duodenum; Figure 20) at 10 mm intervals. It is round and usually firm, smooth and tan to red. Assess for gross abnormalities including neoplasia, pale areas, dark spots (often a sign of presence of trematode eggs), or roughened surfaces. Microscopic changes include inflammatory changes, most of which are centred on spiorchiid trematode eggs and/or bacteria (Figure 24).

Examine the pancreas. The pancreas is closely associated with the spleen and lies distally to the duodenum. It is usually shiny, flat, thin, lobulated and pink to tan. Feel for inconsistencies in texture due to masses, parasites or decomposition. Inspect for areas of colour change and inflammation. It should be noted that the pancreas decomposes particularly rapidly due to the presence of digestive enzymes; inflammatory changes may be difficult to recognise.

Figure 24. Photomicrographs of splenic tissue (a) Pyogranulomas centred on bacterial colonies (basophilic material indicated by arrows). Magnification x 100; (b) Large numbers of granulomas (arrows) centred on brown spiorchiid trematode eggs. Magnification x 40. Haematoxylin and eosin.

Jejunum and ileum: Examine the remaining small intestine. The jejunum and ileum are distal to the duodenum and proximal to the caecum. On both the internal and external surfaces, the transitions from duodenum to jejunum to ileum are difficult to discern grossly or histologically. Assess as per the duodenum.

Caecum
Examine the caecum. It lies distal to the ileum (described by the ileocaecal valve) and can also be termed the proximal colon. Comparatively it has a greater diameter than the rest of the colon (Figure 20). Both internal and external surfaces should be smooth and tan. Inspect for areas of colour change and inflammation.

Colon
Examine the colon. It lies distal to the caecum and proximal to the rectum. Both internal and external surfaces should be smooth and tan to white. Inspect for areas of colour change and inflammation.
Coelomic cavity
Examine the coelomic cavity for excessive fluid (e.g. more small pools on either side of the spine when the animal is placed in dorsal recumbency). Estimate the volume, viscosity, translucency and colour of any fluids present. Account for any vascular seepage of blood post heart removal. Collect samples of the fluid if indicated or if parasites are being sought. Drain the fluid (by rolling the animal on its side and evacuating excess fluid) to allow visual inspection of the internal organs connected to the dorsal surface of the coelom.

Examine the coelomic surfaces for evidence of trauma. Estimate the depth of fat reserves lining the coelomic cavity. A healthy turtle has green to tan coloured adipose tissue. Turtles in poorer condition have mobilised fat reserves giving the appearance of watery to jelly like adipose tissue. Unhealthy turtles may have black fat.

Lungs
Examine both lungs in situ. The lungs lie bilaterally along the dorsal wall of coelomic cavity extending approximately two thirds of cavity length (Figure 25). They are attached to the carapace.

Figure 25. The lungs, observed attached to the underside of the carapace following the removal of other viscera.

The lungs should be pink with a spongy consistency. Serially section each lung at 10 mm intervals. Assess for any abnormalities including neoplastic masses, areas of discolouration, dense consistency, and increased amounts of foam or pulmonary haemorrhage. Examine the bronchi by opening as per the trachea. Assess for abnormalities. The lungs are common sites of
inflammatory lesions including those associated with bacterial or parasitic infection; many may only be identifiable histologically (Figure 25 a,b).

Urogenital system
Examine the urogenital system (kidneys are described separately).

The urinary bladder is a thick walled sac found within the pelvic girdle (Figure 26). It may contain both urine and mucous. Make a ventral incision through the wall. Inspect for volume and consistency of the urine, calculi, and the texture and colour of the mucous. Mucous colour may vary from clear (normal) to fluorescent green (abnormal). The mucosa should be tan but irregular dark mottling may be noted in normal animals. Parasites may colonise the bladder wall. Presence should be noted.

Figure 26. Urinary bladder and kidneys (white arrows) following removal of other viscera.

Trace the ureters from the caudal kidneys to the urinary bladder. Ureters are thin ducts that are white to tan. Assess for any abnormalities. Hyperplasia of mucosa is the most commonly noted microscopic change of the genitourinary tract. Similarly, parasitism may cause granulomatous inflammation (Figure 27).
The gonads of turtles are extensive and may extend from a point cranial of the kidney to the level of the urinary bladder. They tend to be located along the dorsal surface of the coelomic cavity but are mobile depending on breeding status. In both sexes, the gonads are tan, but vary depending on maturity and breeding status. Inspect for texture, colour changes and neoplastic masses. Estimate sex, maturity and breeding status. If blood may be obtained ante-mortem or immediately post mortem, the sex of immature turtles may be determined by hormone assays. Immature male marine turtles have an elevated concentration of testosterone (>20 pg/mL) when compared with females (<10 pg/mL). However, assays are expensive to run. During post mortem examination, anatomical cues offer more accurate sex determination and breeding status techniques.

**Female**

The female reproductive tract has the same structures of other vertebrate species: paired ovaries, oviducts and suspensory (ovarian) ligaments (Figure 28 a,b). In immature female turtles, the ovaries appear tan to pink with a grainy structure formed by tiny follicles, and white straight oviducts lying laterally to each ovary. At sexual maturity, mature (bright yellow) follicles will tend to cluster on the cranial aspect of the ovary (Figure 28 a). These follicles may be greater than 2 cm. Mature female turtles that have nested will have large follicles and scar tissue. Scar tissue is classed as recently atretic corpora luteum (yellow body) becoming corpora albicans (white body).
Figure 28. Female reproductive tract (a) The ovary of a mature female containing immature (small yellow) and mature (large yellow) follicles, and corpora albicans (white bodies); (b) The oviduct (arrows).

Male
Like other vertebrate males, male marine turtles have paired testes, an epididymis, ductus deferens, a suspensory ligament, and a penis. In immature male turtles, the testes appear tan to pink with convoluted tubules. The epididymis tracks along the body wall. At sexual maturity the testes, epididymis and ductus deferens change in shape and size. The epididymis becomes pendulous and distends from the body wall. Mature breeding males have testes grossly filled with white fluid (sperm and accessory gland product).

Kidneys
Examine the kidneys. The kidneys are situated bilaterally on the dorsal surface of the coelomic cavity either side of the vertebral column (Figure 26), caudal to the lungs. They are retrocoelomic and lack a distinct cortex and medulla grossly. Incise the coelomic lining and use blunt dissection to expose the kidneys. The kidneys should be lobular red to dark brown structures. Serially section each kidney at 10 mm intervals. Assess for texture, calculi, colour changes and neoplastic masses. Nonspecific inflammatory lesions and fibrosis are common microscopic changes noted in kidneys.
**Adrenal glands**

Examine the adrenal glands, which are located at the cranial end of the kidneys between the lungs and kidney (Figure 29). They may be located by palpation. The adrenals are small, yellow-orange elongated amorphous structures. Inspect for texture, colour change and neoplastic masses.

**Figure 29. Adrenal glands within adipose tissue just caudal to the lungs (asterisks) and adjacent to the cranial poles of the kidneys (white arrow). In the image on the right, the adrenal glands, following excision of the fatty tissue, are small, yellow-orange structures (asterisks).**
**Distal aortas**

With the gastrointestinal tract removed, the white to tan coloured distal aortas should be clearly visible along the dorsal wall of the coelomic cavity immediately lateral to either side of the vertebral column (Figure 30).

Variation occurs between individuals. The left and right distal aortas may anastomose at any point from immediately caudal of the heart to cranial of the kidneys. The anastomosis forms a single dorsal aorta. Open the vessels longitudinally. Inspect for luminal calcium deposits, nodules (parasites), clots and constrictions. Microscopic changes are discussed under cardio-vascular-respiratory changes.

**Figure 30.** Distal aortas, overlying the lungs from a ventral view. The paired vessels anastomose in the mid-body of this animal (red arrow). The female genitourinary tract is also present in this specimen.
**Eyes**
Examine the eyes for fibropapillomas, corneal changes or trauma. Remove by placing a circumferential incision through the skin then cutting the retractor muscles using curved scissors. Cut through the optic nerve and remove the eye. For histological examination, make sure the eyelids and ocular muscles have been removed, and place the globes into 10% neutral buffered formalin whole.

**Central nervous system and brain**
Secure the head manually or in a vice so that the cranium is facing up. Using a saw or a hacksaw, make a transverse cut immediately caudal to ocular orbits. The cut should align at the rostral edge of the frontoparietal scale (Figure 31 a, b).

**Figure 31. Incision of the head to for removal of the brain (a) The incision site, immediately caudal to the orbits and at the rostral edge of the frontoparietal scale; (b) Extending the cut.**

Extend the cut ventrally to the mandible. Remove the rostral section of the cranium. The brain and salt glands should be exposed; the brain lies midline with two large salt glands laterally on either side (Figure 32). The brain should be firm, homogenous, white to tan and relatively small in comparison to the size of the skull.
Remove the brain by carefully elevating it and cutting the cranial nerves using curved scissors. Every attempt should be made to include the pituitary gland (within the infundibulum). Inspect for evidence of infarction, abscesses or granulomatous inflammation (the latter usually associated with spirorchiid parasite eggs that themselves are not large enough to be grossly visible).

Remove the head by locating the intervertebral disc space between C3-C4, and circumferentially incising all muscles and ligaments around this joint. Disarticulate the joint by cutting intervertebral attachments while rotating the head.

Remove a section of the cervical spinal cord at the site of disarticulation and examine for inflammatory changes (typically associated with spirorchiid parasites).
Salt glands
Examine the salt glands; these should be the predominant structure visualised when examining the skull rostro-caudally. Salt glands function to aid in osmoregulation. They should be firm, lobular and pink to brown. Assess for abnormalities such as pale spots or calculi. Mild granulomatous inflammation associated with spirorchiid eggs is the most common histological change noted in this structure (Figure 33).

Figure 33. Salt gland lobules. Inflammatory changes centred on spirorchiid trematode eggs (arrows) are often noted in stromal tissue around central ducts. Magnification x 20. Haematoxylin and eosin.

A final checklist for samples required for histological examination is provided in Appendix V.
### Appendices

**A-I Basic terminology used in this guide**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Axillary scale</td>
<td>The first scale on the proximal caudal aspect of each forelimb.</td>
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<tr>
<td>Caecum</td>
<td>A pouch located at the proximal end of the colon for the retention and digestion of digesta; also referred to as the proximal colon.</td>
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<tr>
<td>Carapace</td>
<td>The dorsal aspect of the shell of the turtle. Comprised of moulded scutes.</td>
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<tr>
<td>Caudal margin of the suture of the post-central scutes</td>
<td>Posterior aspect of the joint between the last left and right scute of the carapace.</td>
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<tr>
<td>Coelomic cavity</td>
<td>The internal area of the thoraco-abdomen encased by the carapace, plastron, and pectoral and pelvic girdles. Marine turtles do not possess a diaphragm. Thoracic and abdominal organs are distributed within this space. It is bounded by soft tissue. Referred to as body cavity in some references.</td>
</tr>
<tr>
<td>Crop</td>
<td>A muscular evagination of the distal oesophagus devoid of mucosal spines and digestive glands. Found in some green turtles only. It is assumed to be a reservoir for food. Its function is not known.</td>
</tr>
<tr>
<td>Fibropapilloma</td>
<td>A cutaneous tumour found on skin, eyes, keratinised tissue, in the oral cavity and occasionally internally. They are strongly associated with a herpes virus, but Koch’s postulates have not been proven. This disease is currently present in epidemic proportions throughout many regions of the world, including Australia. (putative cause: Chelonid Fibropapilloma-associated Herpes Virus- FPHV)</td>
</tr>
<tr>
<td>Marginal scute</td>
<td>The lateral scutes of the carapace</td>
</tr>
<tr>
<td>Plastron</td>
<td>The ventral aspect of the shell of the turtle. Comprised of moulded scutes that are less keratinised to that of the carapace scutes.</td>
</tr>
<tr>
<td>Scute</td>
<td>Keratinised plates found on the carapace and plastron overlying dermal tissue of all the species of marine turtles, except the Leatherback; which has thickened areas of the dermis.</td>
</tr>
<tr>
<td>Skin/carapace junction</td>
<td>The interface between the dermis and the keratinised scutes forming the carapace.</td>
</tr>
</tbody>
</table>
A-II Relevant authority for recovered tags

Australia

Queensland (East Coast)
Environmental Protection Agency/ Queensland Parks and Wildlife Service
PO Box 15155
Brisbane, Queensland 4001, Australia

Western Australia (West Coast)
Department of Environment and Conservation
Locked Bag 104
Bentley Delivery Centre, Western Australia 6983, Australia

United States of America
Department of Commerce, NOAA National Marine Fisheries Service
Silver Springs, MD, USA

Department of Interior, US Fish and Wildlife Service
Washington, DC, USA

South Pacific Islands
South Pacific Regional Environment Programme (SPREP)
PO Box 240
Apia, Samoa
# MARINE TURTLE NECROPSY DATASHEET

**Date/Place Collected:** __/__/__  
**Date/Place Necropsied:** __/__/__

### Data

<table>
<thead>
<tr>
<th>Clinician:</th>
<th>Clinic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tel. no.:</td>
<td>Fax no.:</td>
</tr>
<tr>
<td>File number:</td>
<td>Animal ID:</td>
</tr>
<tr>
<td>Species:</td>
<td>Breed:</td>
</tr>
<tr>
<td>Age:</td>
<td>Sex:</td>
</tr>
<tr>
<td>Weight:</td>
<td>CCL:</td>
</tr>
</tbody>
</table>

### History


### Necropsy Examination Findings: (make notes where appropriate)

<table>
<thead>
<tr>
<th>Body Condition:</th>
<th>Post Mortem Condition:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>External Examination</strong></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td></td>
</tr>
<tr>
<td>Celomic cavity</td>
<td></td>
</tr>
<tr>
<td>Heart &amp; Vessels</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Trachea, Lungs</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
</tr>
<tr>
<td>Kidneys, Adrenal Glands</td>
<td></td>
</tr>
<tr>
<td>Gonads</td>
<td></td>
</tr>
<tr>
<td>Thyroid Gland, Thymus</td>
<td></td>
</tr>
<tr>
<td>Oral Cavity</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td></td>
</tr>
<tr>
<td>Crop</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
</tr>
<tr>
<td>Small Intestine</td>
<td></td>
</tr>
<tr>
<td>Large Intestine</td>
<td></td>
</tr>
<tr>
<td>Urinary Bladder</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>Salt Glands</td>
<td></td>
</tr>
</tbody>
</table>

**OVERALL GUTFILL (%):**

### SAMPLES & PHOTOS: (list and/or circle)

<table>
<thead>
<tr>
<th>Formalin</th>
<th>Frozen</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photos</td>
<td>Ventral</td>
<td>Dorsal</td>
</tr>
</tbody>
</table>
A-IV Equipment required
Before commencing the post mortem examination, ensure that the following equipment is available. For certain samples (e.g. microbiology, toxicology), it may be necessary to have a separate set of tools available to prevent cross-contamination.

- PPE- disposable/rubber gloves, safety goggles, mesh gloves, overalls, apron, steel capped boots
- Pencil and indelible marker
- Paper and labels
- Post mortem examination datasheet
- Digital camera
- Graduated reference scale that animal identification number may be written on
- Fibreglass measuring tape- 150 cm minimum length
- Vernier or standard ruled callipers
- Needle nosed pliers
- Microchip scanner
- Scales- 50 kg, 100 kg, 500 kg
- Scalpel blades and handles
- Forceps- surgical and dissecting (rat-toothed)
- Scissors- gut, surgical and curved
- Post mortem knives- boning and pointed
- Hook
- Bone saw
- Secateurs
- Cutting board
- Sample jars (50 mL and 500 mL) with 10% neutral buffered formalin
- EDTA and serum-clot vacutainers, microscope slides, 21 & 18 G needles, 10 mL syringes
- Small esky containing ice
- Aluminium foil and chemically clean glass jars
- Plastic bags (15 cm x 17 cm)
A-V Checklist of samples required for histopathological examination

At the conclusion of the post mortem examination, ensure a minimum dataset of the following tissue samples has been collected.

- Heart
- Aortic tissue
- Adrenal glands
- Thymus
- Thyroid gland
- Liver
- Gall bladder
- Tongue
- Oesophagus
- Crop
- Stomach
- Duodenum
- Spleen
- Pancreas
- Jejunum
- Ileum
- Caecum
- Colon
- Trachea
- Lung
- Urinary bladder
- Kidneys
- Gonads
- Spinal cord
- Brain (entire including pituitary gland)
- Salt glands
- Eyes
- Skeletal muscle
- Skin
References


