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Pathological findings in wild harvested dugongs *Dugong dugon* of central Torres Strait, Australia

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ABSTRACT: The dugong *Dugong dugon* is classified as Vulnerable to extinction but may be endangered in some regions. Cause of death in stranded dugongs has not been determined in a large proportion of animals examined, with investigations hindered by limited information on dugong health and diseases, and paucity of knowledge of common or endemic pathological findings. Here we describe pathological findings in harvested dugongs from the relatively pristine area of central Torres Strait, and we characterise lesions attributable to drowning. Other recorded lesions were mild and predominated by host reaction to the presence of trematodes within the gastrointestinal tracts, liver and pancreas. Ascarid worm burdens were low in comparison to dugongs from developed coastlines. Hepatocellular lipofuscin and ferritin pigmentation were commonly observed, more pronounced in livers of older animals and concurrent with periportal and bridging fibrosis. Lesions attributable to drowning included incomplete collapse of lungs, dorsal or diffuse pulmonary congestion, mild intra-alveolar haemorrhage and oedema, mild interstitial oedema and rupture of peripheral alveolar septae with acute myofibre fragmentation and degeneration. No accumulation of foam or aspiration of water or particulate matter was observed, suggesting that dugongs 'dry drown'. Morphometric features of normal spleen are also presented. Characterisation of common pathological findings and those attributable to drowning in this species will aid in the interpretation of post mortem findings for the significant number of dugongs found deceased along urbanised coastlines.

KEY WORDS: Pathology · Sirenia · Necropsy · Drowning · Parasitology · Organs

INTRODUCTION

The dugong *Dugong dugon* is classified as Vulnerable to extinction by the International Union for Conservation of Nature (IUCN 2013), but may be endangered at the regional level (Marsh et al. 2011), with populations declining in at least a third of its global range (Marsh & Kwan 2008). The present distribution of dugongs in Australia extends from urbanised southeast Queensland north and west to the Shark Bay World Heritage site in Western Australia; however, small numbers of dugongs may be sighted farther south along the east coast during the summer months (Allen et al. 2004).

A variety of anthropogenic threats (e.g. coastal development, netting operations), as well as natural and climatic events (e.g. coastal flooding, cyclones, and freshwater discharges), have contributed to degradation of seagrass habitat and/or declines in dugong numbers in Australia (Marsh et al. 2011, Meager & Limpus 2014). In response to population decline, health assessments are conducted in Queensland to monitor live populations of dugongs along the most developed coastlines (Lanyon et al.

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2010). These are complemented by opportunistic analysis of stranded carcasses (Eros et al. 2007, Greenland & Limpus 2010). Carcass salvage is inherently biased, identifying the incidence of mortality and not health-related morbidity (Wobeser 2006); however, a number of natural and anthropogenic threats to dugong health have been identified from this approach. The most obvious and widespread health problem in recovered dead dugongs appears to be starvation after incidents of food shortage (Marsh et al. 2002, Marsh & Kwan 2008). On several occasions, higher than usual mortalities of dugongs have followed severe weather events (including cyclones, storms and widespread coastal flooding; Meager & Limpus 2012) that have impacted seagrass abundance (Preen & Marsh 1995) and/or quality (Longstaff & Dennison 1999). Gross indicators of starvation including poor body condition, wasting and atrophy of blubber stores have been documented in recovered carcasses (Meager & Limpus 2012).

Pathological surveys conducted in Queensland have identified trauma (particularly boat strike and net entanglement), bacterial pneumonia, septicaemia and salmonellosis as primary or contributing causes of death (Elliott et al. 1981, Owen et al. 2012, Nielsen et al. 2013). A wide spectrum of trematode-associated disease is frequently reported in dugongs (Blair 1981, Ladds 2009), along with confirmed incidences of *Cryptosporidium parvum* (Hill et al. 1997, Morgan et al. 2000) and *Toxoplasma gondii* (Greenland & Limpus 2010, Owen et al. 2012).

Apart from these few studies, cause of death has not been determined in a large proportion of dugong carcasses examined (Greenland & Limpus 2010, Owen et al. 2012); investigations can be hindered by advanced decomposition of salvaged carcasses, and are often hampered by limited available information on dugong health, physiology and response to diseases (Elliott et al. 1981, Eros et al. 2007, Ladds 2009), as well as a paucity of knowledge of lesions commonly found in otherwise healthy dugongs. In some cases, extrapolations of symptoms have been made to closely related species within the order Sirenia (Rowlatt & Marsh 1985, Ladds 2009). Baseline or benchmark information on health and disease indices for dugongs in non-urban areas is required.

The objectives of this study were to determine the common pathological findings in dugongs harvested from the relatively pristine area of central Torres Strait, Australia, with the aim of establishing accurate baseline gross morphological and histopathological datasets from normal, apparently healthy, wild dugongs in non-urban waters. An additional objective of this study was to characterise lesions associated with drowning, to assist in investigations of mortalities in stranded dugongs. Some discussion is also provided on the microanatomy of dugong respiratory tract and lymphoid organs, complementing previous anatomically focused studies on the normal macroscopic and histological features of dugong anatomy (Engel 1962, Marsh et al. 1977, 1984a,b, Rowlatt & Marsh 1985).

MATERIALS AND METHODS

Post mortem examination

Six dugongs hunted legally for human consumption were examined on Mabuiag Island, a Western Island located approximately 100 km north of Thursday Island in central Torres Strait (9° 57′ S, 142° 11′ E). Necropsies occurred between 19 and 26 April 2011. The on-site research team was notified by local Traditional Owners (rangers or hunters) of the availability of fresh carcasses. All animals were hunted by current indigenous methods: during a night-time hunt, dugongs were harpooned with a detachable 3-pronged head (wap) from a boat, secured by rope and then drowned. The dugongs died from drowning within a few (3–5) minutes.

Body condition and external body measurements were assessed by standard methods (Eros et al. 2007, Lanyon et al. 2012). Carcass decomposition was assessed on the basis of categories (fresh to decomposed) outlined by Geraci & Lounsbury (2005), and field post mortem technique for carcass processing by traditional hunters was based on published guidelines (Eros et al. 2007). Tissue samples from the major organ systems and any lesions detected were collected into 10% neutral buffered formalin within 9 h of death for the purpose of histopathology; these included trachea, lung, pulmonary lymph node, oesophagus, stomach, small and large intestines, caecum, mesenteric lymph nodes, pancreas, liver, kidney, spleen, skeletal muscle, skin, reproductive tract and brain. Heart was not available for histopathological examination. When permanent tusks were available, minimum age (years) was determined according to counts of growth layer groups, following Marsh (1980).

Histopathology

Formalin-fixed tissues were trimmed to 5 mm thick sections and embedded in paraffin blocks by routine histopathological techniques. Tissues were sectioned at 5 µm and stained with Harris's haematoxylin and eosin (HE). Selected sections were also stained with periodic acid Schiff (PAS), Gram, Trichrome, Giemsa, Reticulin, Masson PTAH, Ziehl-Neelsen (ZN) or Miller's Elastic stain using standard techniques. Images of sections were taken using an Olympus DP25 camera mounted on an Olympus BX43 microscope and captured with labSensTM software (Olympus).

Immunohistochemistry

Immunohistochemistry on spleen and lymph node tissue samples was performed using lymphocyte markers CD3 (polyclonal rabbit anti-human, Dako), CD79a (monoclonal mouse anti-human, Dako) and CD20cy/L26 (monoclonal mouse anti-human, Dako) using a 3,3-diaminobenzidine (DAB) immunoperoxidase technique. Positive control tissue was human lymph node. For negative controls run in parallel, the primary antibody was omitted or replaced with homologous sera.

Histomorphometric analysis

Splenic white pulp and follicle size were assessed using a method described for evaluation of lymphoid depletion in harbour porpoises *Phocoena phocoena* (Beineke et al. 2007). Area and perimeter of lymphoid follicles within spleen sections were measured using a free-hand polygon tool with the LabSensTM software package. All visible white pulp in each section of spleen was evaluated. Mean follicle area, mean follicle perimeter measurements and standard deviations were calculated using Microsoft Excel 2010®.

Parasitology

Gastrointestinal parasites visible at post mortem were collected into 70% ethanol. Faecal floats for the detection of parasitic ova were performed using 5 g of fresh rectal contents from each dugong and processed using the Wisconsin technique with Sheather's saturated sugar solution (Zajac & Conboy 2012).

RESULTS

Six dugongs comprising 1 pregnant female, 2 adult males, 2 sub-adult males and 1 male calf (Table 1) were examined by necropsy. All carcasses were scored as 1 (fresh). All dugongs were in good to excellent body condition. The skin of all animals was clean and there were no algae, and only a few ($n \leq 5$) small barnacles were attached to the skin of one of the adult males. All dugongs had variable numbers of linear healed tusk rake scars distributed over the dorsal and/or lateral body (Athousis 2012). In the 3 adults, coalescing blotchy congestion or petechial and ecchymotic haemorrhages were present throughout the skin and subcutis of ventral neck, abdomen and peduncle.

Respiratory system

Grossly, the lungs of all 6 dugongs were incompletely collapsed, pale pink and sponge-like on palpation, and sections floated when placed in 10% neutral buffered formalin. No water, foreign material or foam was identified in the larynx, trachea or primary bronchi. In 1 animal, the tracheal and bronchial mucosa were expanded by extensive haemorrhage, congestion and oedema, and covered by a white pale fibrinous exudate (dugong 2, Fig. 1a). Mucosal con-

Table 1. Sex, maturity class, body measurements (straight body length [BL], fluke width [FW], girths at each of peduncle, anal, maximum [umbilicus] and axilla), body condition (BC) and maximum post mortem (PM) interval (i.e. maximum time elapsed since death) for 6 wild dugongs *Dugong dugon* harvested in central Torres Strait, Australia. All carcasses were fresh (condition score = 1; Geraci & Lounsbury 2005). nd: not determined

Dugong ID	Sex	Maturity class	Erupted tusks	BL (cm)	FW (cm)	Girth (cm) Peduncle	Anal	Max.	Axillary	BC score	Max. PM interval (h)
1	F	Adult, pregnant	No	246	78	46	112.5	193	153	Good	6
2	M	Adult	Yes	242	92	47	109	196	154	Good	7
3	M	Adult	Yes	224	77	44	99.5	168	nd	Excellent	9
4	M	Calf	No	144	54	38	66	116.5	nd	Excellent	6.5
5	M	Sub-adult	No	191	80	42	95	164.5	nd	Very good	5.5
6	M	Sub-adult	No	210	75	42	90.5	nd	nd	Good	5.5

gestion and petechiae were noted in the trachea and primary bronchi of 2 other individuals. Mild to moderate dorsal or diffuse pulmonary congestion was noted in 5 of 6 dugongs (Fig. 1b).

Histologically normal dugong tracheal mucosa was arranged in elongate villous-like folds that projected into the tracheal lumen, supported by a fine fibrovascular lamina propria. In the primary bronchi, villous projections were reduced to an undulating mucosa lined by pseudostratified ciliated columnar epithelium interspersed with low numbers of mucus-secreting goblet cells, and a prominent muscularis layer of longitudinally and circularly oriented smooth muscle bundles. Mild to moderate eosinophilic, heterophilic and lymphoplasmacytic infiltrates were present in the tracheal and primary bronchi mucosae of all dugongs examined, and mild to moderate epithelial hyperplasia and squamous metaplasia was observed in dugong 6. In dugong 2, tracheal lesions seen grossly were characterised by a moderate to marked exudative fibrinosuppurative and haemorrhagic tracheitis (Fig. 2); however, no aetiological agents were observed.

In all dugong lungs examined, there was mild to moderate diffuse pulmonary congestion, mild interstitial oedema, infrequent diapedesis or intra-alveolar haemorrhage, and rupture of alveolar septae with club-like contraction of septal myofibres, particularly in large air sacs located at the lung lobe periphery, i.e. alveolar emphysema (Fig. 3a), with distortion of myofibres and rare pyknosis of myocyte nuclei at ruptured septal tips, interpreted as myocyte fragmentation and degeneration (Fig. 3b). Multifocal minimal to mild infiltrates of macrophages, eosinophils and lesser heterophils were identified within bronchiolar and alveolar luminae or septal interstitium in all dugongs. Lungs with heavier heterophilic inflammatory infiltrates as seen in dugongs 3 and 5 were associated with prominent bronchiolar-associated lymphoid tissue. No infectious agents were observed in lung sections examined.

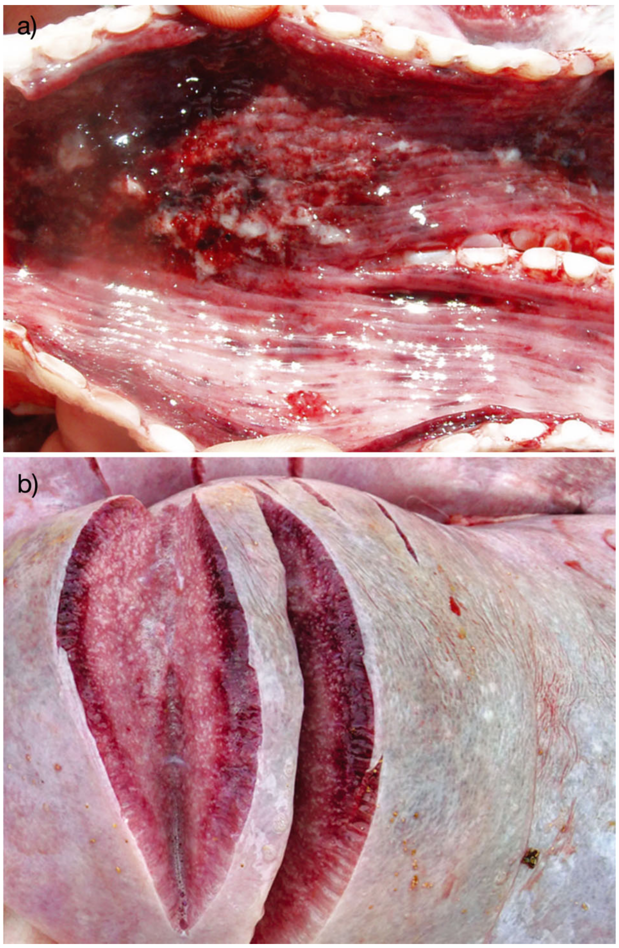

Fig. 1. (a) Dugong 2, trachea: the mucosal lining of the trachea is expanded by haemorrhage, congestion and oedema and covered by a white fibrinous exudate, suggesting pre-existing subacute or chronic-active inflammation. (b) Dugong 6, lung: dark red discolouration (congestion) of dorsal lung parenchyma, an acute change

Gastrointestinal system

The stomachs of all dugongs examined were filled with fresh, partially digested seagrasses. Between 30 and 50 large ascarid nematodes, interpreted as *Paradujardina halicoris*, were observed grossly in the

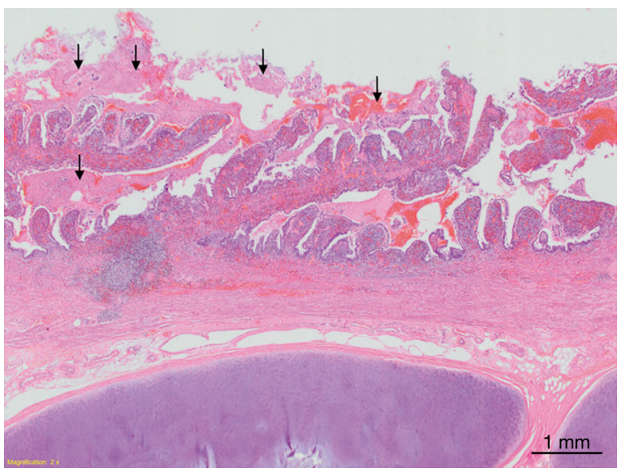

Fig. 2. Dugong 2, histopathological findings in trachea. There is mucosa infiltration by eosinophils, heterophils, lymphocytes and plasma cells, indicating pre-existing chronic and active inflammation. Overlying the mucosa is a layer of fibrin, protein, exfoliated epithelial cells, haemorrhage and inflammatory cells (arrows)

gastric lumen and/or cardiac gland (the gastric acid-secreting portion of the stomach) of 3 dugongs (pregnant female 1, and sub-adult males 5 and 6). Faecal parasitological floats for dugongs 1, 5 and 6 identified low numbers (<2 eggs g^{-1} of faeces) of large embryonated thick-walled ascarid eggs measuring approximately 135 µm in diameter. A single ascarid was observed in the gastric lumen of adult male 2 (no eggs observed in faecal egg float), and no ascarids were seen in either the gastric lumen or cardiac gland of each of adult male 3 and the male calf 4. The presence of parasites in the gastric or cardiac gland mucosae was not associated with inflammation in the adjacent mucosa. Submucosal lymphoid follicles were prominent, and mild interstitial lymphoplasmacytic infiltrates were seen in the gastric mucosa of all dugongs examined.

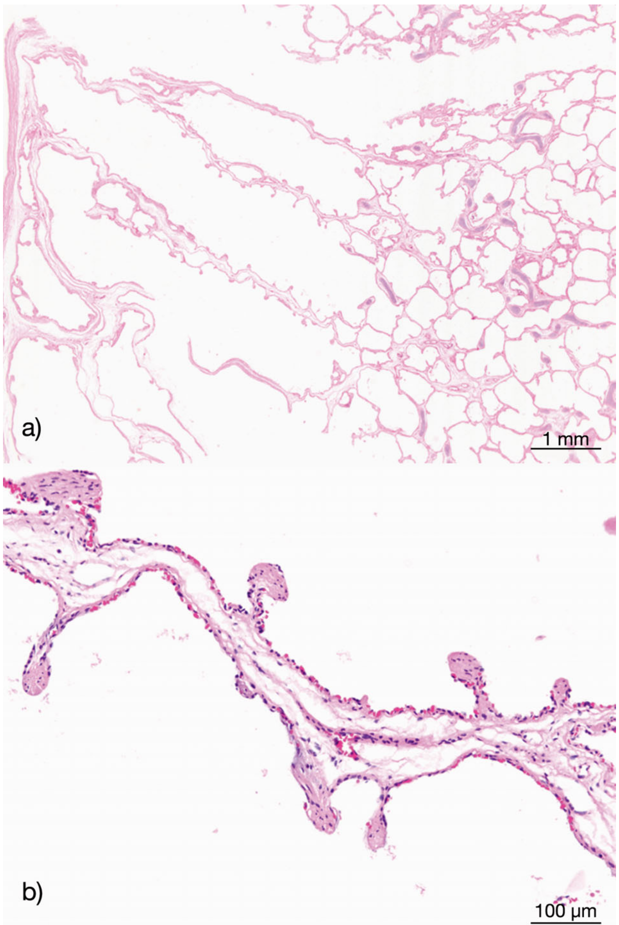

Fig. 3. Dugong 3, lung. (a) Acute rupture of alveolar septae with club-like contraction of septal myofibres, particularly evident in sections from the edges of lung lobes. (b) Nuclear condensation (pyknosis) of myocyte nuclei at ruptured septal tips, interpreted as acute myocyte degeneration

Individual or paired flattened trematode parasites measuring up to 500 µm in cross section and containing many operculated thin-shelled eggs (10 × 15 µm) or testes with spermatozoa were seen in the cardiac glands of 4 dugongs (1, 2, 5, 6; Fig. 4), consistent with *Lankatrema* spp., most likely *L. minutum* (Blair 1981). In dugongs 3 and 4, rare ectatic and attenuated duodenal mucosal glands contained individual trematodes up to 150 µm in diameter, thought to be *L. mannarense* (Blair 1981). Mild, multifocal to diffuse eosinophilic infiltrates were seen in the submucosa and/or mucosa of intestinal sections taken from all levels of the gastrointestinal tract.

Multifocal (up to 10) thick-walled conical transmural abscesses (up to 2 cm height, 2 cm diameter) protruded from the antimesenteric serosal surfaces of the ileum in dugongs 2 and 4 (Fig. 5). Abscesses opened to the intestinal lumen through a 1–3 mm diameter pore, and were filled by gritty caseous material. There was moderate mesenteric lymph node hyperplasia in affected dugongs. Histologically, abscesses contained trematode parasites with ovaries containing light brown pigmented, thin-shelled operculated eggs (23–28 µm long, 8–11 µm wide), testes with developing spermatozoa, and vitellaria, all consistent with *Faredifex clavata* (Blair 1981). In the mesenteric lymph nodes of dugongs with ileal abscesses, vascular sinuses were congested and there was reactive hyperplasia of lymphoid follicles.

Multiple thick-walled granulomas up to 2 cm in diameter expanded thickened, hyperplastic and fibrosed bile ducts in the liver of 1 sub-adult male (6, Fig. 6a). Granulomas were restricted to large hepatic ducts, and the adjacent hepatic parenchyma appeared grossly

Fig. 4. Dugong 1, cardiac gland luminae. Individual or paired flattened trematode parasites measuring up to 500 µm in cross section

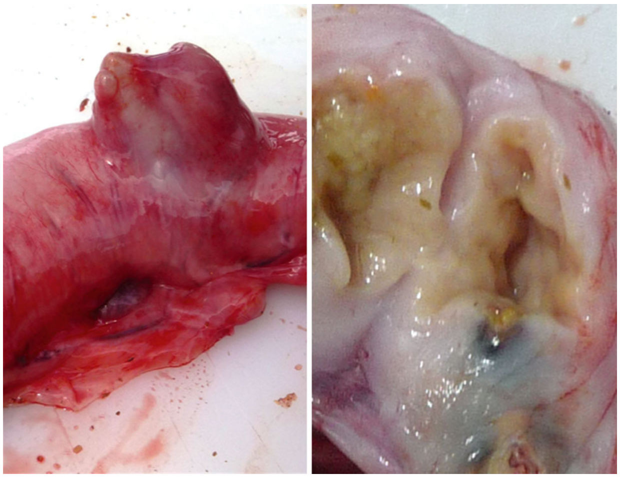

Fig. 5. Dugong 4, small intestine. Thick-walled conical transmural abscess ~2 cm in height and 2 cm in diameter protruding from the antimesenteric serosae of the ileum. Abscesses opened to the intestinal lumen through a 1 to 3 mm diameter pore, and were filled by gritty caseous material

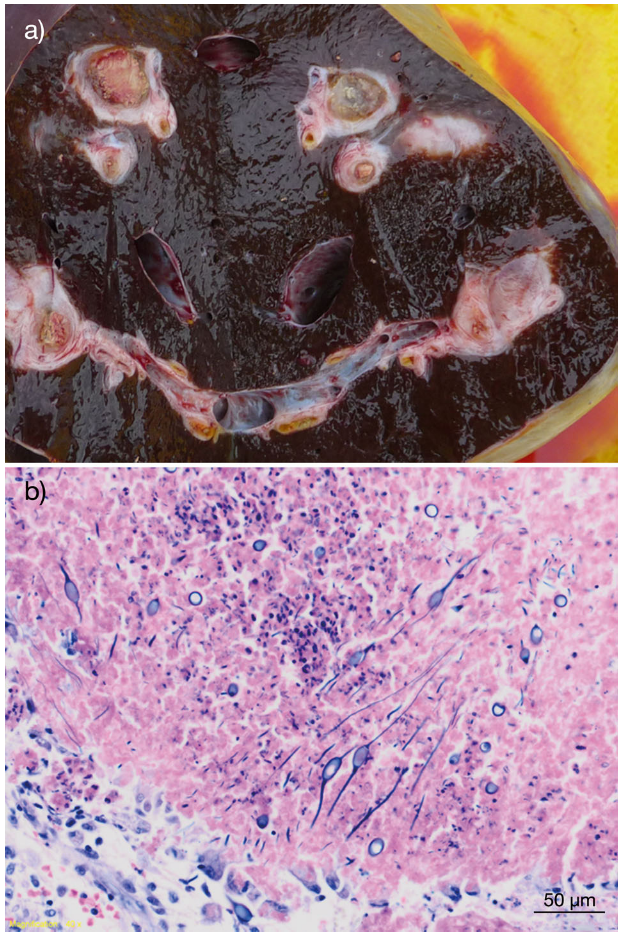

Fig. 6. Dugong 6, liver. (a) Thick-walled granulomas, expanded thickened and fibrosed hepatic ducts. (b) Histologically, granulomas contained operculated ova (18 × 8 µm) with long thin filamentous structures up to 100 µm long extending from each pole (Giemsa stain)

normal. Histologically, granulomas contained operculated parasitic ova (18 × 8 µm) with long thin filamentous structures up to 100 µm long extending from each pole (Fig. 6b). Additionally, within the granulomas, we observed ovoid or folded thin-walled ova (15 × 5 µm), with a brightly eosinophilic wall and finely granular basophilic internal structures (not shown). Additional findings in the liver of dugong 6 were moderate periportal to bridging fibrosis and bile ductule proliferation. Periportal and bridging fibrosis and lymphoplasmacytic periportal hepatitis were seen in the pregnant female; however, no parasites were present macroscopically or histologically.

Two types of cytoplasmic pigmentation were found in dugong livers. In all dugongs, there was minimal to moderate diffuse finely granular light brown cytoplasmic pigmentation of hepatocytes that was most concentrated in periacinar to midzonal regions (Fig. 7a). This pigment stained blue by Schmorl stain (Fig. 7b) and pink by PAS, but did not stain by Perls′, Zn or Rhodanine stains, consistent with lipofuscin. Additionally in all dugongs except the calf, there was mild to moderate multifocal cytoplasmic pigmentation of Kupffer cells by coarse, clumped, dark brown material that obscured nuclear detail (Fig. 7a). Pigment stained dark blue by Perls′ stain (Fig. 7c) and did not stain by PAS, Rhodanine, Schmorl or Giemsa stains, consistent with ferritin. Ferritin pigmentation was most marked in the adult male with the largest tusks (estimated to be 18 yr of age or older) and in the sub-adult male with concurrent hepatic abscesses (dugong 6); it was not observed in the calf liver.

Mild, moderate or marked periductular and interstitial pancreatic fibrosis with follicular lymphoid hyperplasia was seen in 4 of the 5 pancreases examined (from dugongs 2, 3, 5, 6). In dugongs 2 and 3, trematodes measuring up to 2.8 mm long by 0.6 mm in cross section containing uteri with refractile ova and cirrus sac containing spermatozoa were present in pancreatic duct and interpreted as *Lankatrematoides gardneri* (Blair 1981). Trematodes were associated with ulcerative and fibrinosuppurative inflammation in dugong 2 (Fig. 8).

Lymphoid organs

Mild reactive follicular hyperplasia with variable medullary granulocytic infiltrates were seen in the mesenteric (Fig. 9) and/or pulmonary lymph nodes of

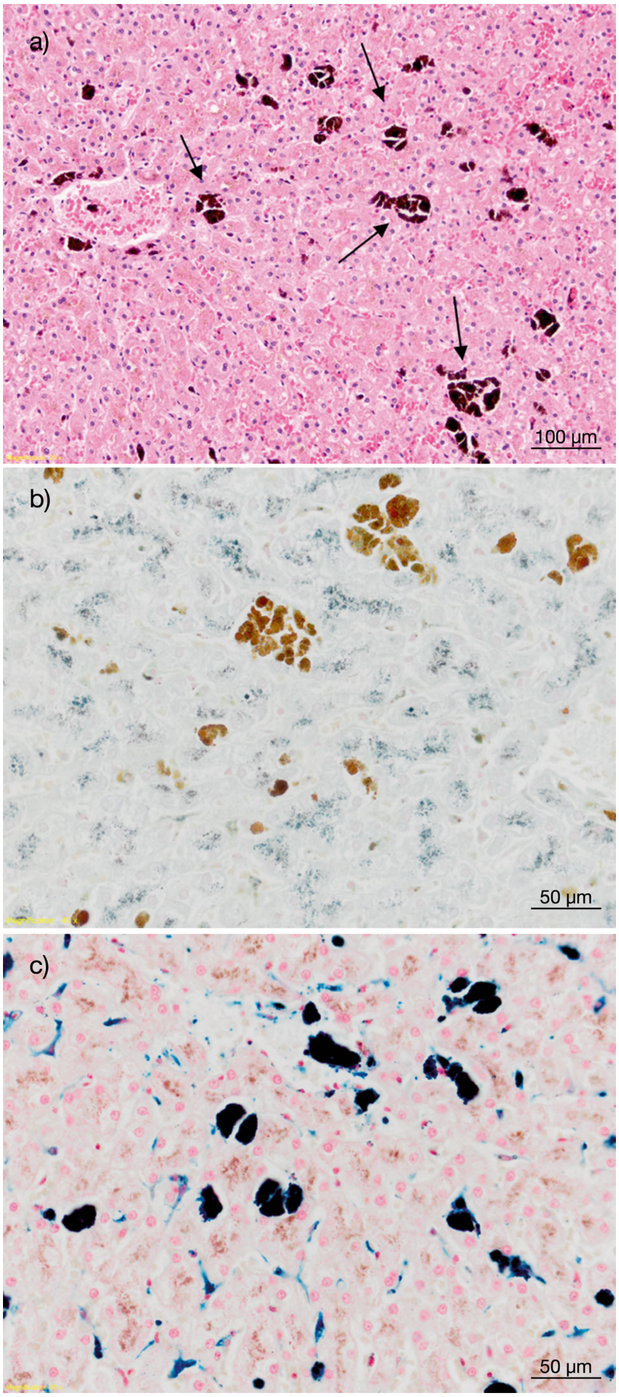

Fig. 7. Hepatic pigmentation seen in dugong *Dugong dugon* livers. (a) Minimal to moderate diffuse finely granular light brown cytoplasmic pigmentation of hepatocytes most concentrated in periacinar to midzonal regions was observed in all dugongs. A second coarsely clumped dark brown-black granular pigment was identified as Kupffer cells (arrows) in all dugongs except for the calf. (b) Finely granular light brown cytoplasmic pigment in periacinar to midzonal hepatocytes stains blue by Schmorl stain, consistent with lipofuscin. (c) Coarsely clumped dark brown-black pigment in Kupffer cells stains dark blue with Perls' stain, consistent with ferritin

all dugongs. Hyperplastic follicles were characterised by prominent germinal centres populated by large immunoblastic B lymphocytes with large vesicular nuclei and scant basophilic cytoplasm, rimmed by a prominent B lymphocyte-rich mantle zone and lesser scattered T cells (Fig. 9). Paracortical and medullary zones were dominated by T lymphocytes. B lymphocytes stained positive by CD20 immunohistochemistry and negatively by CD79a and CD3 (Fig. 9b). T lymphocytes stained positively by CD3 immunohistochemistry and negatively by CD79a and CD20 (Fig. 9c).

Spleens from 5 dugongs (1, 3, 4, 5, 6) were examined histologically. Mean splenic follicle area was variable between animals, ranging between 47 000 and 250 000 µm^2 (Table 2). The largest lymphoid follicles were seen in the adult and sub-adult males (means between 220 000 and 262 000 µm^2), and were much larger than the follicles of the male calf and pregnant female (mean follicular area of 47 030 and <70 881 µm^2, respectively). Large splenic follicles in the adult and sub-adult males were characterised by prominent germinal centres populated by large immunoblastic CD20 positive B lymphocytes with large vesicular nuclei and scant basophilic cytoplasm, rimmed by a prominent CD20 positive B lymphocyte-rich mantle zone and lesser scattered CD3 positive T cells, consistent with lymphoid hyperplasia and formation of secondary follicles. Periarteriolar lymphoid sheaths were less frequently observed, and were composed of CD3 positive-staining T lymphocytes. Prominent splenic extramedullary haematopoiesis was also seen in the adult and subadult males and the pregnant female.

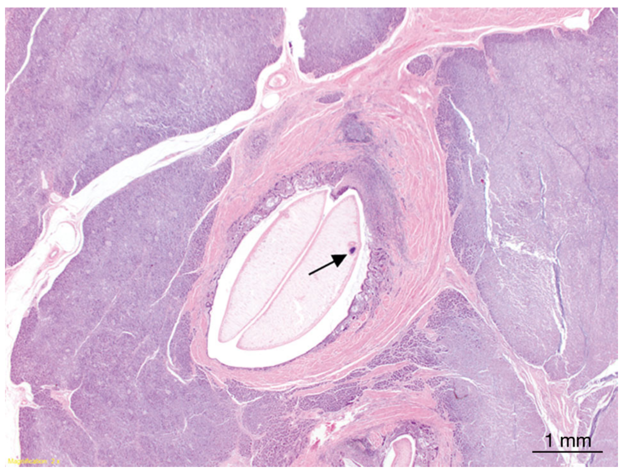

Fig. 8. Dugong 2, pancreas. Trematodes (*Lankatrematoides gardneri*) in pancreatic duct associated with epithelial ulceration and inflammation. Cirrus sac containing spermatozoa is visible (arrow)

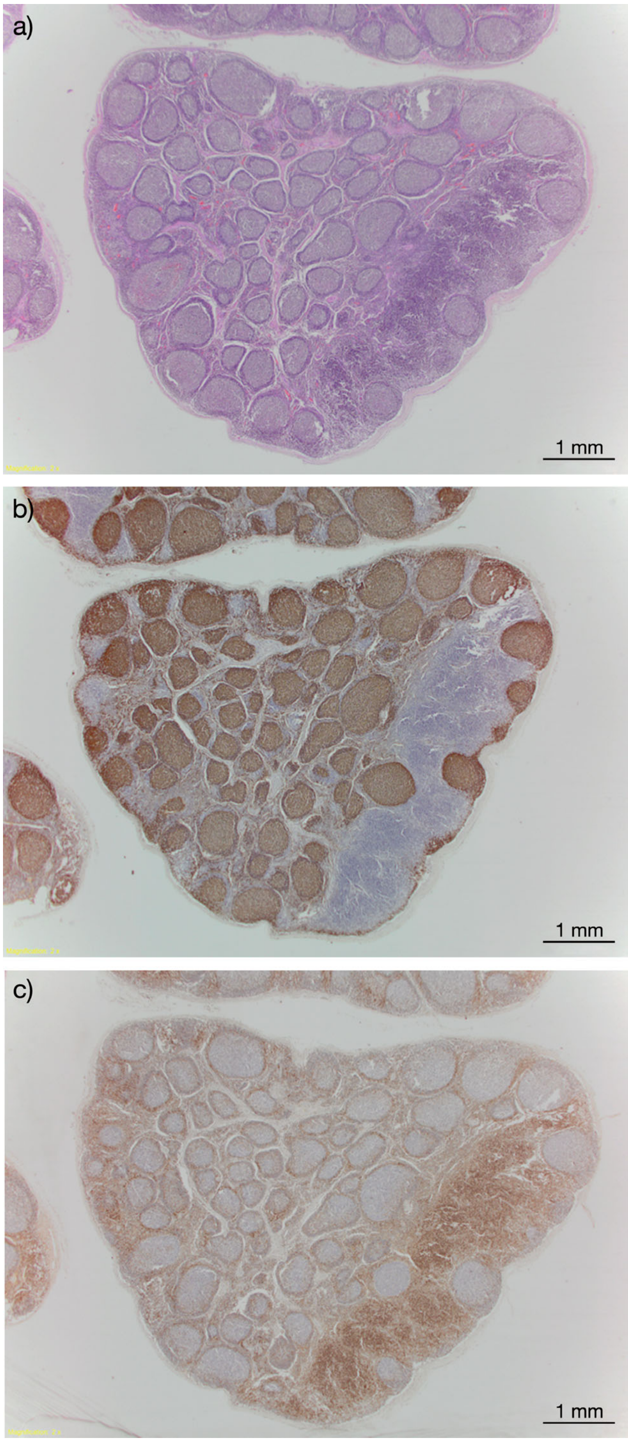

Fig. 9. Dugong 4, mesenteric lymph node. (a) Mild to moderate follicular hyperplasia with formation of germinal centres (secondary lymphoid follicles; haematoxylin and eosin). (b) CD20 immunohistochemical reaction highlighting rich germinal centre and mantle zone B-lymphocytic populations (DAB and haematoxylin counterstain). (c) CD3 immunohistochemical reaction highlighting medullary, paracortical and mantle zone T-lymphocytic population (DAB and haematoxylin counterstain)

Table 2. Mean ± SD follicle area for 5 dugong *Dugong dugon* spleens

Dugong ID	No. follicles counted	Area (μm^2)
1	125	70881 ± 25518
3	93	249168 ± 92886
4	238	47030 ± 31140
5	130	220728 ± 171111
6	100	262019 ± 122263

Other organ systems

Limited skeletal muscle samples were collected and heart muscle was not examined during post mortem examinations due to the use of these tissues as traditional food. Skeletal muscle from the sternum was collected from 2 dugongs (1 and 2), from the peduncle of 3 dugongs (3, 4, 5) and from the body wall of dugong 6. No significant pathological findings were found in these muscle groups using HE or PTAH staining for demonstration of fibre striations.

The vulva of the pregnant female was swollen and the mucosa congested and oedematous. The vulval opening was dilated 13 cm and the vaginal mucosa protruded at the cranial margin. A female foetus of total body length 55 cm was present. Histological examination of the endometrium was consistent with pregnancy and otherwise unremarkable. Testes from 2 dugongs (2 and 3) were collected and examined histologically, and there were no significant findings.

Brains from 2 dugongs (1 and 3) were examined grossly and by histopathology; there were no significant findings. No significant histological findings were identified in sections of skin examined from each dugong. Heart and adrenal, thyroid and pituitary glands were not examined in this study.

DISCUSSION

This study catalogues pathological findings in 6 wild dugongs harvested from the Torres Strait region of Australia for the purpose of human consumption, describing both pre-existing common or incidental lesions, and acute lesions associated with death by drowning. These findings further our histopathological knowledge of dugong health and disease, which up until now has been limited to opportunistic sampling of stranded carcasses, and will assist in the interpretation of similar findings in stranded animals.

Current methods for hunting dugongs used by indigenous Torres Strait communities involve pursuit by water craft, followed by capture and restraint under water until death by drowning. In all of the drowned dugongs examined in this study, lungs were incompletely collapsed, with mild to moderate dorsal or diffuse pulmonary congestion, and airways were clear of water, particulate matter or foam. Hypostatic pooling of blood in the dorsal lungs due to placement of dugongs in dorsal recumbence prior to processing may have contributed to congestive changes observed. Acute microscopic pulmonary abnormalities in these animals included congestion, mild intra-alveolar haemorrhage and oedema, mild interstitial oedema and rupture of peripheral alveolar septae (alveolar emphysema) with acute myofibre fragmentation and degeneration. No aspirated particulate matter was observed microscopically. The absence of gross or histological evidence of aspirated water or foreign material in the lungs of drowned dugongs, in conjunction with absence of foam in the airways, support the current hypothesis that dugongs 'dry drown', that is, asphyxiate following laryngospasm (Eros et al. 2007), rather than 'wet drown', a process through which there is aspiration and flooding of airways by water.

The pathophysiology of drowning is well described in humans (Lunetta & Modell 2005); in 85 to 90% of cases, there will be active inhalation of a variable volume of liquid during stages of drowning (i.e. 'wet-drowning'). Morphological changes associated with liquid inhalation include external foam around the mouth and nares, frothy liquid in airways and lung overexpansion; histologically, foci of acute lung emphysema with over-dilation of alveoli, thinning and lacerations of septa, capillary congestion, interstitial and intra-alveolar oedema, and haemorrhages and exogenous particles in the airways may be seen (Delmonte & Capelozzi 2001, Lunetta & Modell 2005). In the other 10 to 15% of human cases, 'dry drowning' is reported, a process whereby death by prolonged laryngospasm and asphyxiation or vagally-mediated cardiac arrest occurs before liquid is inhaled. Lesions of dry drowning may be similar to those of wet drowning, complicating the diagnosis, with the important exception that in the case of these dugongs, inhalation of water into the lungs has not occurred (Lunetta & Modell 2005).

Like dugongs, cetaceans and pinnipeds are thought not to aspirate water during drowning, with death occurring secondary to laryngospasm and asphyxiation (Hare & Mead 1987, Jepson et al. 2000, Knieriem & García Hartmann 2001, Duignan et al. 2003a,b). Gross and microscopic pulmonary changes associated with drowning due to asphyxia in these taxa include pulmonary congestion, stable froth in airways, pleural congestion, proteinaceous oedema, alveolar haemorrhage, alveolar or bullous emphysema and alveolar myofibre fragmentation (Jepson et al. 2000, Duignan et al. 2003a,b). Alveolar emphysema, as observed in these dugongs, most commonly results from airway obstruction or spasm, with trapping of air in alveoli and failure of the lung to deflate normally (Caswell & Williams 2007). Whilst pulmonary lesions associated with drowning in dugongs in this study were subtle, they are similar to those observed in other drowned marine mammals and may reflect airway spasm with resultant alveolar over-distension and rupture, respiratory distress and exertion.

Dugong hearts were not available for examination in this study, which may have precluded detection of additional lesions that have been associated with drowning in other mammalian taxa, such as endocardial and epicardial haemorrhages, hyper-contraction of myofibres with fibre fragmentation and vacuolation and contraction banding in the media of coronary arteries (Factor & Cho 1985, Lunt & Rose 1987, Duignan et al. 2003b). Similar myocardial findings in dugongs that have been attributed to a possible cold stress-type syndrome (Owen et al. 2013), as is known to occur in manatees (Bossart et al. 2003), may in fact be associated with drowning, and not directly attributable to thermoregulatory stress.

Pathological evidence for capture myopathy, such as acute rhabdomyolysis (Spraker 1993), was not observed in dugongs in this study; however, limited skeletal muscle samples were examined due to their intent for human consumption. Therefore lesions suggestive of myopathy may have been missed.

The majority of lesions identified in dugongs here were not associated with drowning and were of subacute to chronic duration (i.e. pre-existing). Many lesions related to helminth parasitism. Fibrinosuppurative tracheitis in 1 dugong and mucosal granulocytic and lymphoplasmacytic infiltrates in the tracheal mucosa of other dugongs suggest respiratory parasitism, such as with *Cochleotrema indicum,* a trematode found in the nasal passages and lungs associated with tracheal mucosal congestion and haemorrhage, or bronchopneumonia (Blair 1981, Blanshard 2001, Ladds 2009). Although no parasites were observed grossly or histologically in the lungs of these animals, it is possible that respiratory parasites may have been missed if the burden was particularly low. Alternatively, other respiratory pathogens not specifically examined for in this study, e.g. viral agents, may underlie the changes observed here. Mild to moderate (rarely) eosinophilic infiltrates were observed in the airways and pulmonary interstitium of most dugongs examined.

Paradijuana halicoris (Owen 1833, Baird 1859, Sprent 1980), commonly found in dugongs examined elsewhere, was identified in the stomach and cardiac gland of 4 of the 6 dugongs. Worm burdens and faecal egg counts were greatest in the 2 sub-adult males and the pregnant female, although these were all quite significantly low when compared with dugongs in waters along urbanised coastlines of south east Queensland, where gastric ascarid loads are uniformly high in 100% of dugongs (numbering generally in the 100s and as high as 6000; Owen et al. 2012, authors' unpubl. data), and faecal ascarid egg counts in live animals as high as 170 eggs g^{-1} (authors' unpubl. data). High worm burdens in other mammalian taxa have been associated with environmental factors such as heavy environmental contamination and high pasture stocking densities, poor nutritional status, concurrent disease, host immunological naivety or suppression and genetic background (Paterson et al. 1998, Taylor et al. 2007). Although the sample size in this study is small, apparent disparity between parasite burdens in dugongs from urbanised versus non-urbanised waters may suggest that similar phenomena exist for the dugong. Particularly high nematode loads in dugongs sampled along the urban Queensland coast are often concurrent with poor body condition in affected animals (J. M. Lanyon pers. obs.); therefore, higher nematode burdens may be secondary to factors such as nutritional stress, high population densities, pollutant contamination of seagrass or other factors. Once established, these parasitic burdens may further contribute to poor body condition and health. The higher ascarid burdens in sub-adult males may reflect absence of age-acquired resistance, a finding common in juvenile domestic livestock thought to reflect an incompletely evolved host–parasite relationship (Taylor et al. 2007), or increased social and or nutritional stress for males. The higher worm burden in the pregnant female compared with the males in this study may reflect peri-parturient relaxation in immunity; ewes, sows, female goats and cows are more susceptible to helminthiasis during late pregnancy (Lloyd 1983, Taylor et al. 2007).

The lifecycle of *P. halicoris* is presently unknown (Beck & Forrester 1988); many ascaridoid nematodes of aquatic vertebrates typically have free-swimming larval stages initially, and require various intermediate hosts to complete their lifecycle (Bowman 2009); however, a direct life cycle analogous to ascarids of terrestrial mammals cannot be excluded. Due to their exclusive seagrass diet, infestation of dugongs may occur through grazing of seagrass beds contaminated with larvae or through inadvertent digestion of a larvally-infected intermediate host within the seagrass community. There was no evidence for ascaridiasis in the dugong calf in this study. Relatively lower rates of infection with the stomach ascarid *Heterocheilus tunicatus* have been reported in manatee calves compared to adults (Beck & Forrester 1988), presumably due to lower grazing rates and/or shorter time for exposure to helminths.

A number of previously characterised trematodes were identified in dugongs in this study. *Faredifex clavata* were present within transmural abscesses of the ileum in both an adult male and the male calf. These large spoon-shaped digenean trematodes, which reside in large caseous abscesses in the ileal wall, are one of up to 16 trematode species known to parasitise dugongs (Blair 1981, Cribb 1998, Ladds 2009), most of which do not appear to be associated with detrimental effects on host health. *Lankatrematoides gardneri* occurs in the pancreatic ducts (Blair 1981) and was identified in 2 individuals in association with chronic ductal fibrosis, mild pancreatitis and lymphoid hyperplasia. *Lankatrema mannarense* was seen in the intestinal mucosa of 2 dugongs with minimal host response, although this parasite has been associated with variable inflammation and fibrosis in the stomach and cardiac gland (Crusz & Ferdinand 1954, Blair 1981, Ladds 2009). Trematodes in the cardiac gland of 4 dugongs were not associated with any host response and were thought to be another *Lankatrema* spp. (e.g. *L. minutum*, after Blair 1981). In the liver granuloma, operculated parasitic ova with long filaments from each pole are thought to belong to the digenean trematode *Folitrema jecoris,* as described by Blair (1981). The identity of the other parasite forms is uncertain.

The zoonotic potential or impact of dugong parasites is presently unknown, and identification of zoonotic diseases in the dugong population may have implications on the sustainability of harvesting dugong meat for human consumption. The small intestine is sometimes harvested for consumption, but is reportedly discarded by hunters if grossly visible lesions are detected (as occurred for the dugong with *Faredifex clavata* abscesses in this study). However, ingestion of microscopically-infected intestine remains a risk. Prociv et al. (1990) detected unidentified trematode eggs in the faeces of Torres Strait Islander and Aboriginal children, but those authors were unsure whether these were cases of spurious parasitism or true infection. Food-borne trematodiases is a public health problem worldwide (Fried et al. 2004), and at this stage it would be prudent to avoid consumption of obviously infected meat or offal.

All parasite-associated lesions reported in this study, predominantly by trematodes and 1 nematode parasite, were associated with mild pathological changes and not thought to be of detriment to their otherwise healthy hosts. This finding is expected and consistent with previous studies of these parasites in dugongs and in other members of Sirenia, such as Florida manatees, which are also parasitised by a large number of trematodes that appear to have little effect (Dailey et al. 1988, Dailey 2001). More highly pathogenic parasites in sirenians, such as *Toxoplasma gondii* (Buergelt & Bonde 1983, Owen et al. 2012), were not identified in this study. Infection with *Eimeria* spp., as reported in manatees (Dailey 2001), was similarly not identified in this study.

Mild eosinophilic infiltrates were seen histologically along the length of the gastrointestinal tract of the dugongs, predominating in the basilar layers of the mucosa. Eosinophils may be normal residents in the healthy gastrointestinal mucosa of humans and horses, often located basilar and close to the muscularis mucosae, and present in variable densities at different sections of the gastrointestinal tract (Lowichik & Weinberg 1996, Rötting et al. 2008). In contrast, diffuse mucosal eosinophilic infiltration is associated with gastrointestinal disease including hypersensitivity reactions, parasitism or idiopathic hypereosinophilic syndromes (Collobert-Laugier et al. 2002, Zachary & McGavin 2012). In these dugongs, the mild eosinophilic infiltrates observed are likely due to the presence of intestinal parasites and/or as is the case in humans and horses, normal intestinal residents for this species.

Diffuse hepatocellular lipofuscinosis was observed in all dugongs examined, and the amount of pigmentation was greatest in the 3 adult dugongs, suggesting temporal accumulation. Pigmentation was concentrated in mid-zonal to periacinar regions, consistent with age-related cumulative lipofuscinosis in other species (Stalker & Hayes 2007). Lipofusin is a 'wear and tear' pigment formed through intracytoplasmic metabolism of lipid-rich cell and organelle membranes, and the functional significance of this pigmentation is thought to be minimal (Zachary & McGavin 2012).

Ferritin pigmentation was identified histologically in all dugongs with the exception of the calf. Histologically, accumulation of ferritin pigment appeared to be greater in adult than sub-adult dugongs. These observations suggest progressive accumulation of iron with age, as dugongs consume seagrasses with high iron content (J. M. Lanyon pers. obs.). High levels of hepatocellular iron storage (haemosiderosis) is reported as a common finding in dugongs; previous studies have found inherently high levels of iron in dugongs, and these are thought to have little functional or pathological significance (Denton et al. 1980, Rahman et al. 1999). However, it cannot be excluded that iron accumulation may have contributed to periportal and bridging fibrosis observed in 2 of the adult dugongs in this study with visually high levels of ferritin observed in the liver.

The significance of examining lymphoid structures in healthy dugongs was to establish a normal reference by which to assist the detection of morphological changes that may suggest an underlying disease state in stranded animals, as has been performed in studies with other marine taxa (Romano et al. 1993, Beineke et al. 2007). In the adult and sub-adult male dugong spleen and in the gastrointestinal lymph nodes from all dugongs, lymphoid follicles were large, and morphologically and immunohistochemically consistent with reactive hyperplasia. Lymphoid hyperplasia is a common response to antigenic stimulation, and may suggest localised or systemic inflammatory disease. In the case of these dugongs, antigenic stimulation of the spleen and mesenteric lymph nodes due to the presence of gastrointestinal parasitic infestations is most likely. Despite small follicles in the pregnant female and calf spleen, lymphocytolysis was not seen, which may otherwise suggest lymphoid depletion. Lymphoid depletion may be observed in sepsis, with acute viral disease and/or in response to significant stresses (Snider et al. 1996, Thomas et al. 2001, Felmet et al. 2005, Beineke et al. 2007, Valli 2007), and lymphoid depletion is described as a feature of cold stress syndrome in manatees (Bossart et al. 2003) and fatal morbillivirus infections in cetaceans (Kennedy-Stoskopf 2001, Stone et al. 2012).

Immunohistochemistry (IHC) is a necessary tool in the analysis of lymphoid organs, allowing the identification of cell populations based on the expression of cell surface or cytoplasmic antigens by specific lymphocyte subsets. IHC markers were applied to dugong tissues to enable further characterisation of lymphoid structure. In this study, L26 (also known as CD20; a B cell marker) and CD3 (a T cell marker) were used to successfully identify B and T lymphocyte subsets in tissue sections. The relative locations of the B lymphocyte and T lymphocyte populations were typical of other mammalian species. The IHC marker CD79a was used but no positive immunostaining was observed, possibly due to a lack of species specificity for this antibody. This marker has been used successfully in the diagnosis of lymphoma in a related sirenian species, the West Indian manatee *Trichechus manatus* (Hammer et al. 2005).

Due to the small sample size, it is important to avoid drawing too many conclusions; however, our findings provide a useful benchmark against which to compare stranded animals. Descriptions of commonly observed or incidental lesions in apparently healthy dugongs is important for interpretation of the significance of similar lesions detected in moribund or deceased dugongs, particularly from urbanised areas. The presence of mild tracheitis, hepatic abscessation, enteric inflammatory infiltrates and intestinal granulomas in otherwise healthy animals suggests that these lesions had low functional significance for the animal, and tracheal mucosal papillary folds present in all animals are thought to be a normal anatomical feature rather than a hyperplastic response. These findings contrast with previous studies which have attributed many of these lesions to pathological features of a possible cold stress syndrome (Owen et al. 2013). The dugongs examined in the present study were all sampled in warm tropical waters (latitude ~9.5° S) so that physiological and pathological temperature stress was not a consideration. It should be noted that the type and extent of pathology found in this population may be reflective of local endemic factors not fully determined in this study. These potential co-factors and findings may vary in other populations and ecosystems. In addition to descriptions of pre-existing common or incidental lesions, characterisation of acute lesions likely attributable to drowning will assist investigators examining stranded dugongs in determining cause of death, which remains an often elusive objective in this species.

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