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Evidence of sirenian cold stress syndrome in dugongs *Dugong dugon* from southeast Queensland, Australia

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ABSTRACT: Cold stress syndrome (CSS) is the term used to describe the range of clinical signs and chronic disease processes that can occur in Florida, USA, manatees *Trichechus manatus latirostris* exposed to water temperatures below 20°C for extended periods. Although no cold-related adverse events have been described in the closely related dugong *Dugong dugon* thus far, it has been established that they make movements in response to water temperatures lower than about 17 to 18°C. In this study, archive reports for dugong carcasses submitted to The University of Queensland School of Veterinary Science for post mortem examination during 2010 to 2012 were examined. These animals had been recovered from Moreton Bay, southeast Queensland, Australia, and 10 out of 14 fulfilled the criteria for 'potential cold stress cases.' Epidermal hyperplasia and secondary bacterial infection, serous atrophy of pericardial adipose tissue, and multisystem abscessation were features commonly noted in these cases. Water temperature data were correlated with the time of year that carcasses were submitted for examination. Higher numbers of carcasses diagnosed with potential CSS were noted during sustained periods in which water temperature was below 20°C. Given the pattern of increased submission of non-specifically, chronically unwell animals in the colder months and evidence that environmental conditions known to precipitate CSS occur in southeast Queensland, it is probable that, like manatees, dugongs in this area are affected by CSS. Further investigation to confirm and to better characterize the syndrome is recommended to refine management practices and improve treatment of affected animals.

KEY WORDS: Dugong · Sirenia · Cold stress syndrome · Disease · Temperature

INTRODUCTION

Cold stress syndrome (CSS) is the term used to describe the range of clinical signs and acute and chronic disease processes that can occur in Florida manatees *Trichechus manatus latirostris* exposed to water temperatures below 20°C for extended periods (Bossart et al. 2003). The largest population of this family of the Order Sirenia lives in the waters around Florida, USA (Walsh et al. 1987, Bossart et al. 2003), where CSS has been described (Bossart et al. 2003). Such a disease manifestation in an otherwise sub-tropical environment has been speculated to be due to sirenians having a relatively low metabolic rate and poor thermal conductance, probably an adaptation to feeding on a relatively low energy food resource (Worthy 1998). These features limit their ability to tolerate cold temperatures (Marsh et

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al. 2011). When temperatures decrease during the normal winter cycle or 'cold-snap' events, such as those seen in Florida over the winters of 2009–2010 and 2010–2011, numbers of manatees diagnosed with CSS significantly increase (Barlas et al. 2011; and see 'Rescue and mortality response' at http://research.myfwc.com/manatees/; accessed 12 October 2012).

Sirenia are diagnosed with acute and chronic CSS primarily by clinical and pathological changes, including history (where available), emaciation, and serous atrophy of fat stores. Marked epidermal thickening with extensive fissuring and pustule or abscess formation, myocardial degeneration, and enterocolitis are among other common findings (Bossart et al. 2003). However, to date, no definitive diagnostic protocol has been established, and diagnosis is based on cumulative presenting signs associated with cold water temperatures.

The manatee is a close relative of the dugong *Dugong dugong*. Dugongs are strictly herbivorous marine mammals that are widely distributed in the Indo-Pacific region (Marsh et al. 2011). Moreton Bay, Queensland, supports the most southerly year-round foraging population in eastern Australia (Lanyon 2003). Listed as Vulnerable (IUCN 2010), dugongs are of conservation concern, yet little is known about baseline normal clinical indices or the non-anthropogenic causes of pathology in this species. Off the coast of southeast Queensland, dugong mortality due to boat strike and netting is commonly diagnosed, but the cause(s) of a significant proportion of deaths have not been identified (Greenland & Limpus 2007).

To date, no extreme cold-related adverse events have been described in the Australian dugong population. However, it has been established that dugongs make movements in response to water temperatures lower than about 17 to 18°C (Preen 2004, Marsh et al. 2011) and have been observed to rest in an area outside Moreton Bay which was 2 to 5°C warmer because of an eddy of the warm offshore current. In colder weather, dugongs make regular journeys of 8 to 20 km from this rest area into the bay in order to eat (Preen 2004, Marsh et al. 2011). In addition to the behavioral data, elevated mortalities regularly occur during late winter to early spring (Greenland & Limpus 2007), which suggests that dugongs, like manatees, are sensitive to cold temperatures (Marsh et al. 2011).

In an attempt to address this deficit and assess the potential contribution of CSS to overall mortality, we retrospectively examined dugong case files submitted to The University of Queensland School of Veterinary Sciences' Department of Pathology (Owen et al. 2012). The causes of mortality were investigated by standard port mortem examination for all carcasses submitted through the *Veterinary*-Marine Animal Research, Teaching and Investigation unit by the Queensland Department of Environment and Heritage Protection's (DEHP) StrandNet program since 2008. This retrospective study describes changes exhibited by dugongs during colder environmental temperatures and proposes that CSS is a contributing factor for the previously unexplained mortality.

MATERIALS AND METHODS

Dugong carcasses were collected from Moreton Bay (27.33° S, 153.38° E), southeast Queensland, by Queensland Department of Environment and Heritage Protection staff and Queensland Parks and Wildlife Rangers. The carcasses were assessed for post mortem degradation prior to transport to The University of Queensland School of Veterinary Science's Department of Pathology. Carcasses were classified into 6 categories: (1) observed alive but subsequently died, (2) carcass in good condition (fresh/potentially edible), (3) carcass fair (decomposed but organs intact), (4) carcass poor (advanced decomposition), (5) mummified carcass (skin holding bones), or (6) disarticulated bones (no soft tissue remaining). Only carcasses in Categories 1 to 3 were submitted for post mortem examination, which was performed using a previously described approach (Eros et al. 2007).

Body condition was subjectively determined externally by estimation of soft tissue mass around the dorsal aspect of the neck, ventral abdomen, and dorsal aspect of the vertebrae at the peduncle and was internally based on the amount of subcutaneous and visceral adipose tissue and the degree of serous atrophy. Tissue was collected for histological examination from a standard set of organs, preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, and routinely stained with hematoxylin and eosin (H&E).

All dugongs submitted during 2010 to 2012 were included in the study. Animals were considered as potential cold stress cases if there was no obvious alternative cause of death, death had occurred during or soon after a period of cold weather (water temperature <20°C), and a combination of 2 or more gross or histopathological lesions was present, as defined by Bossart et al. (2003). These lesions included, but were not restricted to, hyperplastic and infected

skin, serous atrophy, probable secondary infections, lymphoid depletion, enterocolitis, and myocardial degeneration (Bossart et al. 2003).

Water temperatures outside Moreton Bay (outside the Gold Coast Seaway, 27.95° S, 153.43° E) and adjacent to the Eastern Banks inside the South Passage (27.36° S, 153.43° E) into Moreton Bay were compared using 10 yr of monthly median surface temperatures from standard Department of Information Technology, Innovation and the Arts' Healthy Waterways and Ecosystem Health water monitoring stations. Data were compiled from 4 sampling sites off the Seaway and 3 sampling sites for the Eastern Banks. For the sites measured outside the Seaway, tidal mixed seaway and oceanic water that flowed south along the coast was measured as representative of water temperature outside the passages into Moreton Bay. Measurement sites for the Eastern Banks were representative of the seagrass beds along the eastern side of the bay, which support regular foraging by large herds of dugongs. For a 6 mo period (March to September 2012), water temperatures were continuously recorded at 30 min intervals using a Minilog-II temperature datalogger set at approximately 10 cm above sand in the seagrass pasture of the inter-tidal/sub-tidal flats of the Eastern Banks of Moreton Bay (27.36° S, 153.39° E), about 1 km north of the Rous Channel inside Moreton Bay, where dugongs regularly feed. These latter recordings were representative of temperatures at the height of seagrass on which the dugongs feed.

Proportion, percentage, and exact 95% confidence intervals (CI) were used to quantify clinical and pathological findings and their relationship to water temperature. Descriptive statistics were calculated using Stata version 10 (Stata Corporation).

RESULTS

Thirty-nine dugongs were reported to have stranded over the period between January 2010 and January 2012. One of these was in carcass condition 1, 13 in 2, 9 in 3, 12 in 4, and 4 carcasses were not classified (StrandNet, https://www.derm.qld.gov.au/strandnet; accessed 23 November 2012). Fourteen of these dugongs with carcass conditions ranging between 2 and 3 were submitted to our institute for post mortem examination between January 2010 and January 2012 (Table 1). Of these, 10 (71%; 95% CI 0.42–0.92) fulfilled the criteria for consideration as 'potential CSS' cases, and 8 of these (80%; 95% CI 0.44–0.97) occurred during periods when water temperatures were persistently below 20°C.

Features of potential CSS animals

Epidermal and dermal pathology

Nine of the 10 (90%; 95% CI 0.55–1.00) animals had markedly thickened and fissured areas of skin. This was distinct from the healed skin resulting from rake marks (caused by tusks during social fighting), a normal finding in dugongs (Fig. 1a). In most animals, the more substantial fissures were discharging pus and blood (Fig. 1b), and in one animal, cutaneous and subcutaneous abscesses were noted. Multifocal areas of light grey to cream pallor were also described on the skin of 4 of the animals.

The skin lesions were not histologically examined in all animals; however, in 1 animal with hyperplastic dermatitis, the stratum spinosum consisted of keratinocytes with expanded, pale, feathery cytoplasms and pyknotic nuclei. There were frequent, multifocal to coalescing aggregates of serum and granulocytes within stratum corneum layers and superficial bacterial colonies.

Table 1. *Dugong dugon.* Date of submission, sex, life stage, and cause of death for 14 dugongs submitted to the University of Queensland Veterinary School's Department of Pathology for post mortem examination between January 2010 and January 2012. CSS: cold stress syndrome

Date	Sex	Life stage	Cause of death
Apr 2010	Female	Adult	Intestinal volvulus/torsion, subsequent peritonitis and toxemia
Jul 2010	Female	Juvenile	Potential CSS
Jul 2010	Male	Adult	Potential CSS
Aug 2010	Male	Adult	Blunt trauma
Aug 2010	Male	Adult	Potential CSS
Aug 2010	Male	Dependent calf	Potential CSS
Sep 2010	Female	Adult	Potential CSS
Sep 2010	Male	Adult	Potential CSS
Apr 2011	Male	Juvenile	Unknown
Jul 2011	Female	Adult	Potential CSS
Jul 2011	Male	Juvenile	Potential CSS
Sep 2011	Male	Adult	Potential CSS
Oct 2011	Female	Adult	Potential CSS
Jan 2012	Male	Adult	Intestinal volvulus/torsion, subsequent peritonitis and toxemia

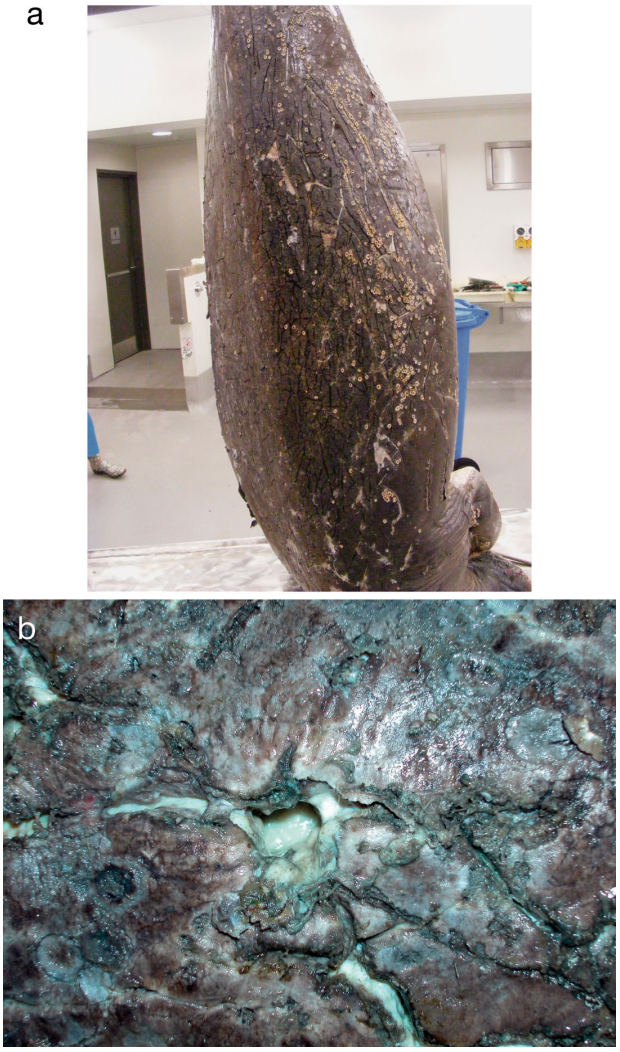

Fig. 1. *Dugong dugon.* (a) Dorsum of adult dugong demonstrating hyperplastic, fissured, and infected skin lesions in addition to multiple rake marks (caused by tusks during social fighting), a normal finding in dugongs. (b) Hyperplastic, fissured and infected skin of adult dugong

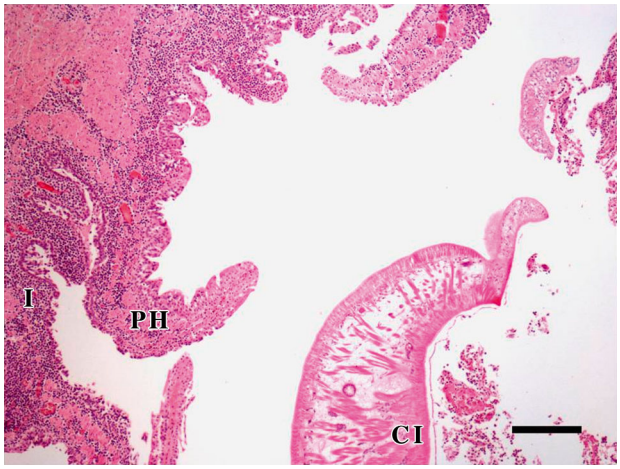

Fig. 2. *Dugong dugon.* Hematoxylin and eosin (H&E)-stained section containing tracheal mucosa and submucosa from a juvenile male dugong. Papillary hyperplasia of the mucosa (PH) is visible, and the submucosa contains a dense population of inflammatory cells (I). Cross section through *Cochleotrema indicum* (CI), bottom right. Scale bar = 250 µm

Systemic pathology

All but one (90%; 95% CI 0.55–1.00) dugong demonstrated serous atrophy of pericardial adipose tissue, and serous atrophy of subcutaneous fat was also described in one (10%; 95% CI 0.00–0.45) animal. Mild, focal to multifocal hepatic and/or pulmonary abscessation was a common finding (70%; 95% CI 0.35–0.93). One (10%; 95% CI 0.00–0.45) individual had a large laryngeal abscess, one (10%; 95% CI 0.00–0.45) animal had pneumonia, and another 3 (30%; 95% CI 0.07–0.65) individuals had tracheitis with small to large numbers of the trematode *Cochleotrema indicum*. All animals had moderate to large amounts of seagrass in their stomachs but minimal intestinal contents (10/10, 100%; 95% CI 0.69–1.00).

Histologically, mild to moderate lymphocytic enteritis, occasionally with a heterophilic component, was a common finding (70%; 95% CI 0.35–0.93). Multifocal granulomas containing many (up to approximately 50) parasite ova were noted in the intestinal submucosa of one animal (10%; 95% CI 0.00–0.45).

The tracheitis in the 3 animals was characterized by generalized, papillary hyperplasia of the mucosa with areas of squamous metaplasia. Within the lamina propria/submucosa, there was a generalized, dense population of plasma cells, lymphocytes, macrophages, and heterophils (Fig. 2).

Histology of the gastrointestinal tract and lymphoid tissue in particular was hampered by the moderate to severe autolysis of many animals.

Water temperatures

For both long-term monitoring areas, water temperatures exhibited consistent seasonal variation over the last 10 yr. Outside of Moreton Bay, average monthly temperatures varied between 18 and 21°C from July to September for each year (Fig. 3a). Inside Moreton Bay, average monthly temperatures varied between 15 and 20°C during July to August for each year, with less than 20°C being recorded in some years from May through to September. Between 2007

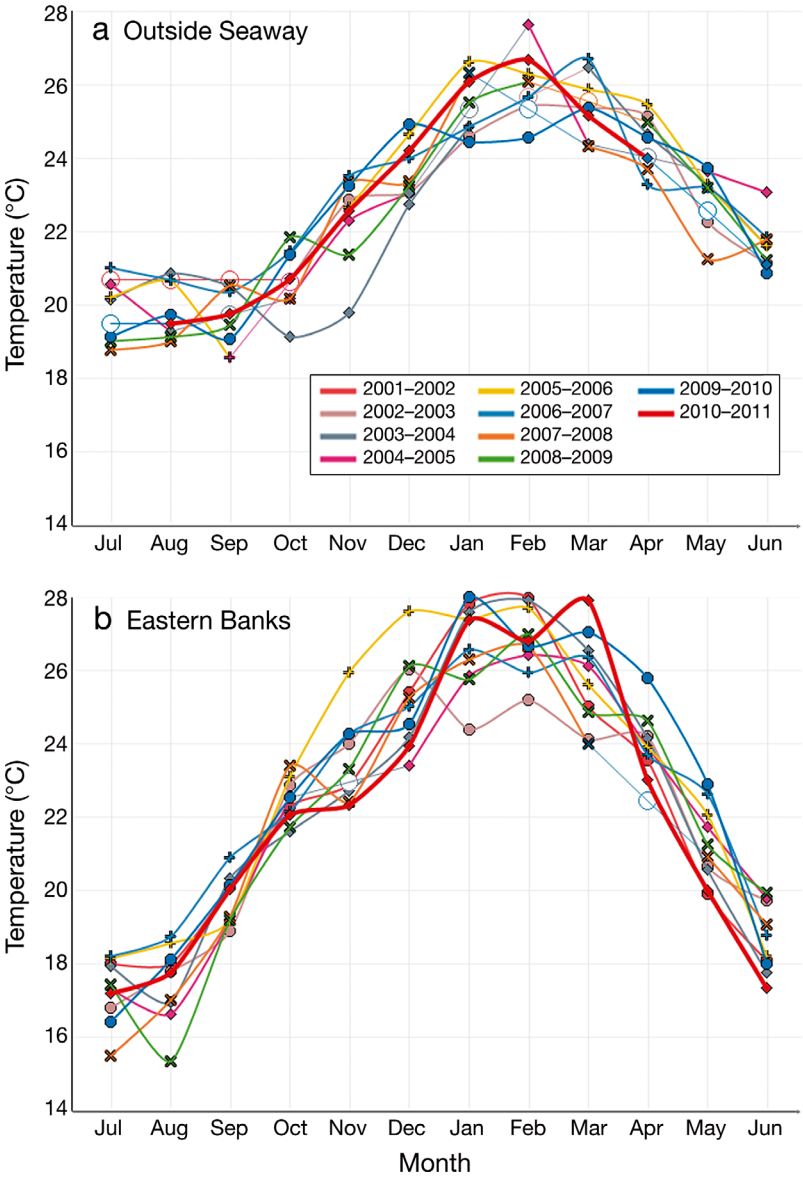

Fig. 3. Average monthly water temperatures (°C) (a) outside and (b) inside Moreton Bay, Queensland, Australia, between July 2001 and June 2011

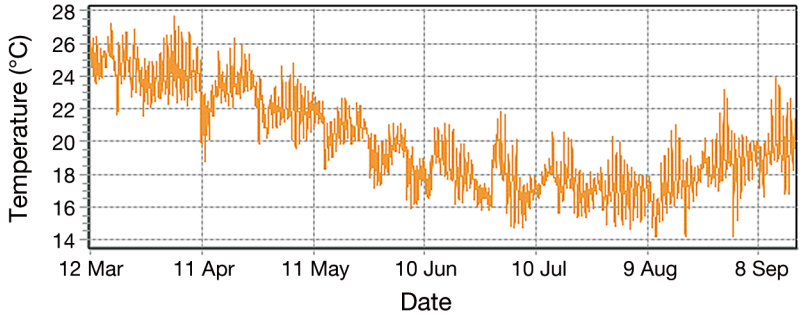

Fig. 4. Water temperatures (°C) recorded at 30 min intervals for Moreton Banks seagrass beds inside Moreton Bay between March and September 2012

and 2009, average monthly temperatures fell to as low as 15°C (Fig. 3b). Outside of Moreton Bay, average monthly median water temperatures were consistently higher than water temperatures inside Moreton Bay.

At the height of the seagrass (approximately 10 cm above the sea floor), water temperatures varied by several degrees in a diurnal pattern. Between late June and late August 2012, average water temperatures were predominantly below 20°C and fell as low as 14.2°C (Fig. 4).

DISCUSSION

CSS is a complex, multifactorial disease process. It is incompletely understood; however, metabolic, nutritional, immunologic, and infectious components are implicated (Bossart et al. 2003). Based on our current understanding, CSS has been identified to cause significant mortality in manatees during periods of winter and adverse cold weather, and as dugongs are closely related and have a demonstrated aversion to cold water, it is likely that they too are affected by this condition (Preen 2004, Marsh et al. 2011).

Pathologic features most commonly noted in the potential cold-stressed dugongs were markedly hyperplastic and fissured skin with secondary bacterial infection, skin depigmentation, emaciation, and serous atrophy. Hyperplastic tracheitis, enterocolitis, and lung and liver abscessation were also noted in some of these animals and may have been due to secondary or opportunistic parasitism and bacterial infections, respectively.

Epidermal hyperplasia is among the most common features of manatee CSS; however, at this stage, its pathogenesis is uncertain. Bossart et al. (2003) offered several theories, including that the hyperplasia is an adaptation to cold temperatures with or without hormonal influence or that

it is a change secondary to malnutrition, specifically protein-calorie, trace mineral, fatty acid, or vitamin deficiency.

The possibility of an infectious etiology has also been considered to explain the epidermal hyperplasia, particularly as the skin lesions had gross and histological features consistent with forms of cutaneous papillomavirus infection, as seen in humans and domestic cats with varying degrees of immunosuppression. A papillomavirus has also been reported as causing disseminated cutaneous papillomatosis in a group of manatees that were likely immunosuppressed (Bossart et al. 2003), even though these animals were maintained in a spring with temperatures approximately 22°C year round; therefore, the immunosuppression was less likely to be the result of CSS. In addition, the papilloma lesions, though larger than other papillomas seen in manatees in rehabilitation centers, were low in number and not associated with a diffuse skin condition.

The etiology of the epidermal hyperplasia in cases in this report is also uncertain, but there is likely to be some overlap with the pathogenesis of the manatee condition. The skin of one dugong showed histological changes (putative cytopathic effects) consistent with a papillomavirus infection and warrants further investigation. Regardless of the pathogenesis, the extensive fissuring is likely to result in a compromised protective barrier against the harsh marine environment, which could result in opportunistic infections, fluid and heat loss, and increased absorption of environmental pollutants (Bossart et al. 2003).

Most of the dugongs examined were underweight and demonstrated varying degrees of serous atrophy. It was interesting that all dugongs had stomachs full of seagrass, in contrast to the manatees that were usually described as having empty stomachs (Bossart et al. 2003). This may be a function of access to seagrass, since dugongs in Moreton Bay have perennial seagrass meadows within their refuge zone, whereas Florida manatees have to leave their refugia to feed. Alternatively, the lower gastrointestinal tracts of the dugong were usually devoid of ingesta, so agonal or terminal ileus may be a consideration.

Immunosuppression is also considered to play a role in manatee CSS. This is evidenced by the common finding of widespread lymphoid depletion and multiorgan inflammatory disease (Bossart et al. 2003). Additionally, significant functional impairment was found in lymphocytes isolated from CSS manatees in a preliminary immunologic study (Walsh et al. 2005). The degree of immunosuppression based on lymphoid tissue cellularity and lymphocyte proliferation was not adequately examined in our study. Further work on the dugong immune system and factors that influence it is necessary to determine the role of immunosuppression in dugong CSS.

The potential link between the observed clinical lesions being exacerbated during periods of colder water temperatures is supported by this retrospective study. The annual occurrence of water temperatures below 20°C, occasionally dropping to less than 15°C, was recorded during periods of up to 3 mo over winter in areas where dugongs are known to graze. Further, water temperatures at the seagrass bed level were lower than surface temperatures, suggesting that dugongs will expose themselves to colder water when feeding on the seagrass. These temporal trends correlated with the higher incidence of carcasses presenting with 2 or more of the lesions used to diagnose Florida manatee CSS.

To avoid prolonged exposure to cold water, Florida manatees have an adaptive strategy of seeking warm-water refuges, only leaving these refuges for short periods of time to feed before returning (Laist & Reynolds 2005). There is some evidence that dugongs use a similar strategy by heading out of Moreton Bay into the warmer coastal waters (Preen 2004, Marsh et al. 2011). However, during the coldest time of the year, water temperatures outside of Moreton Bay may still be below 20°C, preventing escape from persistent exposure to water temperatures that predispose them to the development of CSS.

CONCLUSIONS

Based on this study, like Florida manatees, it appears the dugongs of southeast Queensland develop clinical signs consistent with CSS when water temperatures drop below 20°C, although manifestation of this syndrome is not restricted to winter. This latter finding may be due to chronic cases persisting into the warmer water periods (i.e. summer) or another pathological process causing similar clinical signs. Although only a small number of animals was examined, there appears to be a distinct pattern of increased submission of non-specifically, chronically unwell animals in the colder months that fit the reported signs of CSS in manatees, particularly epidermal hyperplasia and fissuring and emaciation.

A better understanding of sirenian CSS is critically important for optimizing treatment and management strategies. In Florida, manatees rely on a network of natural or artificial warm-water refuges to survive cold winters. They have been observed to return to

the same refuges year after year, and in winter, manatee counts at a single warm-water refuge can exceed 500 animals (Bossart et al. 2003). No such refugia exist for dugongs in southeast Queensland. Maintenance of seagrass pastures in the warmer areas of Moreton Bay during winter may aid in the conservation of this vulnerable species at this southern limit of its normal range in eastern Australia.

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