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NOTE

Cold stress, heart failure, and esophageal occlusion cause the death of a West Indian manatee *Trichechus manatus* in Alabama, USA

Jillian L. Western[1,2,7], Elizabeth E. Hieb[1], Mackenzie L. Russell[1], Cristina Díaz Clark[1,8], David S. Rotstein[3], Sean M. Perry[4], Alexandra Ingrisano[4], Ruth H. Carmichael[1,5], Nicole I. Stacy[6], Jennifer C. G. Bloodgood[1,9,*]

[1]Marine Mammal Research Program, Dauphin Island Sea Lab, Dauphin Island, Alabama 36528, USA
[2]Mississippi State University College of Veterinary Medicine, Mississippi State, Mississippi 39762, USA
[3]Marine Mammal Pathology Services, Olney, Maryland 20830, USA
[4]Mississippi Aquarium, Gulfport, Mississippi 39501, USA
[5]University of South Alabama, Stokes School of Marine and Environmental Sciences, Mobile, Alabama 36688, USA
[6]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32608, USA

[7]*Present address:* Janet L. Swanson Wildlife Hospital, Cornell University College of Veterinary Medicine, Ithaca, New York 14853, USA

[8]*Present address:* Florida Fish and Wildlife Conservation Commission, FWRI Marine Mammal Pathobiology Lab, St. Petersburg, Florida 33711, USA

[9]*Present address:* Cornell Wildlife Health Lab, Department of Public and Ecosystem Health, Cornell University College of Veterinary Medicine, Ithaca, New York 14853, USA

ABSTRACT: Cold stress is the leading cause of mortality in West Indian manatees *Trichechus manatus* in the northcentral Gulf of Mexico. This report describes an adult male manatee that stranded alive with signs of cold stress in Alabama (USA) waters in January 2022 and died during the rescue intervention. Postmortem examination and histopathologic review revealed multiple contributors to death. While the animal had evidence of cold stress syndrome, there was aortic stenosis and concentric left ventricular hypertrophy as well as an esophageal foreign body (plastic bag). Main findings from blood analysis indicated systemic inflammation and possible disseminated intravascular coagulation. Histopathologic findings from the cardiovascular system included aortic fibromuscular dysplasia, mitral and tricuspid valve endocardiosis, left ventricular myocardial hypertrophy, and right myocardial atrophy. A cause of the cardiovascular findings was not determined. This report is the first to document a case of cold stress syndrome complicated by aortic stenosis and esophageal occlusion in this species.

KEY WORDS: Aortic stenosis · Cardiovascular disease · Esophageal foreign body · Cold stress syndrome

1. INTRODUCTION

Due to their evolution as a tropical species with a lower metabolic rate and inadequate insulation for extreme or sustained cold, West Indian manatees *Trichechus manatus* are susceptible to cold stress syndrome when exposed to water temperatures below 20°C for extended periods (Hardy et al. 2019). In the

*Corresponding author: bloodgood.jennifer@gmail.com

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northcentral Gulf of Mexico, where seasonal water temperatures regularly drop below 20°C, manatee sightings and strandings have increased in recent years, and cold stress is the leading cause of mortality (Hieb et al. 2017). Cold stress syndrome is widely variable in effects based on duration of exposure to cold water. Early lesions may be characterized by epidermal hyperplasia and whitening of the extremities, known as bleaching (Bossart et al. 2003); the syndrome may lead to a chronic immunosuppressive state and, ultimately, death (Halvorsen & Keith 2008). While cold stress syndrome has been well-described in West Indian manatees, the prognosis for individuals that do not migrate to warmer waters remains poor throughout their range. This report describes a cold-stressed, adult male manatee that stranded in an industrial canal of Mobile Bay, Alabama, and died from multiple abnormalities, including heart failure and an esophageal foreign body (plastic bag). Understanding the potential comorbidities that may increase susceptibility and complicate the response to and treatment of manatees affected by cold stress syndrome may help improve diagnostic evaluations and treatment outcomes for individuals affected by this condition.

2. MATERIALS AND METHODS

The Dauphin Island Sea Lab's Manatee Sighting Network (DISL/MSN) documents manatee sightings in coastal Alabama and responds to stranded manatees in collaboration with the Alabama Marine Mammal Stranding Network under permits with the United States Fish and Wildlife Service (USFWS). On 24 January 2022, DISL/MSN received a call reporting a live manatee in Theodore Industrial Canal, Theodore, AL (30.5332° N, 88.1416° W). Water temperatures ranged from 10.4 to 14.8°C. Due to the concern for cold stress, responders deployed to monitor the animal. Based on the animal's behavior and signs of cold stress, a rescue was planned for 26 January 2022 under the direction of the USFWS and in partnership with the Florida Fish and Wildlife Conservation Commission and SeaWorld Orlando. Despite a coordinated rescue effort, during which blood was collected, and subsequent resuscitation efforts, the manatee died during the intervention. The carcass was transported to the DISL Marine Mammal Research Center, where an external examination and priority sample collection were performed that evening. The carcass was stored at 4°C until necropsy the following day. During necropsy, representative samples of skin lesions and internal organs were placed in 10% buffered formalin for preservation. Tissues were later sectioned at 5 to 7 µm, stained with hematoxylin and eosin, and examined by a board-certified pathologist (D.S.R.). Sections of the aorta were also stained with Alcian blue–periodic acid-Schiff (AB-PAS), Vehoeff–van Gieson (VVG), and immunohistochemical stain, smooth muscle actin (SMA).

3. RESULTS

3.1. Live animal response and bloodwork

Monitoring efforts on 24 January 2022 (2 d prior to the attempted rescue) revealed that the manatee was tachypneic, remaining underwater for 3–9 min and surfacing to breathe 1–8 times within 1–3 min before diving back down. The following day, the animal showed similar behavior. There was mild skin bleaching on the manatee's snout and evidence of emaciation, including loss of nuchal fat (i.e. 'peanut-head').

On the day of the rescue, the manatee continued to show evidence of distress, including tachypnea, frequent surfacing, and erratic swimming. Between bouts of slow swimming and logging, the animal repeatedly oriented to shallow water at the edge of the canal, slow flipper-walked, and ultimately beached itself prior to capture. Rescue personnel were able to pass a stretcher under the semi-buoyant manatee and move it onto a boat for transport to a rescue truck at 11:30 h. While in transit (~10 min), the animal's heart rate was palpated at ~60 beats min^{-1}, and it took 2 shallow breaths. Stimulation with water on the snout and manipulation of the nares were unsuccessful in stimulating more breaths. The animal was moved into the rescue truck at 11:50 h. The oral cavity temperature during the truck transport was 18.7–18.8°C. During transit, attempted breath stimulation continued, but only 1 additional breath was observed at 12:20 h. The heart rate could no longer be detected via palpation, and auscultation and Doppler were unsuccessful at finding a heartbeat. Emergency drugs were administered during transport, including 5.4 mg and 2.7 mg intramuscular atropine at 12:00 and 12:14 h, respectively (Med-Pharmex Animal Health), 300 mg intranasal/sublingual Dopram at 12:25 h (Hikma Pharmaceuticals), and 10 mg intranasal epinephrine at 12:35 h (MWI Animal Health). Some muzzle twitching and eye movement were noted after atropine administration, after which the animal became unresponsive and was presumed dead.

At 13:05 h, a blood sample was collected from the left brachial vascular bundle for lactate analysis, which indicated lactic acidosis (i-STAT CG4+ cartridge, Abbott; Table S1 in the Supplement at www.int-res.com/articles/suppl/d160p057_supp.pdf). Postmortem blood glucose (CareTouch glucometer, Future Diagnostics) taken at 16:00 h was within published ranges for free-ranging manatees (64 mg dl^{-1}; Harvey et al. 2007). Blood collected at 16:40 h was centrifuged for serum collection and submitted for a serum biochemical panel, serum amyloid A (SAA), and capillary zone electrophoresis at the University of Miami Avian and Wildlife Laboratory (Miami, FL), and blood films were submitted to the University of Florida Aquatic Animal Health Program (Gainesville, FL; Table S1). Primary findings from blood film review and serum chemistry analysis were indicative of systemic inflammation (elevated SAA and beta globulins, severe heteropenia with left shift, lymphopenia, increased rouleaux formation) with concern for disseminated intravascular coagulation (thrombocytopenia; Table S1; Harvey et al. 2007, 2009, 2018). Possible peri- or post-mortem artifacts such as leukocyte and/or platelet clumping could not be ruled out but were absent by blood film review. Considerations for increased blood urea nitrogen without increased creatinine included protein catabolism (starvation), and/or intestinal hemorrhage. Hyperphosphatemia suggested decreased glomerular filtration rate, dietary cause, or myopathy.

3.2. Gross necropsy

Postmortem examination of the fresh carcass revealed that the manatee was a 275 cm (dorsal recumbency, straight length) male weighing 376 kg. Impression smears of the seminal fluid did not reveal mature sperm. The animal was in thin body condition (body condition score 2/5), with flattening of the abdomen and moderate loss of nuchal fat. Subcutaneous fat layers were thin (mid-ventral measurements: 0.9 cm outer fat, 0.5 cm inner fat; mid-lateral measurements: 0.5 cm outer fat, 0.7 cm inner fat). The body condition index of this manatee (BCI = weight/straight length$^{2.578}$) was 27.7, falling in the 10–25% range for wild male Florida manatees (Harshaw et al. 2016). Cold stress lesions were externally evident, including moderate bleaching of the skin on the snout, head, flippers, axillae, and fluke, and patches of ulcerative lesions on the fluke ranging in size from 2 mm to 3 cm (Fig. 1a,b). Ulcerative skin lesions were also observed on the right lateral flipper and left lateral peduncle. Internal examination findings were also consistent with emaciation and cold stress. The intraabdominal fat appeared gelatinous and pink with multifocal, <0.5 cm diameter purpura. The large intestine contained compact, dark green, fibrous, clay-like feces. The pancreas was uniformly dark pink to red and unremarkable upon gross examination.

The pericardium was pale pink and edematous, and the pericardial sac contained ~125 ml of pink, translucent fluid. The heart with greater vessels weighed 1178 g (0.31% of body weight). The left ventricular wall was severely thickened (thicknesses at half the ventricular height: ventral = 3.2 cm, lateral = 3.8 cm, dorsal = 3.0 cm), and the chamber size was decreased, consistent with concentric hypertrophy (Fig. 1c). Aortic stenosis was evident proximal to the heart (Fig. S1b). The internal diameter of the aorta where it exits the heart was 2.6 cm, and the wall thickness was 8 mm. On gross examination, the lungs were pale pink to white and edematous, and there was abundant white foam throughout the bronchi. In the caudal lung lobes, there was multifocal pulmonary hemorrhage.

The distal esophagus was occluded by a twisted plastic bag measuring 35 cm long and 1.3 cm wide (Fig. 1e). When untwisted, the bag was partially deteriorated and off-white to gray with red print (Fig. 1f), similar to a commercial plastic grocery shopping bag. At least 7 nasal trematodes were present around the bag. No natural forage material was present proximal to the bag or in the stomach or duodenum; however, there were ~100 ml of pale-yellow liquid in the stomach and ~700 ml of pale-grey liquid in the duodenum. There was scant digesta in the proximal gastrointestinal tract. The gallbladder was distended with ~20 ml of brown to green bile.

3.3. Histopathologic review

Cold stress lesions demonstrated subacute, multifocal, moderate to marked epidermal necrosis and hydropic degeneration with intraepidermal heterophilic pustules and dermal abscessation, epithelial hyperplasia, vasculitis, and intravascular fibrin thrombi. There was moderate serous atrophy of abdominal, omental, and pericardial adipocytes. There was multifocal extravasation of erythrocytes of the epidermis, dermis, and fat, and moderate congestion of the fat tissues. The pancreas had moderate, diffuse depletion of acinar zymogen granules.

Histopathologic review of the pericardium revealed fibrosis with intimal fibropapillary projections. The heart exhibited left ventricular cardiomyocyte hyper-

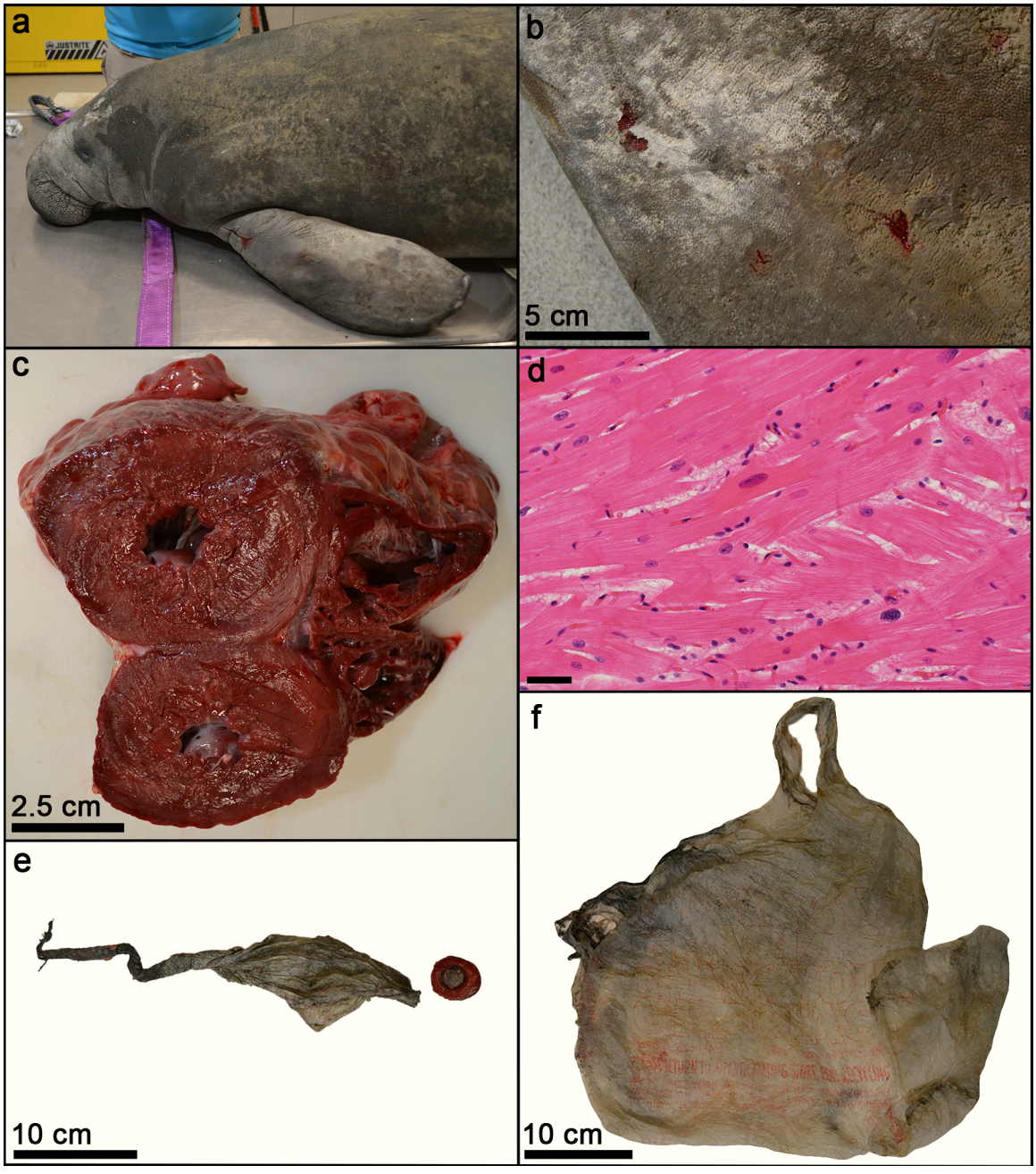

Fig. 1. Multifactorial cause of death in an adult male, 275 cm, West Indian manatee *Trichechus manatus*. (a) Cold stress lesions on the head and flipper, including moderate bleaching of the skin of the extremities. (b) Cold stress lesions (ulcerative skin lesions) on the ventral fluke. (c) Left ventricular concentric hypertrophy. (d) Heart, stained with hematoxylin and eosin (scale bar = 40 µm), showing hypertrophy of the left ventricular cardiomyocytes. (e) Cross-section of the distal esophagus (right side of image), which is occluded by a twisted, plastic bag. Some of the bag was removed prior to photographing (left side of image). (f) Plastic bag that was occluding the esophagus (panel e). When untwisted, the bag is partially deteriorated and has legible, red print

trophy with enlarged nuclei (Fig. 1d), left atrial and right ventricular cardiomyocyte atrophy, and mitral and tricuspid valve endocardiosis. There were also several changes in the aorta (Fig. S1). Fibromuscular dysplasia was evident, collagenous and smooth muscle fibers were irregularly oriented, and there was increased ground substance. In one section of the aorta, there were fibrous extensions. Fibropapillary intimal fronds were present. AB-PAS-stained sections of stenotic aorta had increased ground substance

compared to unaffected aorta (Fig. S1c,d). VVG stain showed reduction and loss of orderly laminar arrangement of elastic fiber (Fig. S1f). Smooth muscle was arranged in a disorderly pattern. SMA immunoreactivity for smooth muscle fibers was attenuated. Histologic findings in the lungs included occasional alveoli containing fibrinous exudate and lightly eosinophilic proteinaceous substance (edema). In the caudal left and right lung, multifocal alveolar spaces contained extravasated erythrocytes. There was also a mild, focal interstitial infiltrate of lymphocytes and plasma cells and multifocal perivascular expansion by clear spaces (edema). Histologically, the esophageal superficial epithelial layers were absent; however, there was no compression, ulceration, hemorrhage, congestion, inflammation, or edema present.

4. DISCUSSION

This report presents an unusual case of cold stress syndrome with comorbidities that contributed to the debilitation of this manatee and consequent death. Cold stress was an expected cause of mortality in this individual given the time of stranding. From 1 to 23 January 2022, prior to stranding, the average maximum and minimum water temperatures in nearby Mobile Bay were 16.6 ± 1.1°C and 12.4 ± 0.84°C, respectively (https://seatemperature.info/january/mobile-water-temperature.html), and there are no known thermal refugia (e.g. warm water outflows) in the area where the manatee stranded. Accordingly, the skin lesions of this manatee demonstrated characteristic gross and histopathologic findings of cold stress syndrome (Bossart et al. 2003, Martony et al. 2019). Depleted fat stores and serous atrophy of fat support that this animal was in chronic poor body condition, a common sequela to cold stress (Bossart et al. 2003). Pancreatic atrophy seen in this case is common in animals with protein-calorie deficiency and has been documented in some cold-stressed manatees (Bossart et al. 2003, Martony et al. 2019). The histologic findings of extravasation of erythrocytes in the skin and fat tissues and congestion of fat tissues along with clinicopathologic evidence of systemic inflammation (i.e. heteropenia with left shift), thrombocytopenia, and acid—base disorder were concerning for disseminated intravascular coagulopathy, which has been previously documented in cold-stressed manatees (Barratclough et al. 2017). The compact, clay-like feces seen in this case are also consistent with other cases of cold stress in manatees exhibiting secondary constipation and dehydration (Bossart et al. 2003, our pers. obs.). While most manatees migrate out of AL waters during the winter months, an increasing number are staying longer into the cold season, potentially reflective of climate-induced increases in water temperatures affecting their migratory phenology (Hieb et al. 2023) and increasing the risk of cold-stress related mortality. Given this consideration, cold stress was an expected finding; however, additional findings of heart disease and esophageal occlusion resulted in a uniquely complex, multifactorial cause of death.

Reports of heart disease in manatees include right-sided heart enlargement (Gerlach et al. 2012), thickened atrioventricular valves (Buergelt et al. 1990), acute myocarditis (Moore et al. 2008), left-sided heart failure (Balik et al. 2021), and congenital malformations including right ventricle hypoplasia and stenosis, ventricular septal defect, mitral valve hemocytes, and left ventricular hypertrophy (Carvalho et al. 2019). Myocardial degeneration has been reported in manatees with cold stress syndrome (Bossart et al. 2003). This case is the first to report aortic stenosis in this species. The cause of aortic stenosis in this case was not evident. An inherited disorder, sequela to changes in vascular flow, nutritional deficiency, and Marfan syndrome were considered. With these conditions, aneurysms can occur, but none were observed in this case. Fibromuscular dysplasia is uncommon and has been reported in humans involving renal and carotid arteries (Baradhi & Bream 2023). The fibrous extensions are likely the result of chronic turbulence. In the normal manatee heart, there is typically a thick-walled expansion of the ascending aorta (Rowlatt & Marsh 1985), as opposed to a narrowing. In this case, stenosis would have led to increased pressure for vascular flow and subsequent mitral valve endocardiosis and left-sided myocardial hypertrophy. The ratio of the heart weight to total body weight in this case was similar to that of manatees without evidence of heart disease (Buergelt et al. 1990). Thickened atrioventricular valves have been previously reported as an incidental finding in neonate West Indian manatees (Buergelt et al. 1990); however, in this case, it was a mechanical response. This would have resulted in adverse effects on systolic and diastolic function and cardiac insufficiency (Badiani et al. 2016). In turn, cardiac insufficiency would have led to the pulmonary edema seen in this case and may have triggered cardiac arrest during rescue. It is additionally possible that the aortic stenosis and subsequent cardiac insufficiency contributed to the failure of this manatee to migrate out of the area despite the seasonally declining water temperatures.

In addition to heart failure, the manatee had a plastic bag in the distal esophagus. Plastic packing or bags have been found in the gastrointestinal tract of 11–14% of manatee carcasses examined in some areas (Beck & Barros 1991, Reinert et al. 2017), but complete esophageal occlusion has not been described in the literature. The lack of pathologic changes seen in the esophagus epithelial layers may indicate that the foreign body was able to move or did not exhibit characteristic obstructive lesions; lesions may also have been segmental and not captured during sampling. It was unclear how long the plastic bag had been present. The lack of digesta in the proximal GI tract indicates that the manatee did not eat for several days, as typical manatee digesta passage rates range from 4 to 10 d (Larkin et al. 2007). Distension of the gallbladder and pancreatic atrophy may also be associated with inanition (King et al. 2013). Whether inanition was related to the plastic bag occluding the esophagus or whether the bag may have been consumed due to lack of appropriate forage or terminally aspirated could not be determined. Regardless, the plastic bag would have added to the animal's stress and was considered a contributing cause of death.

Importantly, these complicating factors, including respiratory and cardiac compromise, necessarily contributed to an inability to successfully treat the manatee for cold stress signs post-rescue. It is also likely that, had the animal survived transport, both of these factors would have challenged successful rehabilitation. This case highlights the importance of multiple factors contributing to manatee mortality, with cold stress and anthropogenic factors potentially of increasing concern for manatee welfare in areas outside of their traditional Florida winter range. Manatees seasonally migrating farther distances such as to the northcentral Gulf of Mexico or north along the US Atlantic coast have a greater risk of exposure to cold temperatures that lead to cold stress syndrome and increased interactions with people (Cloyed et al. 2021). For example, in January 2023, about 1 yr after this rescue intervention, a manatee was found dead due to trauma following a vessel strike in the Mobile River, Mobile, AL. It is thought that cold stress syndrome in this animal may have hindered evasion of the vessel and resulted in a collision, leading to the animal's death (our pers. obs.). Rapid response efforts, comprehensive examination, necropsy, and sampling protocols, and thorough diagnostic testing (including histopathological analyses) will be key to detecting and fully understanding such important and potentially complex, multifactorial causes of death.

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