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Manatee *Trichechus manatus latirostris* calf mortality in Florida: a retrospective review of pathology data from 2009–2017

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ABSTRACT: High maternal investment and extended inter-calving intervals in Florida manatees *Trichechus manatus latirostris* make calf survivorship critical to overall population growth. However, detailed patterns of causes of mortality in calves have not been reported and state agency statistics report portions of perinatal mortality based on body length rather than actual cause of death (COD). The objectives of this study were to categorize COD based on necropsy data and geographical location in Florida for 1209 manatee calf carcasses (<236 cm total length) examined between January 2009 and December 2017 and to describe factors contributing to calf mortality. Results indicated COD was attributed to natural causes (47%, n = 573), cold stress syndrome (38%, n = 457), watercraft injury (13%, n = 155), or other human-related causes (2%, n = 24). Natural causes were the leading COD for small calves <151 cm, with death due to stillbirth or dystocia most frequent (48%, n = 273/573). Enteric trematodiasis contributed to a large proportion of deaths from natural causes in large calves within the southwest region of Florida, with an increasing annual trend. Brevetoxicosis contributed substantially to natural causes within the southwest region exclusively and was commonly comorbid with enteric trematodiasis. Cold stress syndrome was the leading cause of death for large calves (151–235 cm), with the Atlantic region having the highest proportion of cases. Watercraft injury was a sustained threat to large calves, especially within the southwest region. This report provides details on specific health threats and patterns of mortality among manatee calves.

KEY WORDS: Sirenia · West Indian manatee · Necropsy · Perinatal · Pathology · Stranding · Disease

1. INTRODUCTION

The Florida manatee *Trichechus manatus latirostris*, a subspecies of the West Indian manatee, is an herbivorous aquatic mammal native to the southeastern USA, particularly coastal Florida. Currently, the subspecies is listed as threatened by the US Fish and Wildlife Service under the Endangered Species Act (Kurth 2017) and Endangered on the IUCN Red List (Deutsch 2008). Threats to this species include trauma from watercraft (WC)-related injuries or other man-made structures, habitat loss and disrup-

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tion, and natural events such as red tides or prolonged exposure to cold, which may be exacerbated by human activities (US Fish and Wildlife Service 2001, Florida Fish and Wildlife Conservation Commission 2007). Data on manatee mortality have been collected and documented within the state of Florida since 1974 and are currently overseen by the Florida Fish and Wildlife Conservation Commission (FWC). Evaluation of mortality data has been instrumental for the identification of emerging threats and the development of conservation programs (Bonde et al. 2012b).

Initial analysis of mortality data collected from the 1970s to the 1990s revealed WC injuries and other human activities as the largest single cause of manatee mortality (O'Shea et al. 1985, Ackerman et al. 1995, Lightsey et al. 2006), leading to protective measures limiting boater speeds in certain areas (US Fish and Wildlife Service 2001, Calleson 2014). Despite these efforts, WC injury remains a significant cause of mortality; additionally, quantitative models have identified red tide events and loss of warm-water habitat as significant threats (Runge et al. 2017). Most analyses have focused on general population trends by evaluating cause of death (COD), sex, season, and location of carcass recovery (Ackerman et al. 1995), but young calves are generally excluded from population models because their mortality is subsumed under estimates of reproductive rate (Runge et al. 2017). An average of 19% (n = 909/4709) of annual manatee mortality was reported as 'perinatal' (Florida Fish and Wildlife Conservation Commission 2019) from 2009 through 2017 — which includes a wide range of natural and undetermined COD for carcasses that were 150 cm in total length or shorter — without further consideration of other criteria, and a few carcasses that were determined stillborn and longer than 150 cm. This is in contrast to carcasses 150 cm or shorter with overt anthropogenic trauma or those longer than 150 cm in which COD is assigned based on a gross pathological diagnosis. In addition to delineating small calves that did not die from human-related (HR) causes, this 'perinatal' category has historically represented dependent calves unlikely to survive if separated from their mothers and was defined by the upper size limit of the largest known *in situ* fetus at the time.

Manatees are a long-lived species and display high levels of maternal investment. Females are believed to reach sexual maturity as early as 4 yr of age, with calves typically remaining dependent for up to 2 yr, thus prolonging the calving interval in Florida to about 3 yr (Rathbun et al. 1995, Koelsch 2001). Calf survivorship is essential for the long-term success of this species, and while population dynamics in manatees and other long-lived species are generally more sensitive to adult survival rates (Runge et al. 2004), the loss of manatee calves still dependent on their mothers for survival may slow overall population growth (O'Shea et al. 1985, Bonde et al. 2012b). Examining mortality trends in this age grouping could facilitate the development of novel strategies and focused initiatives for advancing conservation management. However, there has been little described on actual COD and health threats in this significant portion of reported manatee mortality, largely due to a classification based on body size for small calves without evidence of HR trauma. The objectives of this study were to categorize known COD based on pathological evaluation and geographical location for 1209 manatee calf carcasses (<236 cm total length) between January 2009 and December 2017 and describe factors contributing to calf death.

2. MATERIALS AND METHODS

Within Florida, the FWC coordinates manatee rescue operations and carcass salvage. The purposes of this program are to characterize and record necropsy information to determine causes of mortality and obtain information on manatee life history and health. A well-publicized, toll-free wildlife alert phone number allows members of the public to report distressed, injured, or dead manatees. Five FWC field stations that are located throughout the state respond to manatee carcass reports, perform necropsies in the field, or transport them to the Marine Mammal Pathobiology Laboratory (MMPL) in St. Petersburg, Florida, USA, where they are examined and processed following standardized procedures (Bonde et al. 1983). Collected necropsy data are entered into a database that includes carcass geographical location and recovery date, sex, length, and category of probable COD. The COD is based on gross findings in addition to ancillary testing as warranted by an examiner's observations of each individual case, including histopathological, microbiological, and/or molecular diagnostic testing.

For this retrospective study, necropsy reports were reviewed for all manatee calf carcasses recovered between 1 January 2009 and 31 December 2017 in the state of Florida. Calves are manatees that are dependent on maternal care for survival, subadults are weaned, independent manatees that are not yet sexually mature, and adults are physically and reproductively mature manatees. Calves were further defined as having a straight-line total length of <236 cm (Bonde et al. 2012a, Runge et al. 2017, Lonati et al. 2019). Small calves were defined as measuring 150 cm or less, per the state's standing characterization of perinatal deaths based on total length and prior categorization of manatees 150 cm or less as a neonatal or early juvenile age class (O'Shea et al. 1985, Ackerman et al. 1995); large calves ranged from 151–235 cm. The latter size category was based on current definitions by state (FWC) and federal (US Geological Survey) agencies and an average length at weaning of approximately 240 cm (O'Shea & Reep 1990). Both categories have been utilized for carcass analyses and manatee population models over the past decade (Bonde et al. 2012a, Runge et al. 2015, 2017, Hostetler et al. 2021).

Small calves were presumed to be less than 1 yr old, and previous evidence suggests that these range from newborn up to 5 or 6 mo of age (Odell 1978, White et al. 1984). Large calves are generally up to 2 yr in age (Runge et al. 2017), but age estimation studies suggest that this group can include individuals up to 3–4 yr old (Schwarz & Runge 2009, Lonati et al. 2019), as age estimations based on length are not precise, and there is variability between sexes and in both age and size at weaning and independence (Schwarz & Runge 2009). Therefore, the large calf category may contain both dependent calves as well as some subadults. For simplicity, this category is hereafter referred to as large calves. Carcass recovery location was used to assign each animal to Atlantic coast (ATL), northwest (NW), southwest (SW), or upper St. Johns river (USJR) regions following previously defined regional management units (US Fish and Wildlife Service 2001). Within each region, manatee deaths were also examined by county. Carcasses that were found outside of Florida were excluded. COD is defined as the condition that most likely resulted in the death of the manatee based on all available information, including gross necropsy and any histopathology reports and ancillary diagnostic tests if available. Contributing factors are conditions which may have led to the manatee's death. Comorbidities are defined as the simultaneous presence of 2 or more contributing factors in the same manatee. All manatees for which COD could be determined were included in our analysis. Manatees with undetermined COD were excluded from this study. Exclusion criteria included advanced stage of decomposition resulting in limited opportunity for necropsy examination or inconclusive findings during necropsy due to lack of gross lesions.

COD was broadly categorized as attributed to natural causes (NC), cold stress syndrome (CSS), WC-related injuries, or other HR causes following established state agency definitions. CSS, a natural phenomenon exacerbated by anthropogenic activities, was added as a stand-alone state COD category outside of the NC category in 1986 due to increased observed frequency and significance to the species (Barlas et al. 2011). Specific contributing factors to mortality due to NC included enteritis, enteric trematodiasis, perinatal death, congenital deformity, umbilical disease, brevetoxicosis, and pulmonary disease. Enteritis was defined based on gross lesions such as gastrointestinal erosions or ulceration, mucosal thickening, hyperemia, increased rugosity, or hemorrhage, the presence of necrotic plaques or foci, segmental ischemia, segmental distension, or discoloration, and possible perforations with peritonitis and adhesions or inflammation of adjacent mesentery. Enteric trematodiasis was characterized by the gross presence of many small, white, pinpoint lesions within the intestinal mucosa and adult *Moniligerum blairi* or *Nudacotyle undicola* trematodes on mucosal scrapings when examined microscopically in direct association with significant gross and, where available, histological intestinal lesions. Overt gross lesions included intestinal wall thickening and increased mucosal rugosity, hemorrhage, and raised nodules with necrotic plaques or foci (Fig. 1). Histological findings were characterized by adult trematodes embedded within intestinal mucosa with associated mixed inflammatory infiltrates, mucosal hyperplasia, erosion or ulceration, necrohemorrhagic and fibrinous debris, villus blunting, and crypt atrophy (Fig. 2). The presence of trematodes in the absence of significant intestinal lesions was considered incidental infection. For the purposes of this study, perinatal deaths were defined strictly as calf deaths due to presumed dystocia or still birth and not in relation to carcass total length. Most perinatal deaths were believed to occur either before, during, or immediately following parturition, but in a few instances may have occurred up to a few days later for calves succumbing to complications such as aspiration or hemorrhage. This category was classified based on factors such as the presence of attached fetal membranes or tissues, nonaerated lungs, lack of ingesta or umbilical tear, and hemorrhage without gross or (when available) histological evidence of inflammation. Supporting evidence included meconium within the intestinal tract or the presence of

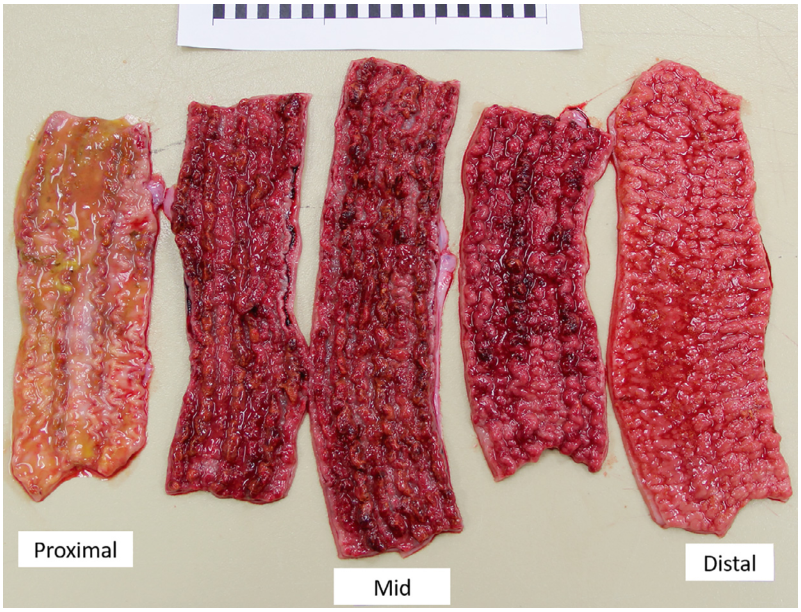

Fig. 1. Gross lesions associated with enteric trematodiasis within the small intestines of manatee calves at necropsy. Proximal, mid, and distal intestinal segments are open revealing thickened mucosa with increased rugosity, hemorrhage, and necrotic plaques. Scale: cm

aspirated sediment. Congenital deformities were defined as grossly observable anatomical abnormalities capable of contributing to or causing death and believed to be present prior to birth rather than acquired postnatally. Umbilical disease was associated with purulent or caseous material within or adjacent to the umbilicus, associated peritonitis or adhesions, umbilical distension, discoloration, or ulceration,

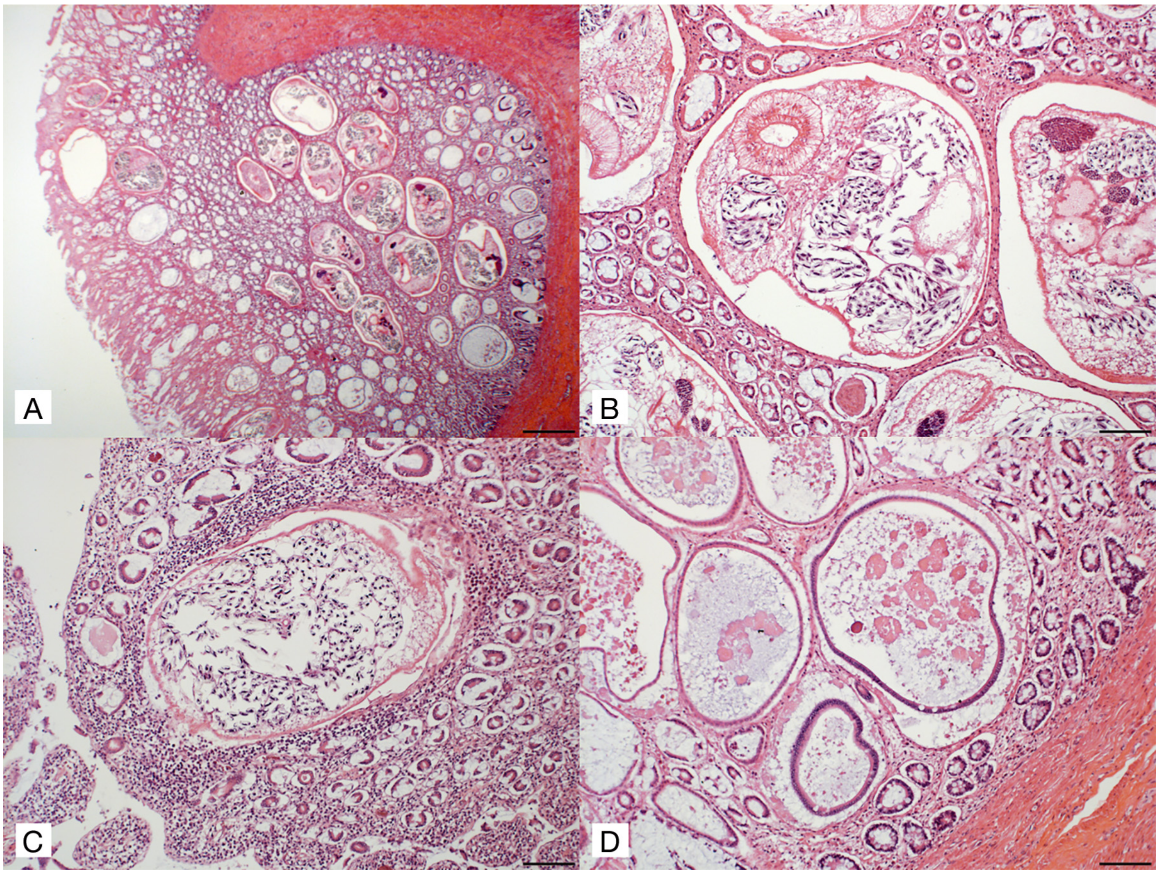

Fig. 2. Histological lesions associated with enteric trematodiasis within the small intestines of manatee calves. (A) Mucosal hyperplasia and encysted trematodes within dilated crypts. Hematoxylin and eosin stain (HE); scale bar = 500 µm. (B) Intestinal crypts are dilated. There is mild inflammation. HE; scale bar = 50 µm. (C) A crypt containing a trematode is partially ruptured and there is moderate inflammatory response. HE; scale bar = 100 µm. (D) There are dilated crypts which are devoid of parasites, but contain proteinaceous aggregates and cellular debris. HE; scale bar = 50 µm

positive bacterial cultures when obtained, and wherever available, histological evidence of active inflammation. Brevetoxicosis was determined by toxicological analysis of ingesta, liver, lung, brain, and kidney tissues or urine for the presence of brevetoxins. Since a lethal threshold of brevetoxins in manatees has not been established to date, brevetoxicosis was considered to have contributed to death if brevetoxins were detectable in more than one tissue or sampling site and there were gross or histological lesions consistent with, but nonspecific for brevetoxicosis (Florida Fish and Wildlife Conservation Commission 2013). Lesions included edematous, and liquid, mucus, or froth-filled airways, significant vascular congestion within the respiratory tract, liver, kidneys, or brain, and meningitis (Bossart et al. 1998, Florida Fish and Wildlife Conservation Commission 2013). Testing for brevetoxins is not regularly performed on all manatee carcasses and was limited to carcasses examined during known harmful algal blooms or red tide events. Pulmonary disease was characterized by pulmonary abscessation, fibrinous pneumonia, infectious pneumonia (i.e. positive bacterial culture or histologically detected bacteria or fungi and associated inflammation), regions of atelectasis, or hemorrhage as well as copious mucus, foam, or airway froth, and/or aspirated sediment. Contributing factors to NC deaths outside of these categories were labeled as 'other.'

Gross and histological lesions consistent with CSS included cutaneous lesions (bleaching, ulceration, hyperplasia, hyperkeratosis, or abscessation) or whole-body fat depletion, hard, dry feces, or abscesses internally (Bossart et al. 2002, Barlas et al. 2011, Martony et al. 2019). Deaths associated with WC were defined by traumatic lesions from either sharp penetrating or blunt force injuries consistent with vessel impact (Lightsey et al. 2006). CODs classified as HR were defined as lesions resulting from human activity other than obvious WC injury and included entanglements, foreign debris ingestion, entrapment in pipes and culverts, or flood gate/canal lock drownings or trauma. The presence of foreign debris within ingesta was evaluated across all COD categories in addition to where it is believed to have been the primary COD.

Confidence intervals (95% CI) were calculated for selected overall COD proportions (e.g. due to CSS or WC injuries) using the software EpiTools (Sergeant 2018). The null hypotheses that annual COD proportions (due to NC, CSS, or WC) were not different between years were tested by using a chi-squared test. The HR category was excluded from annual analysis due to low sample size. Within each region and year, the null hypotheses that COD proportions due to NC, CSS, WC, and HR were not different were examined by using a chi-squared test. The USJR was excluded from annual comparisons due to small sample size. Simple linear regression was used to examine the observed linear relationship between years (2009–2017) and annual proportions of enteric trematodiasis-associated deaths. Values of $p < 0.05$ were considered significant. Statistical analyses were completed in Microsoft Excel 2019 and Statistix 10 for Windows (Analytical Software).

3. RESULTS

From January 2009 through December 2017, a total of 2107 manatee calf carcasses were recovered (annual mean: 234). There were 1121 males, 945 females, and 41 carcasses where sex was not determined. Nearly half of the cases (43%; n = 898/2107) did not meet inclusion criteria and were excluded from further analysis. Most excluded cases were in advanced stages of decomposition (89%; n = 797/898) which precluded diagnostic workup. A total of 1209 cases met the inclusion criteria for this study and was used as the total number for calculation of COD proportions.

3.1. Overall patterns

The main COD during the study period was NC (n = 573/1209 or 47%; 95% CI = 45, 50%) (Table 1). The proportion of annual mortality due to NC was higher during 2012–2017 (52–69%), compared to 2009–2011 (18–38%) ($p < 0.05$) (Table 1). Death due to NC was multifactorial, and certain pathological findings were observed to contribute to mortality more frequently (Table 2). Comorbidities were common and numerators of proportions represent all cases where an individual contributing factor was seen. Enteric disease contributed to death in 37% (n = 213/573) of NC cases and of these, 73% (n = 155/213) were found to have parasitic enteritis from *Moniligerum blairi* or *Nudacotyle undicola* trematodes. Trematode infections contributing to death were most common within the small intestine, followed by the cecum and the colon. Trematodes were never found within the stomach and very rarely extended into the duodenum. The annual proportions of documented enteric trematodiasis contribut-

Table 1. Numbers and percentages of manatee *Trichechus manatus latirostris* calf carcasses recovered along the Florida coast that were examined and categorized by cause of death (COD) and by year from 2009–2017. Data reported in parentheses are %. Proportions for death due to natural causes, cold stress, watercraft, and human-related causes were calculated using annual totals of manatees with determined COD. Within each column, groups (percentages) with different superscript letters are different ($p < 0.05$); within the row for all years, groups (percentages) with different superscript numbers are different ($p < 0.05$)

Year	Age class	Total	COD undetermined	COD determined	Natural causes	Cold stress	Watercraft	Human-related
2009	Small	113	106 (44.5)	47	39	8	0	0
	Large	125		85	11	48	24	2
	All	238		132 (100.0)	50 (37.9)^{b}	56 (42.4)^{c}	24 (18.2)^{b}	2 (1.5)
2010	Small	96	123 (33.6)	56	33	23	0	0
	Large	269		186	11	158	15	2
	All	365		242 (100.0)	44 (18.2)^{a}	181 (74.8)^{a}	15 (6.2)^{a}	2 (0.8)
2011	Small	77	92 (41.8)	33	23	8	2	0
	Large	143		95	15	63	15	2
	All	220		128 (100.0)	38 (29.7)^{b}	71 (55.5)^{b}	17 (13.3)^{b,c}	2 (1.6)
2012	Small	69	78 (45.8)	24	24	2	0	0
	Large	101		68	24	24	17	1
	All	170		92 (100.0)	48 (52.2)^{c}	26 (28.3)^{d}	17 (18.5)^{b}	1 (1.1)
2013	Small	128	137 (50.1)	53	41	11	0	1
	Large	145		83	45	24	10	4
	All	273		136 (100.0)	86 (63.2)^{cd}	35 (25.7)^{d}	10 (7.4)^{a,c}	5 (3.7)
2014	Small	99	97 (50.0)	40	36	4	0	0
	Large	95		57	21	20	16	0
	All	194		97 (100.0)	57 (58.8)^{cd}	24 (24.7)^{de}	16 (16.5)^{b}	0 (0.0)
2015	Small	89	93 (47.9)	39	37	1	1	0
	Large	105		62	28	13	19	2
	All	194		101 (100.0)	65 (64.4)^{cd}	14 (13.9)^{e}	20 (19.8)^{b}	2 (2.0)
2016	Small	111	93 (45.1)	49	42	6	0	1
	Large	95		64	36	13	14	1
	All	206		113 (100.0)	78 (69.0)^{d}	19 (16.8)^{d}	14 (12.4)^{,b}	2 (1.8)
2017	Small	109	79 (31.9)	68	60	8	0	0
	Large	138		100	47	23	22	8
	All	247		168 (100.0)	107 (63.7)^{cd}	31 (18.5)^{d}	22 (13.1)^{b}	8 (4.8)
All	Small	891	898 (42.6)	411	335	71	3	2
	Large	1216		798	238	386	152	22
	All	2107		1209	573 (47.4)^{4}	457 (37.8)^{3}	155 (12.8)^{2}	24 (2.0)^{1}

ing to NC-related death increased from 10% (n = 5/50) in 2009 to 40% (n = 43/107) in 2017, and the frequency of manatee carcasses with a positive diagnosis of mono or co-infections with enteric trematodiasis linearly increased by year during the study period ($R = 0.95$, $R^2 = 0.91$, $p < 0.01$) (Fig. 3). When conservatively including all manatee cases with intestinal lesions characteristic of enteric trematodiasis, but lacking definitive identification of trematodes during necropsy (n = 15), this linear trend persists ($r = 0.95$, $R^2 = 0.93$, $p < 0.01$). Enteric trematode infections were present in 61% (n = 95/155) of WC cases, 38% (n = 173/457) of CSS cases, and 25% (n = 6/24) of HR cases and found most commonly within the cecum and the colon without concurrent severe lesions. Trematode-associated intestinal disease was not seen in any of the HR cases but was detected in some WC and CSS cases. Perinatal death accounted for 48% (n = 273/573) of NC mortalities. Brevetoxicosis contributed to 21% (n = 118/573) of deaths and was a condition frequently observed comorbid with enteric trematodiasis (n = 72). Brevetoxins were detected in at least one tissue in 8% (n = 13/155) of WC cases, <1% (n = 2/457) of CSS cases, and not in any HR cases. Congenital deformities severe enough to contribute to death were seen in 3% (n = 18/573) of NC cases. Deformities seen included under-development of or complete organ agenesis

Table 2. Frequency of specific conditions or diseases contributing to death from natural causes among manatee *Trichechus manatus latirostris* calves recovered along the Florida coast by year and size-class from 2009–2017. E: enteritis; Te: enteric trematodiasis; P: perinatal; Cd: congenital deformity; U: umbilical disease; B: brevetoxicosis; Pu: pulmonary; O: other

Year	Age class	Total	E	Te	P	Cd	U	B	Pu	Te + B	Cd + U	E + Pu	Te + Pu	P + B	Te + B + Pu	Te + Cd + B	O
2009	Small	39	5	0	30	2	1	0	0	0	1	0	0	0	0	0	0
	Large	11	2	3	0	3	0	0	1	2	0	0	0	0	0	0	0
	All	50	7	3	30	5	1	0	1	2	1	0	0	0	0	0	0
2010	Small	33	3	0	26	2	1	0	0	0	0	1	0	0	0	0	0
	Large	11	9	1	0	0	0	0	1	0	0	0	0	0	0	0	0
	All	44	12	1	26	2	1	0	1	0	0	1	0	0	0	0	0
2011	Small	23	2	0	16	3	1	0	0	0	0	0	0	0	0	0	1
	Large	15	9	0	1	0	0	1	0	4	0	0	0	0	0	0	0
	All	38	11	0	17	3	1	1	0	4	0	0	0	0	0	0	0
2012	Small	24	1	0	20	1	1	1	0	0	0	0	0	0	0	0	0
	Large	24	6	2	0	0	0	9	0	4	0	0	3	0	0	0	0
	All	48	7	2	20	1	1	10	0	4	0	0	3	0	0	0	0
2013	Small	41	2	0	28	3	3	2	0	0	0	0	0	3	0	0	0
	Large	45	3	14	1	0	0	16	0	11	0	0	0	0	0	0	0
	All	86	5	14	29	3	3	18	0	11	0	0	0	3	0	0	0
2014	Small	36	1	1	30	1	3	0	0	0	0	0	0	0	0	0	0
	Large	21	5	15	0	0	0	0	0	0	0	0	1	0	0	0	0
	All	57	6	16	30	1	3	0	0	0	0	0	1	0	0	0	0
2015	Small	37	2	0	31	0	2	1	1	0	0	0	0	0	0	0	0
	Large	28	0	16	2	0	0	2	2	5	0	0	1	0	0	0	0
	All	65	2	16	33	0	2	3	3	5	0	0	1	0	0	0	0
2016	Small	42	2	0	30	1	5	0	1	1	0	0	0	2	0	0	0
	Large	36	0	4	1	0	0	5	1	21	0	0	1	0	2	0	1
	All	78	2	4	31	1	5	5	2	22	0	0	1	2	2	0	1
2017	Small	60	2	2	47	0	4	1	1	1	0	0	0	2	0	0	0
	Large	47	3	17	3	0	0	1	0	19	0	0	2	0	1	1	0
	All	107	5	19	50	0	4	2	1	20	0	0	2	2	1	1	0
Total	Small	335	20	3	258	13	21	5	3	2	1	1	0	7	0	0	1
	Large	238	37	72	8	3	0	34	5	66	0	0	8	0	3	1	1
	All	573	57	75	266	16	21	39	8	68	1	1	8	7	3	1	2

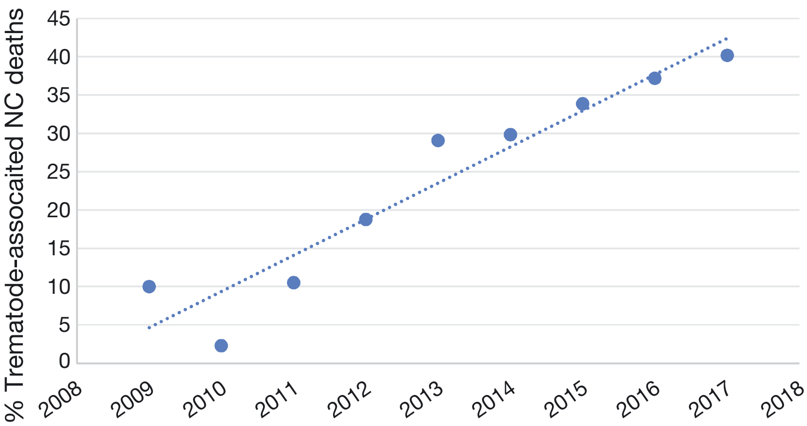

Fig. 3. Proportions of enteric trematodiasis-associated deaths of manatees in the category of natural causes (NC) by year. Trendline (dotted line) R = 0.95. This demonstrates a linear increase in the frequency of manatee carcasses with a positive diagnosis of mono- or co-infections with enteric trematodiasis annually during the study period

(renal and pulmonary), organ malformation (cardiac, pulmonary, renal, gastrointestinal), enterocolonic strictures, and skeletal abnormalities including severe spinal curvature, midline deficits, skull malformation, and skeletal agenesis (pectoral flippers). Other contributing conditions included a single small calf with hepatitis and a single large calf with hepatocellular carcinoma.

Mortality due to CSS was 457/1209 or 38% (95% CI = 35, 41%) during the study period. Annual mortality due to CSS was higher during 2009–2011 (range: 42–75%), compared to 2012–2017 (range: 14–28%) ($p < 0.05$) (Table 1). CSS mortality was highest in 2010, accounting for 75% (n = 181/242) of cases with known COD that year. Overall, mortality due to WC-induced injuries was 155/1209 or 13% (95% CI = 11, 15%) during the study period. The percentage of annual mortality due to WC-induced injuries was consistently above 12% during 2014–2017 (range: 12–20%) compared to 2009–2013 (range: 6–19%), when mortality from CSS and brevetoxicosis was elevated (Table 1). Mortality due to HR causes accounted for 2% (n = 24/1209) of calf deaths. Foreign material consisting principally of monofilament and plastics was found in 13% (n = 155/1209) of calf carcasses and was the presumptive specific COD in 8 cases.

3.2. Size class patterns

Death due to NC was the leading COD for small calves (82%, n = 335/411) but not large calves (30%, n = 238/798) (Table 1, Fig. 4a). Size-class differences were also seen for contributing factors in the NC category (Table 2, Fig. 4b). A greater proportion of enteric trematodiasis (97%, n = 150/155, 95% CI = 93, 99%) was seen in large calves. Perinatal death was seen in 8 animals characterized as large calves (3%, n = 8/273, 95% CI =

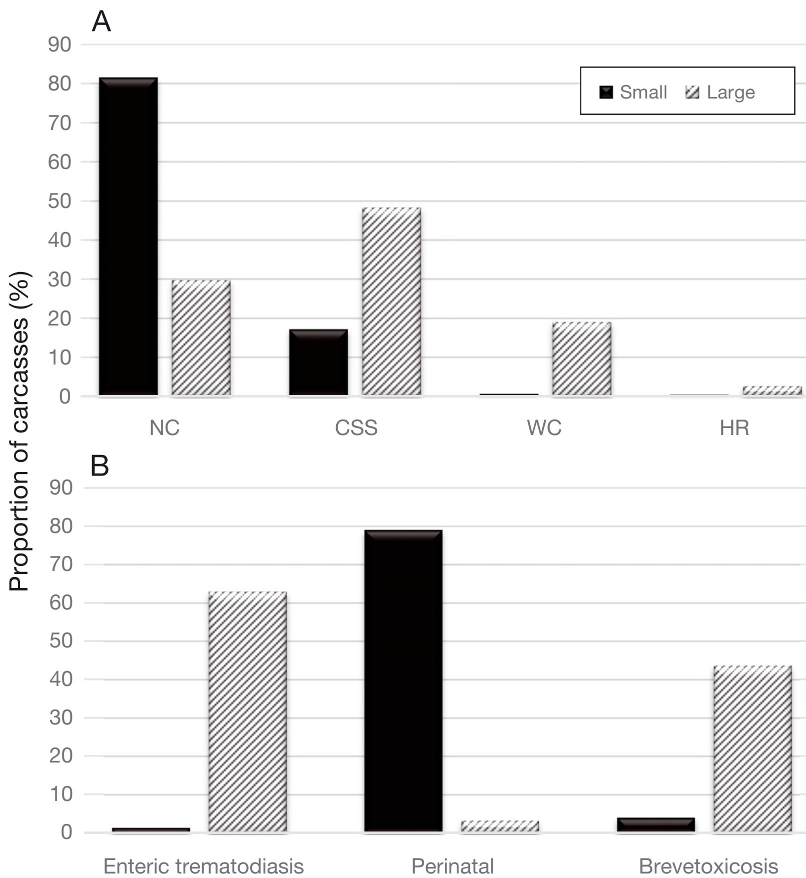

Fig. 4. (A) Proportions of causes of death for manatee calves by size class, excluding unknown causes. NC: natural causes; CSS: cold stress syndrome; WC: watercraft; HR: human-related causes. Total proportion is out of n = 411 for small calves and n = 798 for large calves. (B) Important factors contributing to manatee calf death due to natural causes by size class. Total proportion is out of n = 335 for small calves and n = 238 for large calves

1, 6%). Brevetoxicosis was associated with death more often in large calves (88%, n = 104/118, 95% CI = 81, 93%). CSS was the leading COD for large calves (48%, n = 386/798, 95% CI = 45, 52%) (Fig. 4a). Foreign debris was seen in a greater proportion of large calves (87%, n = 135/155, 95% CI = 81, 91%) than small calves (13%, n = 20/155, 95% CI = 9, 19%).

3.3. Regional patterns

Within Florida, NC was the leading COD in all regions except the ATL, accounting for 69% (n = 50/72), 59% (n = 314/530), and 60% (n = 15/25) of regional cases within NW, SW, and USJR regions, respectively (Table 3). The proportion of deaths due to NC where enteric trematodiasis contributed was

Table 3. Cause of death (COD) in manatee *Trichechus manatus latirostris* calves shown by geographic region of the Florida coast during 2009–2017. ATL: Atlantic; NW: northwest; SW: southwest; USJR: upper St. Johns River. Data reported in parentheses are %. Superscript letters indicate significant differences ($p < 0.05$) in groups (percentages) within each column and within each year

	All regions	ATL	NW	SW	USJR
2009					
Natural causes	50 (37.9)^{c}	28 (36.8)^{c}	2 (50.0)^{a}	20 (39.2)^{b}	0 (0.0)
Cold stress	56 (42.4)^{c}	38 (50.0)^{c}	1 (25.0)^{a}	16 (31.4)^{b}	1 (100.0)
Watercraft	24 (18.2)^{b}	9 (11.8)^{b}	1 (25.0)^{a}	14 (27.5)^{b}	0 (0.0)
Human-related	2 (1.5)^{a}	1 (1.3)^{a}	0 (0.0)^{a}	1 (2.0)^{a}	0 (0.0)
All COD	132 (100.0)	76 (100.0)	4 (100.0)	51 (100.0)	1 (100.0)
2010					
Natural causes	44 (18.2)^{c}	22 (14.9)^{b}	2 (66.7)^{a}	19 (21.3)^{b}	1 (50.0)
Cold stress	181 (74.8)^{d}	120 (81.1)^{c}	1 (33.3)^{a}	59 (66.3)^{c}	1 (50.0)
Watercraft	15 (6.2)^{b}	5 (3.4)^{a}	0 (0.0)^{a}	10 (11.2)^{b}	0 (0.0)
Human-related	2 (0.8)^{a}	1 (0.7)^{a}	0 (0.0)^{a}	1 (1.1)^{a}	0 (0.0)
All COD	242 (100.0)	148 (100.0)	3 (100.0)	89 (100.0)	2 (100.0)
2011					
Natural causes	38 (29.7)^{c}	18 (25.7)^{b}	4 (50.0)^{a}	14 (30.4)^{c}	2 (50.0)
Cold stress	71 (55.5)^{d}	45 (64.3)^{c}	2 (25.0)^{a}	23 (50.0)^{c}	1 (25.0)
Watercraft	17 (13.3)^{b}	6 (8.6)^{a}	2 (25.0)^{a}	8 (17.4)^{b}	1 (25.0)
Human-related	2 (1.6)^{a}	1 (1.4)^{a}	0 (0.0)^{a}	1 (2.2)^{a}	0 (0.0)
All COD	128 (100.0)	70 (100.0)	8 (100.0)	46 (100.0)	4 (100.0)
2012					
Natural causes	48 (52.2)^{c}	11 (30.6)^{c,d}	6 (66.7)^{b}	31 (67.4)^{c}	0 (0.0)
Cold stress	26 (28.3)^{b}	19 (52.8)^{d}	1 (11.1)^{a}	5 (10.9)^{a,b}	1 (100.0)
Watercraft	17 (18.5)^{b}	6 (16.7)^{b,c}	2 (22.2)^{a,b}	9 (19.6)^{b}	0 (0.0)
Human-related	1 (1.1)^{a}	0 (0.0)^{a}	0 (0.0)^{a}	1 (2.2)^{a}	0 (0.0)
All COD	92 (100.0)	36 (100.0)	9 (100.0)	46 (100.0)	1 (100.0)
2013					
Natural causes	86 (63.2)^{c}	19 (35.6)^{b}	7 (70.0)^{a}	58 (86.6)^{c}	2 (50.0)
Cold stress	35 (25.7)^{b}	30 (54.5)^{c}	0 (0.0)^{a}	4 (6.0)^{a,b}	1 (25.0)
Watercraft	10 (7.4)^{a}	1 (1.8)^{a}	3 (30.0)^{a}	5 (7.5)^{b}	1 (25.0)
Human-related	5 (3.7)^{a}	5 (9.1)^{a}	0 (0.0)^{a}	0 (0.0)^{a}	0 (0.0)
All COD	136 (100.0)	55 (100.0)	10 (100.0)	67 (100.0)	4 (100.0)
2014					
Natural causes	57 (58.8)^{c}	24 (50.0)^{c}	4 (66.7)^{b}	27 (65.9)^{c}	2 (100.0)
Cold stress	24 (24.7)^{b}	18 (37.5)^{c}	2 (33.3)^{b}	4 (9.8)^{a,b}	0 (0.0)
Watercraft	16 (16.5)^{b}	6 (12.5)^{b}	0 (0.0)^{a}	10 (24.4)^{b}	0 (0.0)
Human-related	0 (0.0)^{a}	0 (0.0)^{a}	0 (0.0)^{a}	0 (0.0)^{a}	0 (0.0)
All COD	97 (100.0)	48 (100.0)	6 (100.0)	41 (100.0)	2 (100.0)
2015					
Natural causes	65 (64.4)^{c}	25 (59.5)^{c}	7 (77.8)^{b}	31 (67.4)^{c}	2 (50.0)
Cold stress	14 (13.9)^{b}	10 (23.8)^{b}	1 (11.1)^{a}	3 (6.5)^{a}	0 (0.0)
Watercraft	20 (19.8)^{b}	5 (11.9)^{a,b}	1 (11.1)^{a}	12 (26.1)^{b}	2 (50.0)
Human-related	2 (2.0)^{a}	2 (4.8)^{a}	0 (0.0)^{a}	0 (0.0)^{a}	0 (0.0)
All COD	101(100.0)	42 (100.0)	9 (100.0)	46 (100.0)	4 (100.0)

highest within the SW (43%, n = 134/315), which accounts for 87% (n = 135/155) of all enteric trematodiasis cases seen (Table 4). Enteric trematodiasis contributed to 14% (n = 7/50) of deaths due to NC within the NW, 7% (n = 13/194) in the ATL, and 0% (n = 0/15) in the USJR region. The proportion of perinatal deaths contributing to NC was highest within the USJR region at 73% (n = 11/15), but comparable to proportions in the ATL (69%, n = 134/194) and NW (64%, n = 32/50) (Table 4). By contrast, the lowest proportion of perinatal death was seen in the SW (31%, n = 96/314). Perinatal deaths in the ATL accounted for 49% (n = 134/273) of all perinatal cases seen. The SW was the only region where brevetoxicosis contributed to calf mortality, accounting for 38% (n = 118/314) of regional cases.

The highest overall proportion of CSS cases (70%, n = 320/457) originated from the ATL region and CSS was also the leading COD within the region (55%, n = 320/582) (Table 3). The ATL region also had the highest proportion of carcasses with ingested foreign material (15%, n = 87/582), accounting for 56% (n = 87/155) of all foreign debris cases. The proportion of HR deaths was low across all regions, accounting for 3% (n = 17/582) of total calf deaths within the ATL, 1% (n = 1/72 and n = 6/530) within the NW and SW, respectively, and no cases in the USJR.

Across counties, Brevard County had the highest proportion of CSS cases in the state (32%, n = 148/457). CSS was also the leading COD within Brevard County (58%, n = 148/256). Within the SW, Lee County had the greatest overall number of deaths due to NC in the state (10%, n = 126/1209), and death due to NC was also the leading COD in this county (54%, n = 126/233). Lee County also had the greatest number of deaths due to WC within the SW region (60%, n = 53/87) and led the state with the highest number of WC deaths overall (34%, n = 53/155).

4. DISCUSSION

This study provides novel, detailed data on Florida manatee calf mortality, including patterns among COD, geographical location, and size classes, covering almost one decade of pathology data collected at the FWC MMPL. The leading COD for all manatee calves analyzed was due to NC. Death due to NC was also the leading COD in all geographic regions except for the ATL. Significantly higher annual mortality due to NC during later years in this study identifies areas where future research may be needed. Perinatal deaths contributed to the largest proportion of calf deaths within this category. The manatee population has grown over time, including most of the study period (Hostetler et al. 2021), and it is possible that an increased proportion of younger females led to more unsuccessful births (O'Shea & Hartley 1995). Additionally, within the NC category, cases where brevetoxicosis and enteric trematodiasis contributed to death appear to have increased concurrently and may be factors contributing to the observed trend. For the purpose of this study, the FWC COD category 'perinatal death,' which historically has been determined by total carcass length, was redefined so that mortality causes among calves could be better understood. Here, perinatal death was specifically defined as due to stillbirths/fetal death or presumed dystocias. Unsurprisingly, perinatal death is observed more commonly in small calves; however, it is important to emphasize that perinatal death was not restricted to this length-defined age class. Therefore, an assumption of age that is based solely on carcass length is unreliable, as some large calves by our definition had just been born, and is consistent with a previously published perinatal status model published (Schwarz 2008). The SW region had a lower proportion of such perinatal deaths, which might indicate that reproductive rates in this region are

Table 4. Frequency of specific conditions or diseases contributing to death from natural causes (NC) among manatee *Trichechus manatus latirostris* calves in Florida by region. ATL: Atlantic; NW: Northwest; SW: Southwest; USJR: Upper St. Johns River. Totals are inclusive of cases where comorbidities were identified. Numbers in parentheses are the total number of deaths due to NC in each region; percentages are the proportion of each condition by region

Region	Enteritis	Enteric trematodiasis	Perinatal	Congenital deformity	Umbilical disease	Brevetoxicosis	Pulmonary
ATL (194)	28, 14%	13, 7%	134, 69%	6, 3%	8, 4%	0, 0%	7, 4%
NW (50)	4, 8%	7, 14%	32, 64%	2, 4%	4, 8%	0, 0%	4, 8%
SW (314)	26, 8%	135, 43%	96, 31%	8, 3%	9, 3%	118, 38%	8, 3%
USJR (15)	0, 0%	0, 0%	11, 73%	2, 13%	1, 7%	0, 0%	0, 0%
All (573)	58, 10%	155, 27%	273, 48%	18, 3%	22, 4%	118, 21%	19, 3%

lower. Potentially, this region is a generally less desirable birthing locale or the environmental or other stressors associated with stillbirth and dystocia are less commonly encountered. It is also possible that carcasses associated with perinatal death are less recoverable in this location due to less stranding response in remote regions.

After perinatal deaths, enteric disease, comprising enteric trematodiasis and enteritis from other causes, was the second most common contributing factor to death due to NC. Prior estimates of enteritis-related death among manatees when reported have ranged from <1–4 % (O'Shea et al. 1985, Bossart et al. 2004). The higher proportion of cases seen in the present study is consistent with the commonality of gastrointestinal disease encountered during the rehabilitation and hand-rearing of orphaned calves (Davis & Walsh 2018). The proportion of these deaths that were associated with severe parasitic infections was an unexpected finding, as parasites have rarely been associated with death or significant pathological observations in manatees prior to the current study (Buergelt et al. 1984, Beck & Forrester 1988). In 1977, a single calf was reported to have died from severe enteric trematodiasis involving *Moniligerum blairi* (Beck & Forrester 1988). Our findings are in agreement with recent reports that have associated the trematode species *M. blairi* and *Nudacotyle undicola* with histological lymphoplasmacytic, ulcerative enteritis, and grossly visible and variably hemorrhagic, nodular, necrotic, and rugose intestinal mucosa in manatee calves (Arnett-Chinn et al. 2013, Panike et al. 2017). While both species are found within the intestinal tracts of manatees, *N. undicola* is typically identified within the intestinal lumen and *M. blairi* is embedded within the mucosa (Dailey et al. 1988). It is currently unclear which species are capable of causing the observed severe intestinal lesions and associated host response seen in the present study. Although identification of these parasites during necropsies has likely improved since their initial detection, the observed increase in annual cases seen in this study persisted even when cases were evaluated for characteristic intestinal lesions alone and the parasites not noted grossly. This may be related to changes in manatee population dynamics, the environment, or abundance of parasites or intermediate host(s). The observation that most trematode-associated mortalities were seen in large rather than small calves may be due to factors such as age-related immunity or the life-stage transition when weaning off a milk diet and feeding on natural vegetation. The majority of enteric trematodiasis cases were seen in the SW region and may be related to the distribution of the parasite or intermediate stage vector. The life cycle of these parasites remains unknown. Alternatively, concurrent stressors such as red tide events may contribute to the development of severe disease in this area, as brevetoxin-associated calf deaths were restricted to this region. Potential increased susceptibility due to presumed immunosuppression (Walsh et al. 2015) is supported by the frequent observation of concurrent enteric trematodiasis with brevetoxin exposure (Table 2). Further, it is possible that a large proportion of calves have enteric trematodiasis and that severe disease and mortality are more frequently observed with concurrent immunosuppressant diseases. While most trematode infections in other COD categories were likely incidental, in a few cases they were associated with intestinal lesions in WC and CSS manatees. Compromised immune function and general debilitation may be a consequence of WC trauma in animals surviving the initial insult (Bassett et al. 2020), and secondary infections resulting from immunosuppression are a well-recognized component of CSS (Bossart et al. 2002). Some large calves may have experienced sublethal CSS in previous years, which might render them more susceptible to trematode infections. It is currently unclear what the driving force is behind these observations, but contributions from either increasing severe enteric trematodiasis cases, red tide, or cold events are possible. Future research regarding the pathogenicity and epidemiological significance of these trematode species and the role of brevetoxins or other immunosuppressive conditions such as CSS is warranted.

Congenital deformities have been documented infrequently among manatee calves (Watson & Bonde 1986, Walsh & Bossart 1999, Carvalho et al. 2019). Deformities contributing to death in calves in this study varied and included both skeletal and visceral abnormalities. Cleft pectoral flippers have been previously reported in manatees (Watson & Bonde 1986) and was seen in a single animal in this study; however, this finding was considered incidental as it was not believed to have contributed to death. Umbilical disease has previously been reported as a COD in a manatee calf (Walsh et al. 1987) and typically results in sepsis, peritonitis, and pneumonia in neonates across mammalian taxa (Bianchi et al. 2018, Bozukluhan et al. 2018). In manatee calves, omphalitis likely develops for reasons similar to other species and may include inadequate colostrum consumption, maternal infection, or environmental contamination (Clarke-Pounder & Golden 2018).

Deaths due to brevetoxicosis were restricted to the SW region and were not unexpected based on historic red tide events and reported mortalities among marine mammals along the west coast of Florida (O'Shea et al. 1991, Landsberg et al. 2009, Capper et al. 2013). Red tide blooms are common in the Gulf of Mexico, especially along the Florida coast, and their occurrence may be influenced by nutrient run-off or climatic factors such as wind patterns and ocean currents (Walsh et al. 2006, Weisberg et al. 2019). Brevetoxin-associated calf deaths were highest in 2013, 2016, and 2017 and correspond to years where manatee brevetoxicosis repeat mortality events have been declared by NOAA. The primary route of exposure to brevetoxins in manatees is believed to be through ingestion, with inhalational exposures contributing to a minimal extent (Flewelling et al. 2005, Fire et al. 2015). Calves may become exposed through ingestion of toxin-contaminated plants if they are eating solid food, and a higher proportion of brevetoxin-associated mortalities among large calves emphasizes the importance of dietary exposure in this age class; however, their susceptibility to intoxication may be enhanced through additional routes of exposure. Brevetoxins have been detected in manatee milk, which could transfer to dependent nursing calves (Capper et al. 2013, Fire et al. 2015). Additionally, manatees could be exposed to the toxin during fetal development, as placental transport of the toxin has been demonstrated to occur in mice, and toxins have been detected in the tissues of a deceased fetal dolphin (Benson et al. 2006, Fire et al. 2015). The observation of coincident brevetoxicosis and perinatal death seen in this study justifies further investigation into the role of brevetoxins and fetal death or still birth. This is especially important, as the lethal dose of brevetoxins in manatees remains unknown and a correlation between tissue toxin concentrations and severity of clinical signs has not been determined (Florida Fish and Wildlife Conservation Commission 2013, Walsh et al. 2015). The potential impact on calves is not limited to acute exposures and resultant death; chronic, sublethal exposure may result in developmental abnormalities (Kimm-Brinson & Ramsdell 2001) and is likely to have an immunosuppressive and inflammatory effect leading to increased susceptibility to disease (Walsh et al. 2015).

Pulmonary disease among manatee calves that was not directly related to trauma or other obvious underlying cause was predominantly pneumonia and is consistent with previously reported findings for manatees (Bossart et al. 2004, Bonde et al. 2012b). Causative agents were not determined in all cases of pneumonia in calves, but bacterial pneumonia and pneumonia associated with sediment aspiration were commonly reported. *Streptococcus pneumoniae* was confirmed in one case.

CSS occurs in manatees with prolonged exposure to water temperatures below 20°C (Bossart et al. 2002). Physiologically, a low metabolic rate, high thermal conductance, and inefficient heat production restricts manatee distribution to tropical and sub-tropical waters and leaves them vulnerable to the effects of colder temperatures (Gallivan & Best 1980, Irvine 1983). Calves are particularly susceptible to the effects of cold water due to their lower body mass and greater surface area relative to adults, inability to raise their metabolic rates with cold exposure, and relative lack of experience seeking out nutritional resources and warm-water refuges if orphaned or recently independent (O'Shea et al. 1985, Worthy 2001, Bossart et al. 2004). Despite receiving the benefit of higher-energy milk, small calves are expected to be less tolerant of prolonged cold exposure than larger calves and would be more likely to succumb to hypothermia from acute CSS without discernable gross lesions. In the present study, a higher proportion of CSS-associated mortalities occurred among larger calves and is likely explained by the timing of the manatee birthing season. With higher manatee birthing rates during spring and summer months (Rathbun et al. 1995), the majority of calves have likely transitioned to the large-calf category prior to experiencing their first winter. Secondarily, large calves may be inexperienced in independently navigating warm-water sites and foraging areas during the winter, while small calves can rely on their mother for guidance and nutrition (Bossart et al. 2002). Spatial patterns of calf death from CSS were consistent with previous observations. The greatest proportion of CSS deaths occurred in large calves within the ATL region, which coincides with reports of more frequent CSS deaths within the northeastern portion of the state (O'Shea et al. 1985, Ackerman et al. 1995, Barlas et al. 2011). Colder water from south-flowing oceanic currents, fewer high-quality warm-water refuges, and variable power plant operations especially from 2010–2016 might contribute to this geographical correlation and leading COD within the region itself (Ackerman et al. 1995, Laist & Reynolds 2005, Laist et al. 2013, Runge et al. 2017). The peak in calf CSS death in 2010 correlates with 2 consecutive winters of unusually cold weather as well as a record cold wave in January of that year that resulted in the mortality of 480 manatees over the course of 89 d (NOAA 2010, Barlas et al. 2011). Brevard County led the state with the highest calf mortality due to CSS (Table 3). This region of the Indian River Lagoon has previously been recognized as an important overwintering area for manatees (Laist & Reynolds 2005, Barlas et al. 2011). The results of the current study emphasize the importance of this county for manatee calves specifically and provide further justification for targeted conservation.

Historically, WC injury has been recognized as a leading COD for manatees (Calleson 2014, Runge et al. 2017) and has been a primary focus of many manatee conservation efforts, including the implementation of manatee protection zones beginning in the 1970s. Starting in 1991, during the adoption of most protection zones (Calleson 2014) and continuing until 2017, WC-related deaths accounted for 30% (n = 2004/6746) of all manatee mortality where COD was determined (Florida Fish and Wildlife Conservation Commission 2019). In contrast, the proportion of WC death in calves was substantially lower at 13% and is consistent with previous findings of proportionally fewer WC scars on calves compared to older age classes (Bassett et al. 2020). WC trauma was rarely reported for small calves, with nearly all cases occurring in large calves, and may reflect the temporal age differences between small and large calf size categories. Small and large dependent calves likely benefit from maternal experience and guidance, and small calves may receive additional protections due to their greater dietary dependence on milk rather than solid food, thus reducing exposure to WC during nursing. The commonly observed calf echelon positioning relative to their mothers may also provide physical shielding from WC. Additionally, calves are smaller targets relative to adult manatees, thereby reducing the likelihood of WC collisions. It is also important to recognize that calves may suffer the indirect effects of a maternal WC strike, and their subsequent death would be attributed to conditions other than WC. Regionally, Lee County leads the state in number of WC calf deaths, which is in agreement with historic mortality records for manatees in general (Florida Fish and Wildlife Conservation Commission 2019). These observations indicate that despite accounting for a lower proportion of mortality among manatee calves compared to adults, WC trauma remains a persistent and important COD among large calves despite protections.

Foreign debris ingestion including plastics was notable among calves and observed in 13% of all carcasses. Previously, debris was reported within the gastrointestinal tracts of approximately 7% (n = 210/2995) of all manatee calf carcasses examined from 1993 through 2012, including animals where COD was unknown (Reinert et al. 2017). This observation suggests that the overall proportion of calves with debris ingestion has increased over time. Alternatively, detection and reporting of debris findings may have increased. Compared to calves, adults are expected to have higher exposure to debris due to greater time spent feeding and larger quantities consumed. Large calves were found with debris ingestion more often than small calves, which is consistent with large calves devoting more time to foraging and their greater consumption relative to small calves, as well as the likely greater time period encompassing the large calf category relative to the small calf category. The notable proportion of small calves with debris ingestion likely represent orphaned or abandoned animals unable to appropriately meet their nutritional needs who would typically be fully dependent on their mothers at this life stage. Debris ingestion may therefore be an important consideration when caring for rehabilitating manatee orphans. A higher proportion of foreign debris ingestion cases came from the ATL region than others examined and is worthy of further investigation.

Age determination of manatee carcasses is not currently part of standard Florida manatee carcass necropsy procedures. Investigations evaluating the utility of using growth layer groups in manatee ear bones have been pursued, and despite showing promise for aging younger manatees up to about 15 yr old, physiologic bone resorption hinders precise age estimation in older individuals (Marmontel et al. 1996, Lonati et al. 2019). The process is labor- and time-intensive, requires extensive training, and is costly. Age-class assignments of carcasses as calves, subadults, and adults are typically based on measurement of total length, as this is a cost-effective, readily accessible measurement particularly in field settings. Estimates based on lengths, however, are unfortunately imprecise as indicated by the occurrence of some truly perinatal calves being categorized as large calves in the present study and research showing less certainty in manatee ages specifically within the length range of 175–275 cm (O'Shea et al. 1985, Schwarz & Runge 2009). Additionally, recent research in other marine mammals has correlated shorter body lengths with the sublethal impacts of fishing gear entanglements (Stewart et al. 2021). Given the sustained threat WC trauma represents to calves, the high proportion of adult manatees with WC scars (Bassett et al. 2020), and the unknown long-term energetic consequences of sublethal trauma, examination of WC impacts on manatee size may be warranted. Review and further analysis of all available age and size data for Florida manatees may be useful in refining length-based age estimates for this species in the current absence of more precise and practical strategies.

Interpretation of retrospective mortality data should be considered with the limitations inherent to this type of review in mind. While all reported manatee mortalities were considered, this does not represent all manatees that died in Florida waters during the study period since not every manatee carcass is discovered; hence, these manatees are neither reported nor recovered for necropsy. Additionally, for those carcasses that were recovered, inconclusive cases or carcasses with advanced decomposition were excluded because this study focused on patterns of known COD. We acknowledge the limitation associated with such an exclusion of cases, as bias can be created; however, data were used for conservative interpretations of apparent patterns. Further, certain geographical regions may be over- or under-represented due to variability in carcass reporting, ease/logistics of recovery in certain geographical areas, or likelihood of carcass drift due to currents or weather leading to larger or smaller sample sizes across regions. The proportion of carcasses where COD is unknown may also differ among geographic regions or counties. It is also possible that certain age groups or disease conditions make it more likely that a carcass is found. Finally, the category of NC, while relatively comprehensive, is not exhaustive because not every disease is tested for in every animal and certain factors influencing susceptibility may or may not be measurable or detectable. Regardless, retrospective analysis of mortality trends among manatee calves represents an important observational tool for understanding current and potential future threats to this species with the ultimate goal of using this objective data for developing targeted, region-specific conservation efforts and for informing policymakers in their management decisions.

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