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MAJOR PATHOLOGIC FINDINGS AND PROBABLE CAUSES OF MORTALITY IN BOTTLENOSE DOLPHINS STRANDED IN SOUTH CAROLINA FROM 1993 TO 2006

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ABSTRACT: Although cause-of-death information on bottlenose dolphins (*Tursiops truncatus*) can be located in the literature, few citations include mortality data over a long period of time covering a broad geographic region. This study describes major pathologic findings and probable causes of death of bottlenose dolphins over a 14-yr period (1993–2006) for the coastal region of South Carolina. Probable causes of death for 97 cases were determined based on gross pathology and histopathology. In an additional 30 cases, probable cause of death was apparent from gross pathology alone, and carcass condition precluded histopathology. Of the 97 dolphins examined grossly and histologically, 30 (31%) likely died of infectious disease and 46 (47%) of noninfectious disease; the cause of death was unknown in 21 (22%). Bacterial infections accounted for the large majority of fatal infections and emaciation was the leading cause of noninfectious mortality. Twelve dolphins were killed by human interactions. Of the 30 dolphins diagnosed from gross examination alone, 23 likely died from human interaction and seven were killed by stingray-spine inflictions. Although the absence of consistent use of microbiology, biotoxin analysis and contaminant testing decreases the conclusiveness of the findings, this study has broad implications in establishing baseline data on causes of death of bottlenose dolphins for future studies and for the detection of emerging diseases.

Key words: Bottlenose dolphin, cause of death, mortality, South Carolina, strandings, *Tursiops truncatus*.

INTRODUCTION

Information on the causes of mortality of bottlenose dolphins (*Tursiops truncatus*) over many years for a broad geographic region has been sparse. Most of the information in the literature on this topic is limited to mortality of bottlenose dolphins that involve periods of increased mortality or descriptions of pathologic findings in small numbers of individuals (Lipscomb et al., 1994; Krafft et al., 1995; Lipscomb et al., 1996; Bossart et al., 2003; McFee and Osborne, 2004).

Bottlenose dolphins can be regarded as sentinel species of the oceans, providing an indication of disease processes and environmental problems (Bossart, 2006) that could affect marine animal and human health. Several studies have described the presence of high levels of contaminants in bottlenose dolphins as a potential cause of reproductive stress and debilitating disease (Schwacke et al., 2002;

Hansen et al., 2004; Houde et al., 2005; Wells et al., 2005). Because humans and dolphins compete for many of the same food resources (i.e., fish, shrimp, and squid) and reside in close proximity, knowledge regarding dolphin disease could act as an indicator of emerging diseases about which humans may need to be concerned. Likewise, interactions of dolphins with fishery operations have been demonstrated to impact both the health and well being of dolphins and the economics associated with fisheries negatively (International Whaling Commission [IWC], 1994).

Concern over bioterrorism has prompted a change in perspective on biologic and environmental data. In their role of sentinels of the oceans, dolphin strandings and causes of death could aid in the detection of biologic or chemical agents that may be harmful to human health. Federal and state agencies in South

Carolina are developing an Environmental Surveillance Network (ESN), the participants of which will share biologic (e.g., marine mammal mortalities, fish kills, harmful algal blooms) and environmental data (e.g., water quality, chemical spills) to detect environmental risks in near real time (University of South Carolina–Center for Public Health Preparedness [USC-CPHP], 2007). This integration of data at the state level could be directed to national emergency response systems such as the Biological Warning and Incident Characterization System (BWIC) established by the Department of Homeland Security (Sandia National Laboratories, 2006).

The South Carolina coastline consists of numerous estuaries and barrier islands in the lower three-quarters of the state (approximately 301 km) south of Murrells Inlet (33.5312°N and -79.0312°W). The northern quarter of the state (approximately 101 km), commonly known as the Grand Strand, is generally a continuous, gently sloping beach. Resident populations of dolphins have been described in the estuaries in and around Hilton Head Island (Petricig, 1995) and Charleston, South Carolina, USA (Zolman, 2002). However, stock structure of Western North Atlantic (WNA) coastal bottlenose dolphins is believed to be more complex than was previously described (Scott et al., 1988; Hohn, 1997; McLellan et al., 2002). Presently, the National Marine Fisheries Service recognizes seven stocks of WNA bottlenose dolphins ranging from New Jersey to Florida (Waring et al., 2006). These Management Units (MU) are based on genetic analysis, photoidentification studies, and shipboard and aerial surveys. Bottlenose dolphins in South Carolina are believed to be from two of these MUs: the Southern North Carolina MU (SNCMU) and the South Carolina MU (SCMU; McFee et al., 2006). The SNCMU extends from Cape Lookout, North Carolina to Murrells Inlet, South Carolina. Dolphins in South Carolina that are in the SNCMU

are believed to be migratory in nature based on seasonal stranding trends (McFee et al., 2006) and the presumption that no resident estuarine population of bottlenose dolphins has been observed north of North Inlet (33.3258°N and -79.1608°W; Young and Phillips, 2002) in South Carolina. The SCMU extends from Murrells Inlet to the Savannah River (32.0391°N and -0.8853°W) bordering Georgia. As such, migratory dolphins that spend most of their lives along the coast and resident, estuarine dolphins that are exposed to increased pressure from anthropogenic activities have the potential to vary in their disease processes and level of anthropogenic impact.

The main objective of this study was to determine probable causes of death of bottlenose dolphins based on necropsies over a 14-yr period (1993–2006) for the coastal region of South Carolina. In particular, spatial trends were analyzed, as well as age-specific causes of mortality.

MATERIALS AND METHODS

Carcasses of bottlenose dolphins stranded in South Carolina were examined and necropsied according to standard procedures (Geraci and Lounsbury, 1993; McFee et al., 2006). In most cases, carcasses were transported to the National Ocean Service's (NOS) Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, South Carolina, USA. Carcasses that could not be transported to CCEHBR were necropsied on site or moved to a suitable location for necropsy. In general, only tissues from fresh dead (code 2) animals were examined histologically. On occasion, tissues from moderately decomposed (code 3) animals were analyzed histologically if unusual lesions were observed or to investigate further evidence of death caused by interactions with humans (e.g., fishery entanglement).

Tissues of all the major organs and lesions were collected and stored in a fixative solution of 10% neutral buffered formalin (NBF). From 1993 to 2003, the formalin-fixed tissues were subsampled into approximately 1-cm cubes, wrapped in formalin-soaked gauze, placed in individual Ziploc bags, and shipped overnight to the Armed Forces Institute of Pathology (AFIP), Department of Veterinary

Pathology (Washington, D.C., USA) for histologic analysis. Beginning in 2004, collected tissue samples were placed directly into Omnisette tissue cassettes (Fisherbrand), soaked in 10% NBF for at least 24 hr, and then shipped overnight to the AFIP. Sections of the following tissues were collected from most dolphins: lung, muscle, liver, kidney, heart muscle (all four chambers), aorta, pericardium, pancreas, spleen, major bronchi, stomach chamber lining, esophagus, diaphragm, thymus, thyroid gland, cerebellum, cerebrum, brain stem, lung-associated lymph nodes, mesenteric lymph nodes, prescapular lymph node, pulmonary lymph node, sternal lymph node, intestine, tongue, adrenal glands, rectal gland, colon, and reproductive organs.

Formalin-fixed tissues were embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin for examination by light microscopy. Selected sections were stained with Brown and Hopps Gram's stain, Brown and Brenn Gram's stain or by Grocott's methenamine silver nitrate method.

Age was determined from thin sections of postnatal dentine observed in the teeth following Hohn et al. (1989). Age classes were separated into neonate (≤ 0.1 yr), calf (> 0.1 – 1 yr), juvenile (> 1 – 9 yr), and adult (> 9 yr). In cases where a tooth was not available for aging, dolphins were placed in one of the four categories based on their length (< 120 cm = neonate; 121–150 = calf; 151–230 cm = juvenile; > 230 cm = adult).

In formulating our assessment of the cause of death, we used the following definition: "Cause of death—the disease, injury, or abnormality that alone or in combination is responsible for initiating the sequence of functional disturbances, whether brief or prolonged, that eventually ends in death" (Froede, 1990). After determining the probable cause of death, the death was categorized as infectious or noninfectious. The infectious and noninfectious diseases were further defined based on the specifics of the cases.

There were a number of dolphins judged to have died as a direct result of human interaction from which tissues were not collected for histology because of postmortem decomposition or artifacts caused by freezing of the carcass. The diagnosis of death likely due to human interaction by entanglement in fishing gear was based on combinations of the following criteria: fishing gear attached to the body, evidence of net or rope wounds, persistent froth in airways, edematous lungs, evidence of recent feeding, good nutritional condition, and exclusion of other causes of death (Kuiken, 1996). The diagnosis of death

likely due to boat collision was based on combinations of the following criteria: evidence of boat propeller wounds, fractures, contusions, exclusion of other causes of death and (in one case) eyewitness observation of the incident.

Locations of bottlenose dolphins analyzed in this study were plotted on a map with the use of ArcMap 9.1 (Environmental Systems Research Institute [ESRI], 2005). For pathologic differences, dolphins were separated into those that were examined or stranded from two geographic locations, the southern portion of the SNCMU (Little River, South Carolina [33.8686° N and -78.4997° W] to Murrells Inlet) and the SCMU (Murrells Inlet to Savannah River; Fig. 1).

RESULTS

During the period 1993 to 2006, 550 bottlenose dolphins were reported floating dead, stranded dead on beaches or in estuaries, alive and stranded, or alive and entangled. Of these, 453 were inaccessible or in an advanced stage of decomposition and not suitable for histologic examination when reached. Complete or partial necropsies were performed on 302 carcasses. Tissues were sufficiently well preserved for histologic examination in 97 (42 male [M]:55 female [F]) cases (17.6% of the total reported strandings). Data on these dolphins, including significant pathologic findings, likely cause of death, and cause of death category, are summarized in Table 1. Additionally, likely cause of death could be reasonably assessed by gross examination alone in an additional 30 cases. Similar information from these dolphins is presented in Table 2. Cause-of-death information on all 127 dolphins is summarized in Table 3.

Ages were determined based on tooth examination for 98 (77.2%) dolphins. The remaining 29 dolphins were placed into age classes based on their lengths. Nineteen dolphins comprised the neonate class (9 M/10 F), 14 dolphins comprised the calf class (6 M/8 F), 42 comprised the juvenile class (23 M/17 F/2 unknown [U]), and 51 comprised the adult class (16 M/33 F/2 U). One animal (case 112) of unknown sex

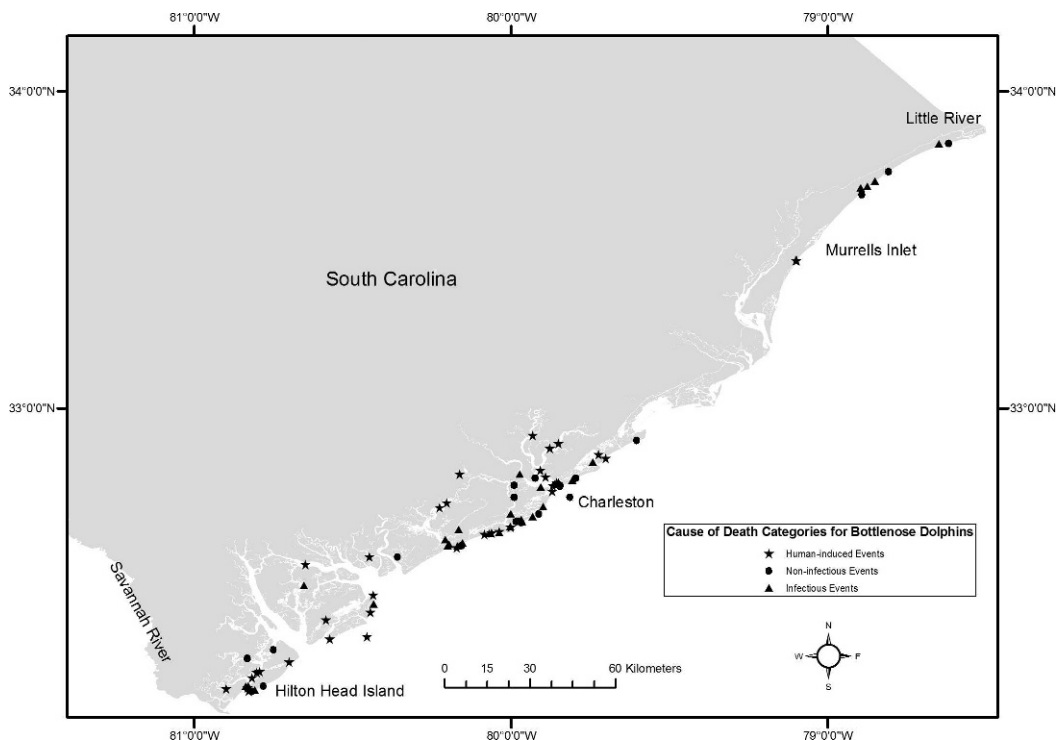


FIGURE 1. Stranding locations of bottlenose dolphins in South Carolina analyzed in the three cause-of-death categories.

and age class was released dead from a crab-pot buoy line but was not necropsied.

Of the 97 dolphins examined grossly and histologically, 30 (31%) likely died of infectious disease and 46 (47%) of noninfectious disease; the cause of death was unknown in 21 (22%). Thus, of the 76 dolphins examined grossly and histologically for which the likely cause of death was determined, 30 (39.5%) died of infectious diseases, and 46 (60.5%) died of noninfectious diseases. The number of dolphins in each age class and in each cause of death category is summarized in Table 4.

DISCUSSION

Bacterial infections comprised the largest group of fatal infections (21 cases) and most of these were septicemias and/or bacterial pneumonias. These diagnoses were based on histopathology; bacterial

cultures were not routinely performed. Bacterial pneumonia has been found to be common in other studies of dolphin mortality (Howard et al., 1983; Baker, 1992). More recent studies suggest that some bacterial infections in dolphins may have their source from sewage outfalls, though this still remains speculative (Parsons and Jefferson, 2000; Greig et al., 2007). There were five cases of verminous pneumonia and one case of pneumonia that was primarily verminous with a lesser component of fungal hyphae. The identity of the fungus was not determined. The lungworms were compatible with *Halicercus lagenorhynchi* based on histomorphology. Lungworm infection is very common in bottlenose dolphins and is generally considered to be of little clinical significance, but the severe pneumonias in these cases appeared to be the causes of death. Evidence of prenatal infection of bottlenose dolphins by these parasites has

TABLE 1. Causes of death based on gross and histologic examination of 97 bottlenose dolphins in South Carolina.

Case no.	AFTP No.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
1	2401990	11 April 1993	J	M	Pulmonary abscess; hepatitis	Septicemia	Infectious disease (bacterial)
2	2416222	21 June 1993	N	M	Vermineous pneumonia; dermatitis with ciliated protozoa	Vermineous pneumonia	Infectious disease (verminous)
3	2432966	16 November 1993	A	M	Disseminated bacterial emboli; bacterial pneumonia; cystitis	Septicemia and bacterial pneumonia	Infectious disease (bacterial)
4	2434964	20 November 1993	J	F	Bacterial hepatitis	Bacterial hepatitis/septicemia	Infectious disease (bacterial)
5	2453733	15 May 1994	A	M	Pneumonia; shotgun pellets in blubber (incidental)	Pneumonia, probably bacterial	Infectious disease (bacterial)
6	2457594	20 June 1994	N	F	Vermineous pneumonia	Vermineous pneumonia	Infectious disease (verminous)
7	2468625	30 September 1994	N	F	Vermineous and fungal pneumonia; bacterial pyelonephritis	Vermineous/fungal pneumonia	Infectious disease (verminous and fungal)
8	2476704	4 December 1994	J	F	Pneumonia, probably bacterial; epicarditis; phlebitis; arteritis; epicardial hemorrhage	Pneumonia and septicemia	Infectious disease (bacterial)
9	2486496	7 February 1995	A	F	Myocarditis, probably bacterial; glossitis, bacterial; lymphadenitis with ciliated protozoa	Myocarditis	Infectious disease (bacterial)
10	2515208	3 October 1995	J	M	Emaciation; bacterial pneumonia; bacterial and ciliated protozoal dermatitis; bacterial glossitis	Bacterial pneumonia and septicemia; emaciation	Infectious disease (bacterial)
11	2525781	10 December 1995	C	F	Viral pneumonia, adenitis, thymitis, endometritis, myocarditis, nephritis, lymphadenitis, splenitis	Disseminated herpesvirus infection	Infectious disease (viral)
12	2531434	1 February 1996	A	M	Bacterial peritonitis	Bacterial peritonitis	Infectious disease (bacterial)
13	2558403	23 August 1996	C	F	Vermineous pneumonia	Vermineous pneumonia	Infectious disease (verminous)
14	2581305	21 March 1997	A	F	Bacterial enteritis, mastitis, adenitis; bacillary emboli in multiple tissues	Disseminated bacterial infection/septicemia	Infectious disease (bacterial)
15	2587274	15 April 1997	A	F	Bacterial pneumonia and lymphadenitis; abscesses in diaphragm, blubber, skin, skeletal muscle; emaciation	Disseminated bacterial infection/septicemia	Infectious disease (bacterial)
16	2610823	13 November 1997	A	F	Bacterial dermatitis; neutrophilic myocarditis; neutrophilic lymphadenitis	Probable septicemia and bacterial myocarditis	Infectious disease (bacterial)
17	2610825	12 November 1997	A	M	Bacterial myocarditis; bacterial orchitis; disseminated bacterial emboli	Bacterial myocarditis, orchitis and septicemia	Infectious disease (bacterial)

TABLE 1. Continued.

Case no.	AFIP No.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
18	2626321	22 March 1998	J	F	Disseminated toxoplasmosis (lung, brain, adrenal glands, liver, lymph nodes, spleen)	Disseminated toxoplasmosis	Infectious disease (protozoal)
19	2629779	11 April 1998	C	M	Shark bite wound with bacterial infection; bacterial hepatitis; disseminated bacterial emboli	Disseminated bacterial infection/septicemia	Infectious disease (bacterial)
20	2641446	17 June 1998	J	M	Bacterial hepatitis, pneumonia and dermatitis; bacillary emboli; myocardial necrosis; emaciation	Disseminated bacterial infection; emaciation	Infectious disease (bacterial)
21	2840855	23 July 2002	A	F	Severe spinal meningitis; severe verminous pneumonia	Meningitis, cause undetermined; verminous pneumonia	Infectious disease (agent unknown)
22	2874150	12 March 2003	A	M	Severe bacterial gingivitis	Debilitation secondary to severe gingivitis	Infectious disease (bacterial)
23	2906669	10 November 2003	J	M	Bronchopneumonia, probably bacterial; lymphadenitis; stomatitis with ciliated protozoa	Bronchopneumonia	Infectious disease (bacterial)
24	2906738	14 November 2003	N	F	Disseminated toxoplasmosis (liver, thymus, adrenal, spleen, diaphragm, brain, lymph nodes); emaciation	Toxoplasmosis	Infectious disease (protozoal)
25	2906987	17 November 2003	A	M	Emaciation; transmural eosinophilic enteritis	Enteritis; emaciation	Infectious disease (verminous)
26	2939467	27 July 2004	N	F	Verminous pneumonia; emaciation; bacterial dermatitis	Verminous pneumonia; emaciation; bacterial dermatitis	Infectious disease (verminous)
27	2969977	24 March 2005	A	F	Bacterial enteritis; peritonitis; renal infarct	Bacterial enteritis; peritonitis	Infectious disease (bacterial)
28	2978240	22 May 2005	A	F	Bacterial placentitis with metritis and peritonitis	Bacterial placentitis with metritis and peritonitis	Infectious disease (bacterial)
29	3004097	12 December 2005	J	M	Myocarditis, bacterial	Myocarditis	Infectious disease (bacterial)
30	3038161	5 October 2006	J	M	Verminous pneumonia; gastritis; enteritis; shark bites	Verminous pneumonia; gastritis; enteritis; shark bites	Infectious disease (verminous)
31	2432968	10 November 1993	A	F	Cataract, unilateral; emaciated	Emaciation; unknown	Noninfectious disease (emaciation)

TABLE 1. Continued.

Case no.	AFTP No.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
32	2444010	28 February 1994	N	F	Intrapulmonary squamous epithelial cells	Unknown	Noninfectious disease (neonate)
33	2460990	22 June 1994	A	M	Necrosis of portion of maxilla; emaciation	Emaciation/starvation	Noninfectious disease (emaciation)
34	2462583	24 June 1994	N	F	Pneumonia with meconium; contusions	Dystocia	Noninfectious disease (neonate)
35	2462579	24 June 1994	N	M	None (PMA ^a)	Unknown	Noninfectious disease (neonate)
36	2482057	15 January 1995	N	M	Pneumonia with squames	Pneumonia	Noninfectious disease (neonate)
37	2490439	18 March 1995	A	F	Emaciation	Emaciation/starvation	Noninfectious disease (emaciation)
38	2493159	9 April 1995	A	F	Emaciation; gastric obstruction by marsh grass; peracute terminal septicemia	Gastric obstruction	Noninfectious disease (alimentary obstruction, natural object)
39	2494220	11 April 1995	A	F	Emaciation; dermatitis with ciliated protozoa; acute centrilobular hepatic necrosis (terminal)	Emaciation	Noninfectious disease (emaciation)
40	2508142	3 July 1995	N	F	Emaciation	Starvation, probable maternal separation	Noninfectious disease (emaciation)
41	2506351	9 July 1995	J	M	Emaciation	Emaciation/starvation	Noninfectious disease (emaciation)
42	2519584	27 October 1995	N	F	Atelectasis	Stillborn	Noninfectious disease (neonate)
43	2531855	20 February 1996	A	F	Emaciation	Emaciation; unknown	Noninfectious disease (emaciation)
44	2543343	17 April 1996	A	F	Emaciation	Starvation, catfish lodged in pharynx	Noninfectious disease (alimentary obstruction, natural object)
45	2543347	05/02/96	N	M	Emaciation	Emaciation/starvation	Noninfectious disease (emaciation)
46	2546274	30 May 1996	J	F	Emaciation; proctitis	Emaciation/starvation	Noninfectious disease (emaciation)
47	2587272	24 April 1997	N	F	Atelectasis; numerous intraalveolar squames	Possible dystocia	Noninfectious disease (neonate)

TABLE 1. Continued.

Case no.	AFIP No.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
48	2600978	15 August 1997	J	F	Emaciation	Emaciation/starvation	Noninfectious disease (emaciation)
49	2716501	27 January 2000	J	F	Steatitis with goutlike crystals; vaginal calculus; emaciation	Emaciation	Noninfectious disease (emaciation)
50	2738313	28 June 2000	J	F	Emaciation	Emaciation	Noninfectious disease (emaciation)
51	2790361	29 May 2001	N	M	Emaciation; verminous pneumonia	Emaciation, maternal separation; verminous pneumonia	Noninfectious disease (emaciation)
52	2790365	7 June 2001	N	M	None	Maternal separation	Noninfectious disease (neonate)
53	2881776	12 May 2003	N	M	Emaciation; bronchial foam and hemorrhage	Maternal separation with terminal drowning	Noninfectious disease (neonate)
54	2915851	11 January 2004	C	M	Emaciation; gliosis	Emaciation/starvation	Noninfectious disease (emaciation)
55	2915856	27 January 2004	A	F	Emaciation; hepatitis; coronary arteriosclerosis	Emaciation; hepatitis	Noninfectious disease (emaciation)
56	2943551	15 August 2004	A	F	Emaciation; pulmonary thrombus	Euthanasia	Noninfectious disease (emaciation)
57	2445679	16 May 1994	A	F	Fishery injuries; dermatitis with ciliated protozoa and bacteria; emaciation; renal cell carcinoma	Entrapment in fishing gear	Noninfectious disease (human induced, fishery)
58	2461797	4 July 1994	N	M	Severe tail wound and infection, consistent with propeller injury; emaciation	Propeller injury	Noninfectious disease (human induced, boat trauma)
59	2538889	14 March 1996	C	M	Pulmonary congestion and edema	Fishery interaction	Noninfectious disease (human induced, fishery)
60	2558045	28 August 1996	J	M	Rope wounds at base of flukes; good nutritional condition; fish in stomach	Entrapment in fishing gear	Noninfectious disease (human induced, fishery)
61	2567123	22 September 1996	C	M	Foam in bronchi; linear marks on rostrum and melon; pulmonary edema; acute cutaneous ulcer	Entrapment in fishing gear	Noninfectious disease (human induced, fishery)
62	2596818	9 July 1997	C	F	Emaciation; rope mark on peduncle; foam in lungs	Fishery interaction; Emaciation	Noninfectious disease (human induced, fishery)

TABLE 1. Continued.

Case no.	AFIP No.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
63	2626314	13 January 1998	J	M	Cutaneous line marks; pulmonary edema; fishery interaction	Fishery Interaction	Noninfectious disease (human induced, fishery)
64	2646243	4 July 1998	A	M	Cutaneous line marks; stomach full of fish	Entrapment in fishing gear	Noninfectious disease (human-induced, fishery)
65	2790426	15 July 2001	C	M	Propeller wounds; lacerations of musculature and internal organs; fractured scapula	Trauma from boat strike	Noninfectious disease (human induced, boat trauma)
66	2847146	22 August 2002	J	F	Skin wounds (net entanglement); acute pulmonary congestion and edema; myocardial edema	Entrapment in fishing gear	Noninfectious disease (human induced, fishery)
67	2897272	17 August 2003	J	M	Peracute skin ulcers (entangled in crab-pot line); acute pulmonary congestion and edema	Entrapment in fishing gear	Noninfectious disease (human induced, fishery)
68	2930382	15 May 2004	J	F	Subdural hemorrhage; skin lacerations on peduncle	Trauma from boat strike (observed)	Noninfectious disease (human induced, boat trauma)
69	2416202	15 May 1993	J	M	Pulmonary congestion and edema	Drowning	Noninfectious disease (drowning, cause not evident)
70	2423048	8 July 1993	J	F	Chest wound through heart	Trauma	Noninfectious disease (trauma, cause not evident)
71	2744107	13 July 2000	A	F	Fluke laceration, skeletal muscle hemorrhage on left side of body; myocardial fibrosis	Trauma	Noninfectious disease (trauma, cause not evident)
72	2747745	4 August 2000	J	M	Fractured ribs on right side	Trauma	Noninfectious disease (trauma, cause not evident)
73	2747747	26 July 2000	C	F	Severe hemorrhage of musculature of right side of body	Trauma	Noninfectious disease (trauma, cause not evident)
74	2765627	6 January 2001	J	F	Severe hemothorax	Trauma	Noninfectious disease (trauma, cause not evident)
75	2779956	5 November 2000	J	F	Lung laceration with hemorrhage	Trauma	Noninfectious disease (trauma, cause not evident)
76	2796778	5 September 2001	J	F	Fractured orbital bone; severe shark bite wounds; emaciation	Trauma; emaciation	Noninfectious disease (trauma, cause not evident)
77	2408808	22 April 1993	A	F	None	Unknown	Unknown
78	2408809	29 April 1993	A	F	None (PMA)	Unknown	Unknown

TABLE 1. Continued.

Case no.	AFIP No.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
79	2416201	11 May 1993	A	F	Dermatitis with ciliated protozoa (PMA)	Unknown	Unknown
80	2423045	31 July 1993	C	F	None	Unknown	Unknown
81	2475439	15 November 1994	A	M	None	Unknown	Unknown
82	2480514	4 November 1994	A	F	None (PMA)	Unknown	Unknown
83	2487855	20 February 1995	A	F	Adrenal fibroma	Unknown	Unknown
84	2543349	30 April 1996	A	F	None	Unknown	Unknown
85	2600980	10 August 1997	A	F	Eosinophilic pneumonia and lymphadenitis (PMA)	Unknown	Unknown
86	2641442	1 July 1998	C	F	None	Unknown	Unknown
87	2662241	17 November 1998	J	M	Hepatic necrosis and lipidosis; cardiomyocyte degeneration	Unknown	Unknown
88	2705452	2 October 1999	J	M	None (PMA)	Unknown	Unknown
89	2744106	20 July 2000	N	M	None (PMA)	Unknown	Unknown
90	2815503	1 February 2002	J	M	Severe bacterial gingivitis	Unknown, possibly septicemia	Unknown
91	2904361	26 October 2003	A	F	Lytic and proliferative lesion of a caudal vertebra with adjacent soft tissue inflammation; PMA	Unknown	Unknown
92	2936386	1 July 2004	J	M	None (PMA)	Unknown	Unknown
93	2958730	27 December 2004	A	M	None (PMA)	Unknown	Unknown
94	2969976	26 March 2005	J	M	Hepatic lipidosis (PMA)	Unknown	Unknown
95	2974993	8 April 2005	A	M	None (PMA)	Unknown	Unknown
96	3001761	19 November 2005	C	F	Bronchopneumonia; tracheitis (PMA); puncture wound to lower jaw (hook)	Unknown	Unknown
97	3041480	15 November 2006	J	F	None (PMA)	Unknown	Unknown

^a PMA = Postmortem autolysis.

TABLE 2. Causes of death based on gross examination only for 30 bottlenose dolphins in South Carolina.

Case no.	Field no.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
98	SC9312	16 April 1993	C	M	Multiple vertical skin lacerations	Boat strike	Noninfectious disease (human induced, boat trauma)
99	SC9521	16 July 1995	J	U	Multiple vertical skin lacerations	Boat strike	Noninfectious disease (human induced, boat trauma)
100	SC9725	18 May 1997	C	F	Multiple dermal abrasions; pulmonary edema and hemorrhage; occipital hemorrhage	Net entanglement	Noninfectious disease (human induced, boat trauma)
101	SC9731	2 July 1997	J	M	Dermal abrasions and hemorrhage; pulmonary hemorrhage	Crab-pot rope entanglement	Noninfectious disease (human induced, fishery)
102	SC9734	2 August 1997	A	U	Not examined; released dead from crab-pot line	Crab-pot rope entanglement	Noninfectious disease (human induced, fishery)
103	SC9737	24 August 1997	A	M	Dermal abrasions and hemorrhage; pulmonary edema and hemorrhage; melon hemorrhage	Rope entanglement	Noninfectious disease (human induced, fishery)
104	SC9922	20 July 1999	J	U	Multiple vertical dermal lacerations and hemorrhage	Boat strike	Noninfectious disease (human induced, boat trauma)
105	SC9913	21 April 1999	A	F	Multiple dermal abrasions and hemorrhage; dermatitis; muscle hematomas; pulmonary edema and hemorrhage; head hemorrhaging	Crab-pot rope entanglement	Noninfectious disease (human induced, fishery)
106	SC9905	7 February 1999	A	F	Hematomas in occipital region; fractured lower jaw and parietal and orbital bones	Boat strike	Noninfectious disease (human induced, boat trauma)
107	SC0006	27 February 2000	J	F	Atrophy; dermal (rostrum) erosion to bone with constricting rope	Fishery entanglement	Noninfectious disease (human induced, fishery)
108	SC0026	14 June 2000	J	M	Multiple dermal lacerations and hemorrhaging with necrosis; pulmonary edema and hemorrhage;	Boat strike	Noninfectious disease (human induced, boat trauma)
109	SC0045	28 September 2000	A	F	Laryngeal dislodgement and constriction by line; pulmonary edema and hemorrhage; enteritis	Hook and line	Noninfectious disease (human induced, fishery)
110	SC0164	20 October 2001	N	F	Multiple dermal lacerations and hemorrhage; head trauma and hematomas; fractured parietal; palate hematoma	Boat strike	Noninfectious disease (human induced, boat trauma)
111	SC0176	2 December 2001	A	F	Maxillary and premaxillary fractures with associated hemorrhage; multiple dermal lacerations (tail)	Boat strike	Noninfectious disease (human induced, boat trauma)
112	SC0179	31 December 2001	U	U	Not examined; released dead from crab-pot line	Crab-pot rope entanglement	Noninfectious disease (human induced, fishery)

TABLE 2. Continued.

Case no.	Field no.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
113	SC0224	23 August 2002	J	F	Multiple dermal abrasions and hemorrhage; sternal hematoma; pulmonary edema and hemorrhage; brain stem hematoma	Shrimp trawl entanglement	Noninfectious disease (human induced, fishery)
114	SC0226	22 August 2002	J	U	Not examined; released dead from trammel net	Trammel net entanglement	Noninfectious disease (human induced, fishery)
115	SC0236	11 December 2002	A	F	Atrophy; cross-hatch dermal abrasions and subdermal hemorrhage; pulmonary edema and hemorrhage	Net entanglement	Noninfectious disease (human induced, fishery)
116	SC0415	05/18/04	A	M	Foreign object stomach obstruction; gastritis	Man-made foreign object	Noninfectious disease (human induced, alimentary obstruction, man-made object)
117	SC0431	11 August 2004	A	F	Multiple dermal abrasions and hemorrhage; encephalomyelitis; bronchopneumonia; myocarditis	Net entanglement	Noninfectious disease (human induced, fishery)
118	SC0606	27 February 2006	J	M	Dermal abrasions (mouth) and hemorrhage; tongue hemorrhage; pulmonary edema and hemorrhage; mandibular hematomas	Rope entanglement	Noninfectious disease (human induced, fishery)
119	SC0630	28 July 2006	A	M	Dermal compression; bronchopneumonia; lymphadenitis; viral condyloma	Shrimp trawl entanglement	Noninfectious disease (human induced, fishery)
120	SC0635	23 September 2006	A	F	Atrophy; multiple dermal lacerations with associated hemorrhage and necrosis; dermal abrasions; rib dislocation	Boat strike	Noninfectious disease (human induced, boat trauma)
121	SC9757	26 November 1997	A	F	Thoracic puncture wound (stingray spine); muscle infection; punctured SVC ^a , aortic arch, and left ventricle	Heart puncture	Noninfectious disease (stingray spine)
122	SC9924	30 July 1999	J	F	Abdominal puncture wound (stingray spine); hypaxial hemorrhaging and edema	Abdominal puncture; probable septicemia	Noninfectious disease (stingray spine)
123	SC0206	7 March 2002	A	M	Abdominal puncture wound (stingray spine); stomach puncture with hemorrhaging and edema	Stomach puncture; probable septicemia	Noninfectious disease (stingray spine)
124	SC0336	21 November 2003	A	M	Liver puncture wound (stingray spine); severe hepatic infection; pneumonia; atrophy	Liver puncture; probable septicemia	Noninfectious disease (stingray spine)

TABLE 2. Continued.

Case no.	Field no.	Date stranded	Age class	Sex	Significant lesions	Cause of death	Cause of death category
125	SC0544	7 December 2005	A	U	Esophageal and trachea puncture (stingray spine); PMA ^b	Tracheal puncture	Noninfectious disease (stingray spine)
126	SC0608	12 March 2006	J	M	Enteritis; intestinal concretion (stingray spine); atrophy; severe thoracic hemorrhaging; pneumonia	Intestinal obstruction; enteritis	Noninfectious disease (stingray spine)
127	SC0638	1 November 2006	J	M	Thoracic puncture wound (stingray spine); lung puncture; severe lung hemorrhaging; pneumothorax	Lung puncture; pneumothorax	Noninfectious disease (stingray spine)

^a SVC = Superior vena cava.^b PMA = Postmortem autolysis.

been reported (Dailey et al., 1991). There was also a case of severe, transmural eosinophilic enteritis that was likely caused by parasitism. Disseminated toxoplasmosis was diagnosed in a juvenile and a neonate. The diagnoses were confirmed by immunohistochemistry testing (Inskeep et al., 1990). There have been several reports of fatal toxoplasmosis in bottlenose dolphins and dolphins of other species (Dubey et al., 2003). There was one case of severe lymphoplasmacytic spinal meningitis that was very likely caused by an infection, but the agent was not detected. One calf died of disseminated alphaherpesvirus infection. This is one of only two reported cases of disseminated alphaherpesvirus infection in bottlenose dolphins (Blanchard et al., 2001). These are the only reported cases of disseminated herpesvirus infection in cetaceans.

The most prevalent noninfectious cause of death was emaciation of unapparent underlying cause. Presumably lack of prey or inability to catch prey for undetected reasons was the underlying problem. Nine dolphins were killed by entanglement in fishing gear and three died from collisions with boats. Thus, of the 76 cases for which the cause of death was determined by gross and histologic examination, 12 (15.5%) died as a direct result of human interaction. Two of the boat-strike victims died as a result of blunt force trauma to the head with associated hemorrhaging and fractured bones and the other one died from a severe wound infection at the site of propeller lacerations on the body.

Neoplasms were detected in two dolphins: a renal cell carcinoma and an adrenal fibroma. Both tumors were small and had not caused significant injury to the organs of origin; metastasis was not detected. They were considered incidental findings. Neoplasia in marine mammals has only recently been described and may reflect important information on carcinogenic contaminants in the marine environment (Bossart, 2006; Newman and Smith, 2006).

TABLE 3. Summary of causes of death of 127 bottlenose dolphins from South Carolina.

Total infectious disease (30)	
19	Bacterial infections (septicemia, pneumonia, myocarditis)
5	Verminous pneumonia
2	Disseminated protozoal infections (toxoplasmosis)
1	Verminous enteritis
1	Inflammatory, unknown agent (meningitis)
1	Mixed verminous and fungal pneumonia (verminous component most severe)
1	Disseminated viral infection (herpesvirus)
Noninfectious disease (46)	
16	Emaciation
12	Human induced (fishery entanglement [9], boat trauma [3])
8	Neonatal
7	Trauma of unknown cause
2	Alimentary tract obstructions by natural objects
1	Drowning of unknown cause
21	Unknown
Gross diagnoses (histology not performed; 30)	
23	Human induced (fishery entanglement [14], boat trauma [8], alimentary obstruction by man-made object [1])
7	Stingray spine wounds

There were eight neonatal deaths of unapparent cause. Two dolphins were killed by alimentary tract obstructions (a catfish lodged in the pharynx in one and gastric blockage by marsh grass in the other). Alimentary obstructions have been noted in previous studies, in particular small cetaceans ingesting prey items that were too large to swallow (Orr, 1937; Gunter, 1942; Hult et al., 1980; Watson and Gee, 2005). Seven dolphins died of traumatic injuries of uncertain cause and one drowned for an undetermined reason.

In those cases in which clear evidence of fatal injury is found by gross examination, a reasonably reliable diagnosis of the likely primary cause of death can be made in the absence of histopathology. The

possibility of an unapparent disease that leads to the fatality, such as encephalitis causing abnormal behavior that results in fatal traumatic injury, cannot be excluded; however, such cases are probably infrequent. Of the 30 cases diagnosed by gross examination only, 14 were attributed to entanglement in fishing gear, eight to boat collisions, seven to injuries caused by stingray spines, and one to alimentary tract obstruction by a manmade object.

Entanglements in crab-pot buoy lines were the most prevalent fishery interaction and are a significant source of bottlenose dolphin mortality in South Carolina (Burdett and McFee, 2004). Three other rope entanglements (cases 103, 107, 118) showed similar wound

TABLE 4. The number of bottlenose dolphins in each cause of death category by age class.

	Neonate	Calf	Juvenile	Adult
Infectious	5	3	9	13
NI ^a	11	2	11	10
Human interaction (NI; gross and histologic)	1	4	5	2
Human interaction (gross only)	1	2	8	11
Stingray (gross only)	0	0	3	4
Unknown	1	3	6	11

^a NI = Noninfectious.

patterns and location of wounds on the body as dolphins entangled in crab-pot lines, but could not be classified as a crab-pot fishery entanglement because the gear was not attached to the animals.

Three of the dolphins (cases 100, 115, 117) showed signs of entanglement in nets with cross-hatched markings and abrasions on the skin, though the source of the fishery was undetermined. South Carolina does not have a substantial net fishery and the last coastal net fishery (shad) was closed on January 1, 2005 (Atlantic States Marine Fisheries Commission [ASMFC], 1999). One dolphin (case 114) was entangled in a trammel net along with case 66 during the course of a South Carolina Department of Natural Resources fish study.

Two dolphins were reported dead during the course of shrimp trawl operations. One of these (case 113) was entangled in the float end of the lazy line and the other (case 119) was found dead in the net when the net was brought on board. Entanglements in the shrimp trawl fishery appear to be a rare occurrence; however, dolphin feeding around the trawls has been documented (Fertl and Leatherwood, 1997) and could make them susceptible to entanglement.

One dolphin (case 109) was observed with a fishing line wrapped around a dislodged laryngeal spout (commonly referred to as the “goosebeak”). The hook was observed in the mouth but was not embedded in tissue. Similar occurrences have been noted by Gorzelany (1998). Depredation of fish by dolphins in recreational fisheries is a growing concern (Garrison, 2007) and hooks have been noted in stomachs of some dolphins (Wells et al., 1998).

Stingray-spine inflictions in marine mammals have been well documented (Walsh et al., 1988; Woodhouse and Rennie, 1991; McLellan et al., 1996; McFee et al., 1997; Duignan et al., 2000) but not all result in death (McFee et al., 1997). The seven cases in this study (see Table 2) all had inflictions of stingray

spines to major organs with the exception of one (case 122), which had a massive infection surrounding the spine. Associated hemorrhage and infection were common in the other six cases. Spines were identified as originating from either the southern stingray (*Dasyatis americana*) or the Atlantic stingray (*Dasyatis sabina*).

Most of the neonate cases involved emaciation, possibly from maternal separation. Verminous pneumonia was a frequent infectious disease of neonates as well. This condition was also described in finless porpoises (*Neophocoena phocaenoides*; Parsons and Jefferson, 2000). Two neonate mortalities were linked with human interaction from boat strikes.

Noninfectious disease in the form of fishery interactions and boat collisions were the most common findings in the calf age class. Infectious causes of death in this age class were diverse, with one case each of viral infection, verminous pneumonia, and bacterial septicemia.

Infectious diseases of juveniles were dominated by bacterial septicemias with individual cases of toxoplasmosis and verminous pneumonia. Human interaction cases were most common in the noninfectious category, with trauma of unknown cause and emaciation also occurring. Juvenile dolphins appear to be susceptible to human-induced injury either out of curiosity or inexperience around fishing gear and boats (Wells and Scott, 1994; Reynolds et al., 2000). Three juvenile dolphins died as the result of stingray-spine inflictions.

Bacterial infections were the only infectious causes of death for dolphins in the adult age class. Most of these were septicemias with the others being pneumonia, meningitis, myocarditis, peritonitis, enteritis, and placentitis. The latter case was associated with a ruptured uterus containing a near-term, decomposed fetus. While the causative agent was unknown in this dolphin, *Brucella*-induced placentitis has been reported in bottlenose dolphins and may have zoonotic potential

(Brew et al., 1999; Miller et al., 1999; Dunn et al., 2001). *Brucella* is also one of the bioterrorism agents of concern (Alibek et al., 2005).

The considerably lower number of cases from the SNCMU in South Carolina compared to the SCMU cases precluded detection of differences in disease prevalence. Of the eight cases from the SNCMU, five involved infectious disease with bacterial infections predominating, which parallels findings in the SCMU. Regional differences in the southeastern United States may exist, however. For instance, dermatologic disease in general and lobomycosis in particular have a relatively high prevalence in the Indian River Lagoon, Florida (Bossart et al., 2003; Reif et al., 2006) but dermatologic disease was infrequent ($\sim 7\%$) in this study and no cases of lobomycosis were found in this study or in Charleston-captured dolphins (Reif et al., 2006).

It should be noted that although this study provides background information for the deaths of bottlenose dolphins in South Carolina, only a small percentage (17.6%) of dolphins that stranded could be analyzed. Thus, other causes of mortality may not have been detected and the causes of the majority of stranded bottlenose dolphins' deaths are still unknown. Future studies on long-term trends in disease processes should provide information on geographic differences that may be helpful in detecting emerging diseases.

It has been noted that "cause of death" is an opinion, not a fact, and that the degree of certainty of that opinion varies from case to case (Froede, 1990). In this study, we have attempted to reach logical conclusions on causes of mortality of dolphins based on the available evidence in each case. The absence of consistent use of microbiology, biotoxin analysis, and contaminant testing is a limiting factor in our study, but pathologic examination remains the primary basis for determination of cause of death. Currently, human environmental influences are pervasive

and include contamination of the oceans with toxic chemicals and trash, alteration of climate, and industrial-scale fishing. There is little doubt that dolphin health is negatively affected by human influences, but the impact of such cannot be assessed in a necropsy-based study such as this, except when the human role is very direct. Thus, this study very likely understates the human contribution to dolphin mortality. In fact, this study does not account for those cases in which evidence of human interaction was found but the cause of death could not be confirmed (see McFee and Hopkins-Murphy, 2002; McFee et al., 2006). Nevertheless, this investigation should be useful because it provides information on probable causes of bottlenose dolphin mortality in a particular region over a 14-yr period during which no increased mortality events were detected. Such background data will be helpful in the recognition of changes in mortality patterns, whatever the cause.

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