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Notable Stingray Spine-Associated Strandings Involving Two Female Bottlenose Dolphins in Florida and Massachusetts, USA, in the Context of Literature and Database Reviews

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Abstract

Trauma from stingray spines (caudal barbs) has been intermittently documented as a cause of mild to fatal illness in odontocetes, particularly bottlenose dolphins (*Tursiops truncatus*), as well as in pinnipeds, sirenians, the loggerhead sea turtle, great white shark, and cobia. Although stingray spines have been noted to cause serious injury to various organ systems, their potential involvement in cetacean reproductive tract pathology has not been previously described. In North America, published descriptions of wild cetacean strandings associated with stingray spines have all involved bottlenose dolphins in the southeastern United States, plus a bottlenose dolphin and a common dolphin (*Delphinus delphis*) in Mexico. In 2018, uterine rupture was observed in a gravid bottlenose dolphin found deceased in Florida with stingray spine penetration into the abdominal cavity. Gross necropsy and histopathology findings were compatible with the stingray spine being involved with the rupture. To provide fuller insight into the case, a scientific literature review was conducted, and results of an unpublished 1998 necropsy involving a coastal female bottlenose dolphin that stranded in the northeastern state of Massachusetts with stingray spine presence was reviewed. Additionally, a query of the nationwide U.S. marine mammal stranding database was conducted for stingray spine-associated cetacean strandings from 1995 to 2019. Of 61 cetacean strandings revealed in the query to involve stingray spine presence, 59 were bottlenose dolphins from the southeastern U.S. states, plus Puerto Rico and Virginia; a bottlenose dolphin in southern California and a short-finned pilot whale (*Globicephala macrorhynchus*) were also noted. Query results did not support a bottlenose dolphin sex predilection for stingray spine-associated strandings; most such strandings involved adults. Behavioral, environmental, anthropogenic, and climatic events may influence marine mammal–stingray interactions. Continued vigilance for stingray spine-associated pathologic changes is warranted during marine mammal stranding investigations at diverse geographic sites.

Key Words: bottlenose dolphin, *Tursiops truncatus*, stingray, uterine rupture, stranding, extralimital

Introduction

Natural history and pathology information collected from marine mammal strandings enhances understanding of marine mammal movements, ecological interactions, disease threats, anthropogenic impacts, and trends in these phenomena over time. Such information may also contribute to the One Health perspective (Hristovski et al., 2010), providing mutual insights into environmental, animal, and human health. Information from strandings primarily contributes to wild marine mammal monitoring, yet also has relevance to facilities that rescue and rehabilitate marine mammals and those that collect animals from the wild for public display, research, or other activities. Performance of a thorough marine mammal necropsy is a fundamental task of stranding investigators and can be strengthened by the investigators' previous knowledge of disease processes that affect particular species, geographic and seasonal factors that may be relevant to strandings, plus vigilance for previously undescribed manifestations of illness or injury. Case reports and database reviews play important roles in alerting and reminding investigators of potential disease manifestations that may be encountered during marine mammal necropsies at diverse geographic sites.

Caudal barbs of stingrays, often and hereafter referred to as "stingray spines," are known to cause mild to fatal pathology in wild marine mammals and other aquatic vertebrates. Worldwide, pinnipeds from sites as diverse as southern Australia and the Pacific North American coast have experienced stingray spine injuries, including the leopard seal (*Hydrurga leptonyx*; Obendorf & Presidente, 1978), Australian fur seal (*Arctocephalus pusillus dorferi*; Obendorf & Presidente, 1978), northern elephant seal (*Mirounga angustirostris*; Obendorf & Presidente, 1978), California sea lion (*Zalophus californianus*; Greig et al., 2005), and New Zealand fur seal (*Arctocephalus forsteri*; Hocking et al., 2020). Published reports from Mexico, the Canary Islands, the Sinai Peninsula, Australia, and New Zealand provide descriptions of stingray spine-associated pathology in odontocetes, including the common dolphin (*Delphinus delphis*; Gallo Reynoso & Aguilar, 1989), bottlenose dolphin (*Tursiops truncatus*; Gallo Reynoso & Aguilar, 1989), false killer whale (*Pseudorca crassidens*; Diaz-Delgado et al., 2018; Puig-Lozano et al., 2020), Indo-Pacific bottlenose dolphin (*Tursiops aduncus*; Spanier et al., 2000), Indo-Pacific humpback dolphin (*Sousa chinensis*; Bowater et al., 2003), and killer whale (*Orcinus orca*; Duignan et al., 2000), respectively. Additionally, as described below, several reports from the United States include descriptions of stingray spine-associated injuries in bottlenose dolphins. Among sirenia, dugongs (*Dugong dugon*) have been known to experience stingray injuries (Haines & Limpus, 2001), and lesions associated with stingray spines are also noted in manatee (*Trichechus manatus latirostris*) stranding data of the Florida Fish and Wildlife Conservation Commission (B. Bassett, pers. comm., 5 January 2023). Non-mammalian aquatic species that have experienced stingray spine-associated pathologic changes include the loggerhead sea turtle (*Caretta caretta*; Bezjian et al., 2014; Vorbach et al., 2019), great white shark (*Carcharodon carcharias*; Obendorf & Presidente, 1978; Flores-Ramírez et al., 2015), and cobia (*Rachycentron canadum*; Huskey, 2021).

In the U.S., journal publications and conference proceedings with descriptions of stingray spine injuries to wild bottlenose dolphins have all involved cases from the southeastern states (Cardeilhac & Jenkins, 1982; Walsh et al., 1988; McClellan et al., 1996; McFee et al., 1997; McFee & Lipscomb, 2009; Burdett & Osborne, 2010; DeLynn et al., 2011; Seguel et al., 2020; Weisbrod et al., 2020; Greenfield et al., 2021; Bassos-Hull et al., 2022). These reports include descriptions of severe injuries to internal organs. Grave illness from stingray spine injury also became manifest in two bottlenose dolphins living at zoological facilities in Florida and New York more than a year after their collection from the wild; the geographic sites of their collection were not mentioned (Walsh et al., 1988). Thoracic and abdominal abscesses associated with stingray spines in bottlenose dolphins from unknown locations were also mentioned in a summary of small cetacean diseases (Sweeney & Ridgway, 1975).

Particularly given that stingray spines may be difficult to detect during gross examination of a stranded marine mammal yet cause substantial pathology, dissemination of novel information related to their presence in stranded animals is warranted. Although an advanced imaging technique, computed tomography, has been employed to reveal stingray spine involvement in a stranded deceased bottlenose dolphin (Weisbrod et al., 2020), and ultrasonography has been utilized to help evaluate stingray spine presence during live bottlenose dolphin health assessments (Bassos-Hull et al., 2022), these diagnostic modalities may not be routinely available to stranding responders. Detection of lesions during field examinations remains fundamental to stranding investigations.

The potential contribution of stingray spine trauma to marine mammal reproductive tract pathology has not been previously described, and a stingray spine-associated bottlenose dolphin stranding has not been previously reported from the northeastern U.S. The two cases described herein shed light on these potential manifestations of stingray spine-associated strandings in bottlenose dolphins, and potentially other marine mammals. Additionally, a query of the U.S. national stranding database for stingray spine-associated cetacean strandings over a 25-y period was conducted and yielded additional information on such strandings throughout the country. The query also informed previous questions regarding any sex predilection that may exist for bottlenose dolphins inflicted with stingray spines.

Methods

Bottlenose Dolphin Specimens

Case 1: Florida Stranding—In May 2018, a deceased adult female bottlenose dolphin in Jacksonville, Florida (Figure 1), was reported to the Florida Fish and Wildlife Conservation Commission biologists for collection and examination. The carcass was assigned alphanumeric identification code TtNEFL1811 and is hereafter referred to as Case 1.

Following gross necropsy, tissue samples from skin, skeletal muscle, lymph nodes, lungs, heart, liver, spleen, esophagus, stomach chambers, intestines, kidneys, and uterus were submitted to the Department of Pathology at the University of Georgia College of Veterinary Medicine for routine histologic examination with hematoxylin and eosin staining.

Case 2: Massachusetts Stranding—Preliminary findings for Case 1 prompted further review of an unpublished July 1998 necropsy record of an adult female bottlenose dolphin that stranded in Gloucester, Massachusetts (Figure 1). The dolphin had been reported swimming in the area the day before it stranded, expired, and was collected for examination by responders from the New England Aquarium. The animal was assigned alphanumeric identification code MH-98-673-Tt and is hereafter referred to as Case 2. Following gross necropsy, tissue samples from skin, subcutis, brain, heart, lung, kidney, bladder, spleen, stomach, colon, liver, pancreas, uterus, mammary gland, lymph nodes, and adrenal and thyroid glands were submitted to Tufts University Veterinary Diagnostic Laboratory for routine histologic examination with hematoxylin and eosin staining. Aerobic and anaerobic bacterial cultures were also submitted to the lab from select grossly abnormal organs. Hemoglobin analysis to determine the morphotype of the animal (coastal vs offshore) was performed at Portland State University via previously described methodology (Hersh & Duffield, 1990).

Literature Review and National Database Query
To enhance understanding of these two cases in the context of stingray spine-associated marine mammal strandings, a review of published literature was performed, which utilized terms including cetacean, odontocete, dolphin, whale, seal, sea lion, walrus, marine mammal, manatee, dugong, stingray, and stranding. Additionally, a query of the U.S. national marine mammal stranding database maintained by the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (NOAA) was made

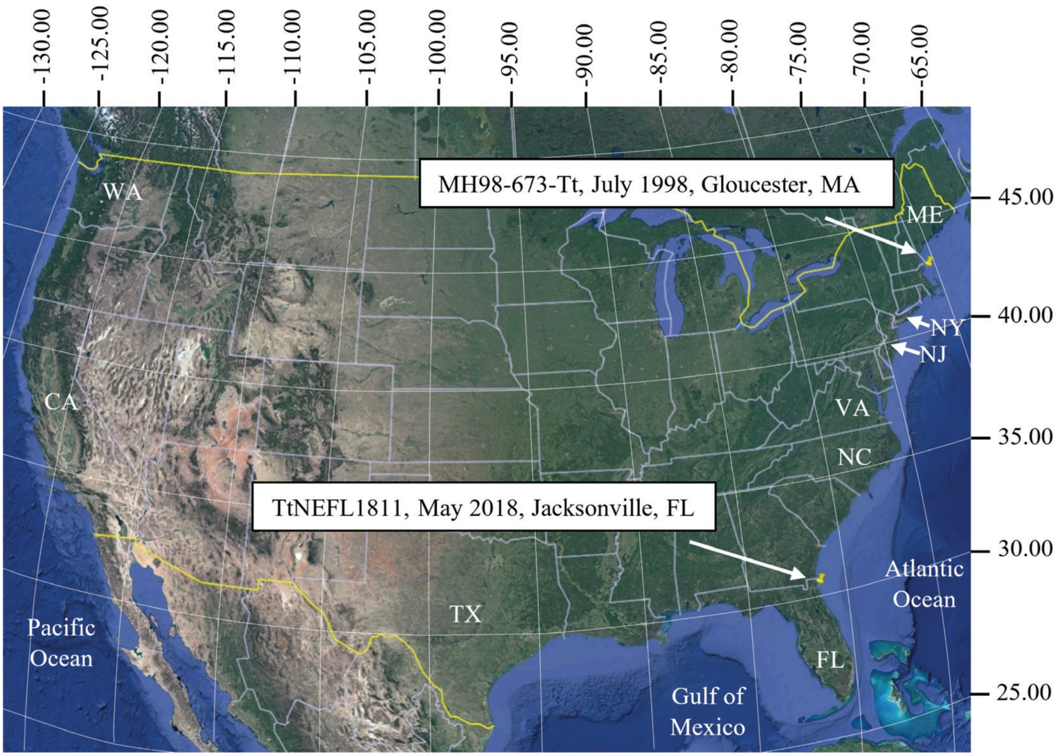

Figure 1. Distant occurrences of two notable stingray spine-associated bottlenose dolphin (*Tursiops truncatus*) strandings in the United States. Stingray spine involvement and gravid uterus rupture were noted in TtNEFL1811. MH98-673-Tt is the northernmost U.S. stingray spine-associated bottlenose dolphin stranding understood from a national database query plus literature review. Select U.S. coastal states referred to in the text: California (CA), Florida (FL), New Jersey (NJ), New York (NY), North Carolina (NC), Maine (ME), Massachusetts (MA), Texas (TX), Virginia (VA), and Washington (WA). Not shown: Alaska, Hawaii, and the Caribbean territory of Puerto Rico.

for stingray-associated cetacean strandings from 1995 to 2019 in all U.S. coastal regions (Figure 1). Basic natural history information, location, and findings from gross necropsy reports are routinely entered into the database for each stranding.

It was acknowledged that the NMFS database query may not reveal all stingray spine-associated cetacean strandings given challenges with detection of stingray spines during field necropsies and potential differences in the amount of detailed information entered into the database from necropsy reports. However, as only some of the numerous cases logged into the database are later published in scientific literature, as determined by the interests of investigators, the database query provided an additional tool to understand stingray-associated strandings.

Results

Gross Necropsy and Histopathology, Ancillary Testing

Case 1: Florida Stranding—The 175-kg carcass measured 239 cm in length and was minimally decomposed. Worn teeth were noted. Overt signs of decreased body condition or evidence of human interaction such as entanglement were not apparent. Most of the epidermis was intact. Milk exuded from the mammary glands, and blood exuded from the urogenital orifice.

Major gross postmortem findings included masses of clotted blood and copious dark red liquid within the peritoneal cavity. A full thickness tear occurred in the right uterine horn through which the head of a 91-cm male fetus partially extended. The edges of the uterine tear were dark purple to black (Figure 2). The tip of a stingray spine was seen to extend into the peritoneal cavity from the right ventrolateral abdominal wall at the level of the gravid uterine horn. Focally, the parietal peritoneum and abdominal wall tissue through which the tip extended were raised and dark red (Figure 3a). No external entry point or scarring of the correlating epidermis or dermis were seen in association with the stingray spine. Aside from its tip, the stingray spine was embedded in blubber and muscle. Firm, irregular presumed reactive tissue encircled the stingray spine anterior to its tip (Figure 3b). The stingray spine measured 6.7 cm and was a fragment of an entire stingray spine. Removal of the tissue surrounding the fragment enabled its further examination (Figure 3c). Absence of the entire stingray spine and other considerations discussed below impeded definitive stingray species identification.

Other gross postmortem findings of the adult bottlenose dolphin were not overtly remarkable and included absence of stomach contents other than scant yellow liquid and presence of parasitic *Braunina* sp. in the second stomach chamber. Gross abnormalities of the fetus were not observed aside from superficial scratches and focal discoloration of the skin in the left cervical region and purple discoloration of the left eye.

Histopathologic examination of tissues showed focal, mild to moderate epithelial hyperplasia with presence of koilocytes in the tongue. Congestion of otherwise unremarkable lung tissue was present. Serosal surfaces of abdominal organs did not display evidence of peritonitis. All other samples, including the uterus, had unremarkable histologic appearance. The uterine rupture was compatible with suspected stingray spine-induced trauma, although this could not be definitively determined histologically.

Case 2: Massachusetts Stranding—The 168-kg carcass measured 245 cm in length and was minimally decomposed. Moderately thin body condition, worn and broken anterior teeth, presence of milk in mammary glands, plus soft barnacles (*Xenobalanus* sp.) on the caudal edge of the dorsal fin were apparent. Raised fluctuant skin lesions, 3 to 4 cm in diameter, containing thick yellow or reddish brown fluid, were noted on the left side of the peduncle. Firm irregular masses were palpable within the skin and soft tissue of the ventral cervical area as well as the left lateral thoracic area below the dorsal fin. Roughly circular areas of dark discoloration with central pinpoint black foci were scattered on skin surfaces of the head, dorsum anterior to the dorsal fin, and peduncle.

A stingray spine fragment, 6.6 cm in length (Figure 4), was discovered completely embedded in soft tissue of the left 4th intercostal space during dissection for thoracic examination. The stingray spine fragment was not associated with a visible external entry wound nor surrounded by grossly abnormal tissue. The fragmented nature of the stingray spine and other considerations discussed below impeded definitive stingray species identification.

Gross abnormalities of internal organs included foci of consolidation and a nodular lesion within the lungs, several milliliters of amber colored fluid in the pericardial cavity, and numerous ecchymotic hemorrhages on the surface of the pancreas. Diffuse areas of the liver were firm and brownish gray, and a fluke-like organism was seen in this organ.

Histopathologic findings included severe necrotizing suppurative dermatitis and steatitis. Numerous fungal hyphae were noted in one section of the dermis and subcutaneous tissue, although similar hyphae were not seen in all skin and subcutis sections examined. Marked myocardial fibrosis existed in some areas of the heart and suggested abnormal heart function. Severe

Figure 2. Dorsal view of a full thickness tear (double arrows) through the right uterine horn of a gravid bottlenose dolphin (TtNEFL1811) found deceased in Jacksonville, Florida, in May 2018. The head of a 91-cm fetus (not shown) protruded through the uterine tear. Scale in cm.

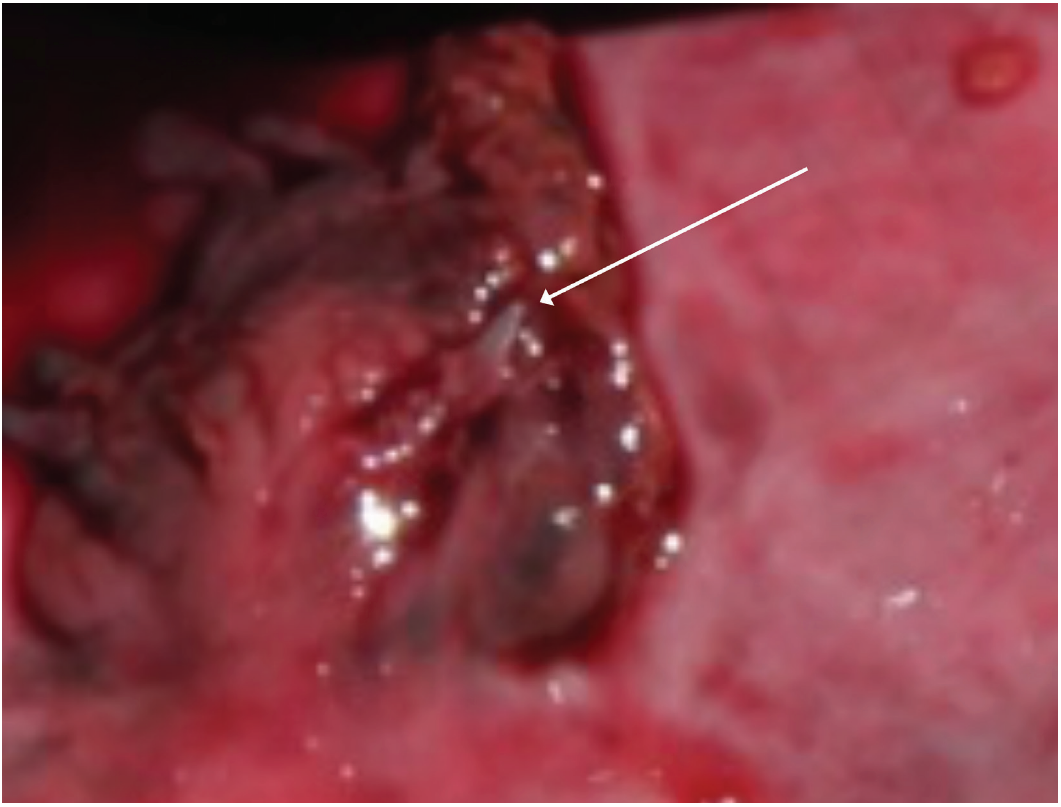

Figure 3a. The tip of a stingray spine (arrow) penetrates the parietal surface of the right ventrolateral body wall at the level of the right uterine horn in a deceased gravid bottlenose dolphin (TtNEFL1811).

Figure 3b. On removal from the body wall of the deceased gravid bottlenose dolphin (TtNEFL1811), the stingray spine fragment (3a) was seen to be surrounded by adherent firm grey tissue, suggestive of fibrosis, consistent with encapsulation and the chronic nature of the infliction. Scale in cm.

Figure 3c. The stingray spine fragment (see 3a & 3b) as it appeared when cleaned of adherent tissue following its removal from the deceased gravid bottlenose dolphin (TtNEFL1811). Scale in cm.

Figure 4. A stingray spine fragment removed from a deceased female bottlenose dolphin (MH-98-673) that stranded in July 1998 in Gloucester, Massachusetts. The fragment was found within intercostal musculature. Scale in cm.

multifocal areas of interstitial and subpleural fibrosis were seen in the lungs as well as mild to moderate perivascular neutrophilic, eosinophilic, lymphoplasmocytic, and histiocytic interstitial pneumonia. Areas of severe bridging portal and periportal fibrosis plus mild to moderate neutrophilic, eosinophilic, and lymphoplasmocytic periportal hepatitis were seen. The chronic fibrotic response in the liver was noted as a possible reaction to the grossly seen trematode parasite. Mild multifocal lymphoplasmacytic pancreatitis existed. Eosinophilic intranuclear inclusions occurred in Islets of Langerhans cells and were noted as potentially indicative of viral infection. Mastitis was observed on cytologic evaluation of mammary fluid. Mild lymphoplasmocytic gastritis, mild membranous glomerulonephritis, and mild to moderate nonsuppurative meningoencephalitis were also seen.

Culture of fluid from skin lesions showed bacterial growth, including *Serratia marcescens* and *Clostridium perfringes*. Fungal culture and further diagnostics to identify pathogens associated with lesions were not performed. Results of hemoglobin analysis confirmed that the bottlenose dolphin was of the coastal morphotype.

Literature Search and National Database Query
Stingray spine-associated lesions in bottlenose dolphins and pinnipeds were documented in scientific journal publications as early as 1975 and 1978, respectively (Sweeney & Ridgway, 1975; Obendorf & Presidente, 1978). Through 2022, no publications were encountered that described uterine pathology associated with stingray spine presence in stranded bottlenose dolphins or other marine mammals. Journal publications detailing stingray spine-associated strandings of bottlenose dolphins in the U.S. have continued to involve cases solely from southeastern states.

There were 61 stingray spine-associated cetacean strandings found in NMFS database query results for the 25-y period 1995 to 2019, including the Case 1 animal. Of those strandings, 59 involved bottlenose dolphins from the southeastern states (including North Carolina through coastal states southward to Florida, and additional states bordering the Gulf of Mexico, including Texas) plus the mid-Atlantic state of Virginia and the Caribbean territory of Puerto Rico. A deceased bottlenose dolphin in southern California, further detailed in an NMFS technical report (Danil et al., 2021), and a short-finned pilot whale (*Globicephala macrorhynchus*) that stranded in Florida as part of a mass stranding were also noted to have stingray spine presence. The Case 2 bottlenose dolphin was logged into the database without notation of the stingray spine. In an additional eight cases, investigators noted wounds that may have been caused by stingray spines, yet the definitive presence of a stingray spine could not be ascertained. Bottlenose dolphin strandings that were *not* associated with stingray spines were reported to the database from states as far north as Maine and Washington (see Figure 1 for state locations).

Uterine pathology associated with stingray spine presence was not noted in query results other than for Case 1. However, investigators involved with a 2003 Florida bottlenose dolphin stranding noted their suspicion that a stingray spine may have caused perforating wounds to the intestine and uterus of the adult animal. They were unable to locate a stingray spine associated with the carcass, which was in moderate to advanced stages of decomposition.

Of the 60 bottlenose dolphin strandings associated with stingray spines that were shown in query results, 28 were male, 29 were female, and three were of unknown sex. Nonlactating and lactating females as well as males were sometimes noted to have severe injuries from stingray spines, such as barb penetration through the heart, lungs, and/or digestive tract. A large majority of the bottlenose dolphins revealed in the database query to have stingray spine presence were listed as adult age class, with sparse numbers of subadults, yearlings, and calves also recorded.

Discussion

Fundamental tasks of stranding investigators include describing lesions encountered during examination of marine mammals such that the information contributes to a body of knowledge regarding potential causes of marine mammal illness, injury, and mortality. Basic life history and location information collected from strandings also provides insight into marine mammal movements, behaviors, and ecological interactions, fortifying animal health monitoring over time in diverse locations. Cases 1 and 2 each contributed novel information related to stingray spine presence in bottlenose dolphins that may also have relevance for strandings of other marine mammal species.

As described below, the stingray spine was believed to be a major contributing factor for fatal uterine rupture in Case 1. Findings in this case enhance understanding of potential causes of reproductive tract pathology in wild bottlenose dolphins and other marine mammals. Uterine rupture has been sparsely documented for odontocetes, and its potential association with stingray spine trauma has not been previously described. The stingray spine in Case 2 may have been an incidental finding, yet its documentation in a species that infrequently inhabits coastal Massachusetts provides a historic baseline for the potential contribution of stingray spines to bottlenose dolphin morbidity in the northeastern U.S. Case 2 alerts current investigators to be aware of the potential for stingray spine-associated pathology in bottlenose dolphins that strand in northern latitudes, particularly given that movements of animal species may change over time amid various environmental factors.

A number of factors suggest that the Case 1 stingray spine was involved in the pathogenesis of uterine rupture, including the dolphin's apparent lack of risk factors for the condition. Uterine rupture is an uncommon but life threatening disorder of humans and animals that is usually associated with risk factors, particularly previous C-section, pharmacologic, or mechanical interventions to facilitate parturition (Hofmeyr et al., 2005; Humm et al., 2010; Ronel et al., 2012; Al-Zirqi et al., 2017). Abdominal trauma, oversized or malpositioned fetus, uterine torsion, obstruction of the birth canal, or other forms of dystocia may also be predisposing factors for the condition (Taylor et al., 1989; Tibary et al., 2008; Ronel et al., 2012). Additionally, hydramnios (Honnas et al., 1985), adenomyosis (Newell-Fugate & Lane, 2009; Nikolaou et al., 2013), neoplasia involving the genital tract (Van Nguyen et al., 2020) or trophoblast (Bruner et al., 2013), and pyometra (McCain et al., 2009; Emergui Zrihen et al., 2017) may contribute to the occurrence of uterine rupture. Forceful exertions of the fetus, such as kicking actions of later term foals, have also been considered as causes of uterine tissue damage and/or torsion that may precipitate uterine rupture (Taylor et al., 1989). Severe convulsions due to domoic acid intoxication were thought to be the cause of uterine torsions in stranded California sea lions (Gulland, 2000). Rarely, uterine rupture may occur without presence of risk factors (Posthumus & Donker, 2017). Regardless of its potential causes, uterine rupture may culminate in fatal hemorrhagic shock.

Limited published information exists on the occurrence of uterine rupture in cetaceans. Two cases of uterine rupture were noted among deceased belugas (*Delphinapterus leucas*) recovered from the St. Lawrence River without mention of potential predisposing factors (Lair et al., 2014). Idiopathic uterine rupture in a deceased shortbeak common dolphin (*Delphinus delphis*) and uterine rupture associated with a mummified fetus in a deceased Risso's dolphin (*Grampus griseus*) were reported from the Canary Islands (Diaz-Delgado et al., 2018). A deceased white-beaked dolphin (*Lagenorhynchus albirostris*) was found to have uterine rupture and presence of a fetus in cephalic presentation (Hart & van der Kemp, 1999). Discussion that cephalic presentation of the fetus for parturition may predispose the dam to dystocia and uterine pathology such as rupture has occurred (Gol'din, 2011; Saviano et al., 2020) as fluke (podalic) presentation of the fetus occurs more frequently in several odontocete species (Robeck et al., 2018). However, several cases of cephalic fetal presentation that culminated in normal bottlenose dolphin births have been reported (Saviano et al., 2020).

Examination of the Case 1 bottlenose dolphin did not reveal risk factors for uterine rupture, and its body condition was not compromised. The animal was producing milk; and its near-term fetus appeared to be positioned for fluke presentation at birth and was not oversized (Mattsen et al., 2006; Neuenhoff et al., 2011). Histologic examination of multiple organs from the adult animal showed virtually no evidence of underlying disease, suggesting overall good health. Focal hyperplastic changes in lingual epithelium suggested oral papilloma virus infection which did not appear to contribute to systemic illness. The presence of copious amounts of clotted blood in the abdominal cavity suggested that substantial antemortem hemorrhage was a primary contributor to the gravid female's death.

The location of the sharp stingray spine tip, protruding into the right side of the abdominal cavity, may have enabled its contact with the expanding gravid right uterine horn. Without more extensive histologic sampling of the edges of the uterine rent, verification of uterine horn sites that may have been compromised by contact with the stingray spine tip could not be made. Alternatively, if the stingray spine did not directly cause laceration and weakening of the uterine horn, it remains possible that inflammation and release of pathogens and/or toxins associated with its presence in the abdominal wall may have contributed to illness, uterine dysfunction, dystocia, and subsequent uterine rupture.

Occult embedded stingray spines are known to cause substantial pain and tissue pathology in people, and similar consequences may occur in non-human animal species inflicted with these objects. Numerous enzymatically active proteins as well as pathogens can be introduced by the retroserrate spine and its associated venom gland. Pathologic consequences include local and/or systemic effects, such as edema, inflammation, vasoconstriction, necrosis, hemorrhage, delayed wound healing, and infection, as well as cardiovascular, digestive, and neurologic abnormalities (Ho et al., 1998; Barber & Swygert, 2000; Germain et al., 2000; Auerbach, 2007; Kumar et al., 2011; Rensch & Elston, 2019).

Penetration of stingray spines into deep tissue layers is viewed as a serious medical condition in people that often necessitates prompt intervention with antimicrobials, debridement, and surgery (Diaz, 2008; Clark et al., 2017; Rensch & Elston, 2019). Serious complications of stingray spine infliction in humans and dolphins may occur weeks, months, or years after the initial injury, particularly as stingray spine fragments may migrate through tissues, causing further injury (Walsh et al., 1988; Saunders et al., 2013; Falk et al., 2019). Thus, concern existed that the deeply embedded stingray spine in Case 1 contributed to morbidity and mortality. Illness during pregnancy may compromise uterine function and precipitate fetal death (Noakes et al., 2001). It is interesting to note that a wild Indo-Pacific bottlenose dolphin, observed to have sustained stingray spine infliction while nursing a calf, became anorectic and listless for several days following the event, and then recovered, although the calf soon died (Spanier et al., 2000).

The raised, discolored appearance of the parietal peritoneal wall surrounding the Case 1 stingray spine tip (Figure 3a) suggested that it may have served as a source of ongoing inflammation and discomfort or as a low grade peritonitis, not revealed via routine histologic sampling. Tissue samples were not specifically taken from this site. Additional sampling of the grossly affected parietal peritoneum, plus tissues directly surrounding the stingray spine, may have provided a time reference for the wound and shed light on the potential presence of focal cellulitis, myositis, or peritonitis in the vicinity of the uterus.

The firm, fibrotic-appearing tissue adhering to the Case 1 stingray spine (Figure 3b), plus absence of a visible entry wound, suggested the presence of the stingray spine within the dolphin for some time. Although healing of skin wounds may occur quickly in dolphins, focal epidermal scarring or discoloration may be present in association with stingray spine presence (Bassos-Hull et al., 2022). Skin blemishes are also known to remain for weeks to months following skin trauma such as via biopsy darting of these animals (Tezanos-Pinto & Baker, 2012). No exterior skin blemish in the vicinity of the stingray spine was seen on the Case 1 dolphin, suggesting that the entry wound had previously healed.

In Case 2, multiple organ system abnormalities contributed to the dolphin's illness and death. Presence of the stingray spine may have been an incidental finding that did not substantially contribute to pathologic processes. The stingray spine was fully sequestered within intercostal muscular tissue and appeared to be separate from other areas of gross pathology. Lack of an entry wound in the vicinity of the stingray spine and lack of grossly apparent reactive tissue around it suggest that the object had been present within the dolphin for a substantial period of time, possibly for years. However, stingray spines may fragment and migrate (Walsh et al., 1988; Weisbrod et al., 2020). The possibility that additional, undetected fragments existed and contributed to chronic infection and/or other pathology cannot be ruled out, particularly as diagnostic imaging and detailed dissection for stingray spine fragments were not performed.

Other findings of interest in Case 2 included severe steatitis within the skin and subcuticular fat as the condition is sparsely reported for wild cetaceans and may have an infectious and/or nutritional etiology (Dawson et al., 2006; Raverty et al., 2006; Soto et al., 2010). Additionally, the dolphin was experiencing a cutaneous infection with filamentous fungi. Cutaneous mycosis involving filamentous fungi has occasionally been reported for wild odontocetes without mention of stingray spine presence (Huggins et al., 2020), yet invasive fusariosis has occurred in association with human stingray injury (Hiemenz et al., 1990).

Case 2 is the sole description of stingray spine presence in a bottlenose dolphin stranding north of the mid-Atlantic state of Virginia as understood via the literature search and the 1995 to 2019 NMFS database query. Case 2 provides historical information for future stingray spine-associated bottlenose dolphin strandings that may occur in the northeastern U.S. and is also notable given the coastal morphotype of the animal. Offshore morphotype bottlenose dolphins are known to inhabit waters off the northeastern U.S. and may strand along that coast (Mead & Potter, 1995), yet occasional movements of coastal bottlenose dolphins into Massachusetts from more southern areas may also have occurred in the past (Wiley et al., 1994). The Case 2 animal may have been part of the coastal bottlenose dolphin stock that winters near Virginia and summers near New Jersey and New York (Waring et al., 2007, 2016; Toth et al., 2010; Figure 1). Vigilance for stingray spine presence in bottlenose dolphins that strand at northern latitudes continues to be warranted as occasional strandings of this species in Massachusetts (Bogomolni et al., 2010) and through the most northern Atlantic and Pacific states (NMFS database query) continue to be documented. Additionally, environmental, anthropogenic, and climatic factors may impact movements of diverse aquatic species (Wells et al., 1990; Hoisington & Lowe, 2005; Toth et al., 2010; Jirik & Lowe, 2012).

The possibility exists for changes in the frequency of coastal bottlenose dolphin movements into more northern areas of the U.S. A recent example of a northern range extension has been described for California coastal bottlenose dolphins, and two of these dolphins traveled some 2,500 km from southern California to Washington (Keener et al., 2023). Additionally, north of the U.S., bottlenose dolphins of undetermined ecotype, likely the offshore form, were fairly recently seen for the first time in offshore Pacific Canadian waters (Halpin et al., 2018). The occasional presence of the presumed offshore morphotype has long been known off the south Atlantic Canadian coast (Gowan & Whitehead, 1995; Waring et al., 2016), and an older summary of bottlenose dolphin occurrences in Canada is also available (Baird et al., 1993).

The stingray spine fragments in these two cases were embedded in the body wall, no longer showed entry wounds, and could have been overlooked during necropsy. Diagnostic imaging techniques such as radiology and ultrasonography may be useful for stingray spine detection. These techniques have facilitated detection of occult stingray spines and/or their associated non-radiopaque material in people (O'Malley et al., 2015; Falk et al., 2019) and in a horse (Riggs et al., 2003). Ultrasound was also utilized to evaluate and retrieve a stingray spine present in a wild live bottlenose dolphin during a health examination (Bassos-Hull et al., 2022), and radiology units specifically adapted for field use with small cetaceans have been designed (Walsh et al., 2018). Magnetic resonance imaging or computed tomography have also been used in humans (Falk et al., 2019) and in a deceased bottlenose dolphin (Weisbrod et al., 2020) to detect stingray spines, yet such techniques may not be as available to stranding investigators as are radiology and ultrasonography. For marine mammals that survive stranding and enter a rehabilitation program, a thorough search for occult stingray spines, potentially utilizing diagnostic modalities described above, may be particularly prudent, such that an appropriate medical intervention plan can be devised.

Southern and Atlantic stingrays (*Hypanus americanus* and *Hypanus sabinus*, respectively; formerly *Daysyatis americana* and *Daysyatis sabina*, respectively), as well as the spotted eagle ray (*Aetobatus narinari*) were noted to be involved in previously reported cases of stingray spine presence in stranded bottlenose dolphins from the southeastern U.S. (McClellan et al., 1996; McFee et al., 1997; Weisbrod et al., 2020).

Examination of the total number and shape of stingray spine serrations and other morphometrics, including total spine length, base, and groove characteristics, have been advised for stingray species identification (Schwartz, 2005, 2008, 2009a, 2009b). Substantial variations in these parameters can occur, even among related species and geographic ecotypes. Stingray life history factors may also affect features such as serration counts. Recently, micro-computed tomography has been used to more fully understand variations in stingray spine characteristics and may be helpful to species identification (Chabain et al., 2018; Shea-Vantine et al., 2021).

The stingray spines encountered in these two cases were each partial segments of an entire spine, and their examination was limited to gross morphology. Life history and specific geographic information for each stingray was not known. Following consultation with elasmobranch biologists and in light of the considerations above, definitive assignment of these stingray spines to specific species was not made. Further attention to stingray spine identification techniques may enhance understanding of interactions between specific stingray species, odontocetes, and other aquatic animals.

Questions have previously arisen as to whether or not female dolphins may be at particular risk of stingray injury such as when these animals enter shallow waters to give birth and rear young (Spanier et al., 2000). Although these two cases involved pre-partum and apparently lactating females, respectively, NMFS database query results did not support a bottlenose dolphin sex predilection for stingray spine-associated stranding. Additionally, a previous study of 97 bottlenose dolphin strandings in South Carolina showed four males, two females, and an individual of unknown sex with stingray spine-associated fatality (McFee & Lipscomb, 2009). Geographic variations in carcass retrieval efforts and in access to some coastal and estuarine areas present further challenges to understanding if any true sex predilection for such injury to bottlenose dolphins exists. Furthermore, as mentioned in the "Introduction," odontocetes and other species that inhabit pelagic waters may also experience stingray spine injuries.

Bottlenose dolphins are known to chase stingrays (Fetterman et al., 2022). They may also toss stingrays with their mouths or encounter stingrays accidentally while traveling or foraging for other fish species (Bassos-Hull et al., 2022). Ingestion of stingrays by bottlenose dolphins is also possible (Burdett & Osborne, 2010; Conway & McFee, 2017). Given the severe pathology that may result from stingray spine infliction, continued awareness of this potential source of marine mammal morbidity and mortality is warranted at diverse geographic sites.

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