Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

Research Article

Cite this article: Ascheri D *et al.* (2024). Post-mortem examination on a striped dolphin (*Stenella coeruleoalba*) reveals a potential fatal interaction with bottlenose dolphins (*Tursiops truncatus*) in Italian waters. *Journal of the Marine Biological Association of the United Kingdom* **104**, e4, 1–7. https://doi.org/10.1017/S0025315423000942

Received: 20 January 2023
Revised: 21 November 2023
Accepted: 7 December 2023

Keywords:
aggressive behaviour; bottlenose dolphin; interspecific interactions; Pelagos Sanctuary; striped dolphin

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Post-mortem examination on a striped dolphin (*Stenella coeruleoalba*) reveals a potential fatal interaction with bottlenose dolphins (*Tursiops truncatus*) in Italian waters

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Abstract

Despite attacks of bottlenose dolphins towards conspecifics and other species of dolphins being reported worldwide, inside the Mediterranean Sea these behaviours are still considered sporadic and have been, to date, only recorded in Spain and France, mostly directed to striped dolphins. In this work, we reported the necropsy outcomes of a dead striped dolphin that suggest a fatal interaction with bottlenose dolphins inside the Italian side of the Pelagos Sanctuary, Western Ligurian Sea. Results from the post-mortem examination showed multiple, multifocal external rake marks spaced 1–1.2 cm and several internal injuries, consisting of subcutaneous and muscular haematomas and haemorrhages, multiple vertebral and rib fractures, haemothorax and lung laceration. The inter-tooth distance and all the internal findings were consistent with a fatal traumatic interaction with bottlenose dolphins as reported in similar cases in other parts of the world where the same results were found. No other significant gross and microscopic findings and concurrent significant pathogens were detected. This case highlights the existence of negative interspecific interactions in an area where they have never been reported before and adds new important information to the literature for understanding their occurrence, expanding the geographical range of observation in the western Mediterranean Sea.

Introduction

Interactions and competition between sympatric species of cetaceans have often been recorded in the wild (Jefferson *et al.*, 1991; Ross and Wilson, 1996; Weller *et al.*, 1996; Herzing and Johnsonz, 1997; Cotter *et al.*, 2012). Mixed-species groups can coexist for some time within the same area (Boys, 2015), sharing and partitioning resources and providing reciprocal benefits in foraging techniques (Norris and Dohl, 1979). Competition, on the contrary, can potentially lead to aggressive behaviours and interactions between individuals (Shane, 1995; Herzing *et al.*, 2003), causing serious injuries and sometimes even resulting in the killing of competitors (Patterson *et al.*, 1998; Dunn *et al.*, 2002; Díaz-Delgado *et al.*, 2018).

Attacks towards both conspecifics ('intraspecific interactions') and other cetacean species ('interspecific interactions') attributed to common bottlenose dolphins (*Tursiops truncatus*), hereafter referred to as 'bottlenose dolphin', have been reported worldwide, some of which resulted in a fatal outcome (Ross and Wilson, 1996; Cotter *et al.*, 2012; Crespo-Picazo *et al.*, 2021).

According to several authors, possible explanations proposed for bottlenose dolphins' aggressivity towards allospecificity are competition for food and feeding interference (Spitz *et al.*, 2006; Cotter *et al.*, 2012; Methion and Díaz López, 2021), enhancing fighting and infanticide skills (Patterson *et al.*, 1998; Cotter *et al.*, 2012), sexual frustration during periods of limited access to females and physiological response to stress (Ross and Wilson, 1996; Cotter *et al.*, 2012). On the contrary, predation is not usually considered a valid hypothesis due to the lack of evidence in the reported cases (Cotter *et al.*, 2012). Infanticide in bottlenose dolphins was observed in different areas worldwide such as Scotland (Patterson *et al.*, 1998; Robinson, 2014), Virginia (Dunn *et al.*, 2002), Spain (Díaz López *et al.*, 2018) and Belize (Ramos *et al.*, 2022) but this behaviour is normally described towards conspecifics. Currently, the most accredited hypothesis of aggressive behaviour is a consequence of competition for available resources in areas regularly used by bottlenose dolphins for feeding (Cotter *et al.*, 2012; Methion and Díaz López, 2021).

Despite few direct observations of interspecific attacks by bottlenose dolphins (e.g. Methion and Díaz López, 2021), the majority of available data on interactions has been collected through the post-mortem analysis of stranded cetaceans (Boys, 2015). Direct observations and necropsies revealed a wide range of cetacean species being attacked by *T. truncatus* including harbour porpoises (*Phocoena phocoena)* (Ross and Wilson, 1996; Patterson *et al*., 1998; Cotter *et al*., 2012; Boys, 2015), estuarine dolphins (*Sotalia guianensis*) (Wedekin *et al*., 2005), Commerson's dolphins (*Cephalorhynchus commersonii*) (Coscarella and Crespo, 2010), Atlantic spotted dolphins (*Stenella frontalis*) (Herzing and Johnsonz, 1997; Herzing *et al*., 2003), Risso's dolphin (*Grampus griseus*) (Barnett *et al*., 2009), long-finned pilot whale (*Globicephala melas*) (Barnett *et al*., 2009), striped dolphin (*Stenella coeruleoalba*) (Barnett *et al*., 2009) and short-beaked common dolphins (*Delphinus delphis*) (Barnett *et al*., 2009; Methion and Díaz López, 2021).

In the Mediterranean Sea, episodes of interspecific attacks appear as sporadic behaviours, and were reported only in Spanish and French waters, addressed towards both striped and Risso's dolphins (Dhermain, 2020; Puig-Lozano *et al*., 2020; Crespo-Picazo *et al*., 2021; Gannier, 2021).

In the Ligurian Sea, inside the Pelagos Sanctuary (northwestern Mediterranean Sea), bottlenose dolphins are regularly seen all year round in coastal waters and use this area for feeding and nursing their calves, both in the eastern (Gnone *et al*., 2011) and western sides of the region (Ascheri *et al*., 2022). The striped dolphin, on the contrary, is considered mainly a pelagic species, even if it can occasionally occur in coastal waters and in the deep coastal submarine canyons characterising the western Ligurian Sea (Moulins *et al*., 2008; Panigada *et al*., 2008).

This area, in fact, presents a very narrow continental platform and the coastal and pelagic habitats are strictly interconnected through the morphology of the seafloor. This allows an ecological overlap between the two species, as described in French and Spanish waters (Norris and Dohl, 1979; Crespo-Picazo *et al*., 2021; Gannier, 2021).

In this study, we report the pathologic features observed in a stranded dead striped dolphin in the Pelagos Sanctuary, Italy, related to external and internal traumatic lesions compatible with a traumatic interaction with bottlenose dolphins. Reporting this event is highly significant to add new valuable information to the literature regarding the occurrence of these behaviours, which are rarely observed in the wild (Boys, 2015) and often difficult to evidence through necropsies due to the lack of specific diagnostic protocol (Puig-Lozano *et al*., 2020).

Material and methods

On 1st November 2020, an adult female striped dolphin was found dead on Bergeggi beach (SV) (44.15N, 8.26E), along the Western Ligurian Sea coast. These waters are part of a marine protected area, named Pelagos Sanctuary, established in 1999 for the protection and conservation of cetaceans and classified as a Specially Protected Area of Mediterranean Importance (SPAMI) in 2002.

A complete post-mortem examination according to standard protocols (Ijsseldijk *et al*., 2019) was carried out the day after the stranding at the Istituto Zooprofilattico Sperimentale Diagnostic Laboratory of Imperia. Twenty-eight morphometric measures were taken following the Italian National Stranding Data Bank protocol (BDS – Banca Dati Spiaggiamenti) developed by the University of Pavia and the Natural History Museum of Milan following Cagnolaro *et al*. (1986). The age class was categorized based on total body length, according to Geraci and Lounsbury (2005) and Carlini *et al*. (2014).

External and internal structures and abnormal findings were fully described, photographed and sampled. Furthermore, external lesions compatible with rake marks were further analysed to assess any possible inter- and intraspecific interaction. For this purpose, measurements of the space between parallel wounds were evaluated from pictures taken perpendicularly to the marks. Using Photoshop, the distance between the parallel wounds and the height of the dorsal fin was calculated and then rescaled through a proportion with real measurements of the dorsal fin taken during the necropsy. Measures obtained were later compared with the inter-tooth distances of different cetacean species reported in the literature.

During the necropsy, tissue samples of all the major organs and lesions were collected and split into aliquots for subsequent analyses: one was kept frozen at −20 °C for microbiological and toxicological investigations; one was kept frozen at −80 °C for biomolecular analyses, and the other was preserved in 10% buffered formalin for histological and immunohistochemical (IHC) investigations. Gastric content was collected and frozen to be preserved for later analysis (weighed, filtered and sorted).

Blood serum, aqueous humour, pericardial fluid and cerebrospinal fluid (CSF) were collected and kept frozen at −20 °C for serological investigations.

Representative tissues including the brain, lung, heart, liver, spleen, kidney, mammary gland, adrenal gland and stomach were collected and fixed in 10% neutral buffered formalin. These tissues were then embedded in paraffin, sectioned at 4 ± 2 μm, stained with haematoxylin and eosin and examined through a light microscope. Different areas from the brain were sampled and examined, including the basal nuclei, thalamus, mesencephalon, pons, obex, and frontal, parietal, occipital and cerebellar cortex. IHC for *Morbillivirus* was performed on tissue sections including the brain, lung, spleen and kidney, using a monoclonal anti-Canine distemper virus antibody (VMRD, Pullman, WA, USA) (Di Guardo *et al*., 2010). *Toxoplasma gondii* IHC was carried out on the nine aforementioned brain tissues using a polyclonal serum of caprine origin (VMRD) (Di Guardo *et al*., 2010).

Tissue samples including those of the brain, lung, prescapular lymph node, liver, spleen, tonsils, kidney and bladder were processed for standard aerobic, anaerobic and microaerobic (5% CO_2) bacterial culture and identification, by biochemical and/or molecular analyses. Following international recommendations (OIE 2018) samples from target tissues underwent specific bacteriological procedures to screen *Listeria* spp., *Salmonella* spp. and *Brucella* spp. The presence of anti-*T. gondii*, anti-*Brucella* spp. and anti-*Morbillivirus* antibodies was investigated in blood serum, aqueous humour, pericardial fluid and CSF (Hernández-Mora *et al*., 2008; Di Guardo *et al*., 2010). Molecular detection of *Dolphin morbillivirus* (DMV) (Verna *et al*., 2017), *Herpesvirus* (HV) (Vandevanter *et al*., 1996), *T. gondii* (Vitale *et al*., 2013) and *Brucella* spp. (Bounaadja *et al*., 2009) was routinely achieved on target tissues available, including the brain, lung, tonsils, prescapular lymph node, liver, spleen and kidney for DMV, brain, lung, prescapular lymph node, spleen and kidney for HV, brain, prescapular lymph node, liver, spleen, heart and muscle for *T. gondii*, and brain, lung, tonsils, prescapular lymph node, spleen and kidney for *Brucella* spp.

Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB) and dichlorodiphenyl trichloroethanes (DDTs) as well as extracted organic material per cent (EOM%) were measured in the blubber. Organochlorine compound (OC) analyses were made according to the Environmental Protection Agency method 8081/8082, with modifications (Marsili and Focardi, 1997). The OC levels are expressed in ng/g dry weight (d.w.).

Finally, toxicological stress was evaluated using a theoretical model where the value of the canonical variable (CAN) represents

https://doi.org/10.1017/S0025315423000942 Published online by Cambridge University Press

the potential hazard from the sum of DDTs and PCBs and is evaluated with a canonical analysis procedure (Marsili *et al.*, 2004).

Results

The carcass, in a post-mortem condition code 2 (fresh), weighed 74 kg and its total length measured 200 cm, thus resulting in its classification as an adult. It was a lactating female, with a moderate nutritional status.

Severe multifocal acute skin lesions, consisting of three to ten linear and parallel fresh lacerations (rake marks), spaced 1–1.2 cm apart were found (Figure 1). These lesions were localized on the posterior part of the body, the dorsal fin, the fluke and around the genital area (Figure 1A–C). Two additional lesions of around 10 cm in length showing blood leaking were present transversally with respect to the anal opening (Figure 1D).

Internal macroscopic examination revealed multifocal severe vascular changes consisting of haematomas and haemorrhages in the subcutaneous space and muscles (left supraorbital region, left temporomandibular region, dorsal and thoracic regions), along with multiple tears within the intercostal muscle tissue (Figure 2A, B, E). Moreover, multiple bilateral contiguous vertebral and rib fractures (last 5–6 ribs and transverse processes of the thoracic vertebrae) were found in the medial-caudal side (Figure 2C, D), along with a haemothorax associated with a perforation of the pleural and pulmonary parenchyma, more evident in the right lung (Figure 2F, G). Other macroscopic findings included a moderate parasitic infection *by Clistobothrium delphini* and *C. grimaldii merocercoids*, especially around the genital area, bloody milk in the mammary gland, enlargement and readness of prescapular lymph nodes (4.5 cm in length), splenic sub-capsular haemorrhages, five nodules (diameter of 1–5 cm) of *Pholeter gastrophilus* at gastric level in the main stomach and haemorrhagic CSF. The pyloric stomach contained scanty material (0.55 kg), represented by bones and otoliths, correlated to a non-recent meal. Microscopically, diffuse oedema in the white matter and microhaemorrhages were observed in the brain. A severe pulmonary haemorrhage and moderate granulomatous bronchopneumonia of parasitic origin (nematodes of the family *Pseudaliidae*) were evident in the lungs. Furthermore, a moderate bronchiolar sphincter contraction was observed. A mild multifocal chronic cholangitis, large haemorrhagic areas, predominantly subcapsular, with lymphoid depletion of the germinal centres with hyalinosis in the spleen, and marked hyperaemia with haemorrhagic foci in the myocardium, were also detected. Vascular congestion and small corticomedullary haemorrhages of the adrenal gland, and a moderate diffuse congestion of the vessels and glomerular capillaries in the kidney were additionally found.

A Gram-negative bacterium, *Empedobacter brevis*, was isolated by aerobic and microaerobic culture from the mammary gland, liver, lung, spleen and prescapular lymph node. No other significant bacteria, including *Brucella* spp., *Listeria* spp. and *Salmonella* spp., were isolated. No IHC evidence of morbilliviral and *T. gondii*-specific antigens were found in the tissues examined.

The levels of HCB, PCBs and DDTs, expressed in ng/g dry weight (d.w.) (HCB: 94.43; PCBs: 66541.03; DDTs: 2898.37) and the canonical variable value (CAN) = −0.16, did not reveal the presence of hazardous levels of OC pollutants (CAN > 0.47). The EOM% was 70.71, showing a mild depletion of the blubber layer. No biomolecular evidence of DMV, HV, *T. gondii* or *Brucella* spp. was found. No evidence of anti-*T. gondii*, anti-*Brucella* spp. and anti-*Morbillivirus* antibodies was demonstrated in aqueous humour, serum, pericardial fluid and CSF.

Discussion

The carcass of the striped dolphin reported in this work showed multiple, multifocal external and internal injuries, respectively

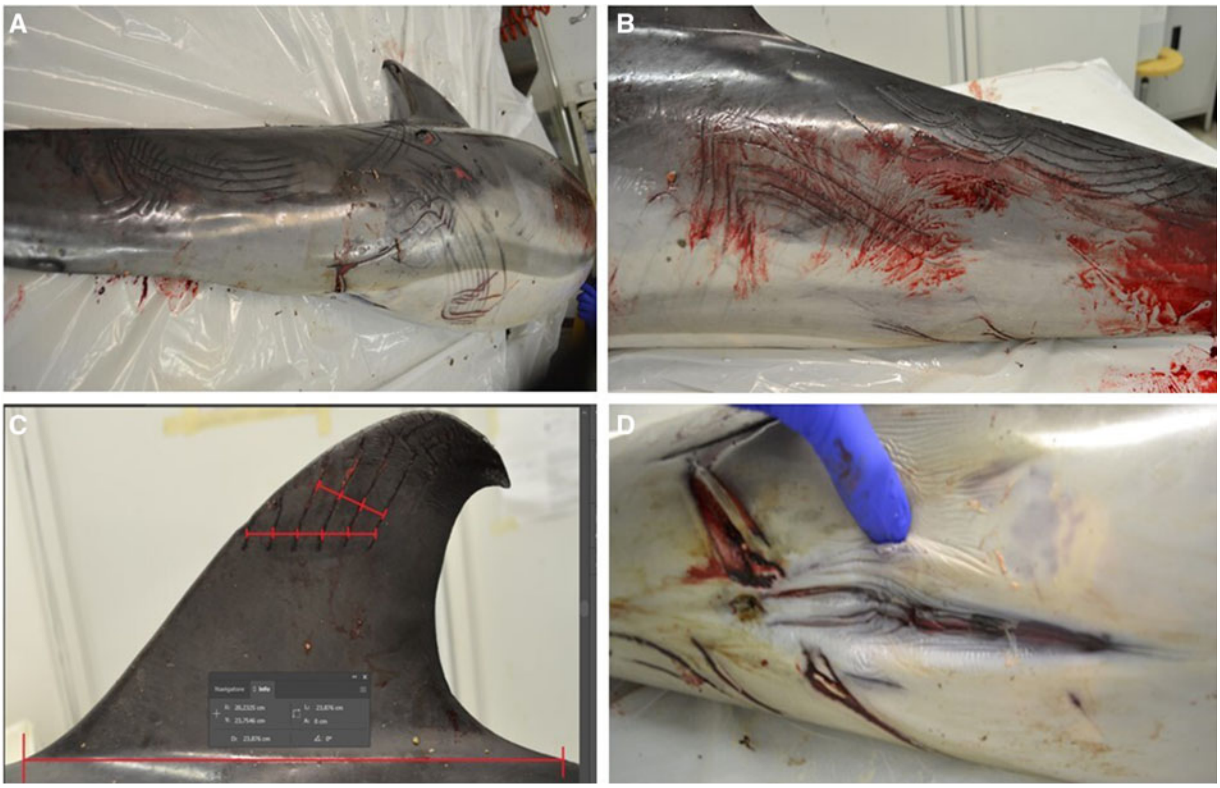

Figure 1. External findings of traumatic interspecific interaction in a mature female striped dolphin. (A) Right side of the body showing severe multifocal tooth-rake marks; (B) right ventrolateral view (mid-flank-belly-ventral peduncle), with severe multifocal tooth-rake marks; (C) left lateral view of the dorsal fin, parallel incisions, spaced 1–1.2 cm; (D) genital area: lacerations transversally with respect to the anal opening, showing blood leaking.

https://doi.org/10.1017/S0025315423000942 Published online by Cambridge University Press

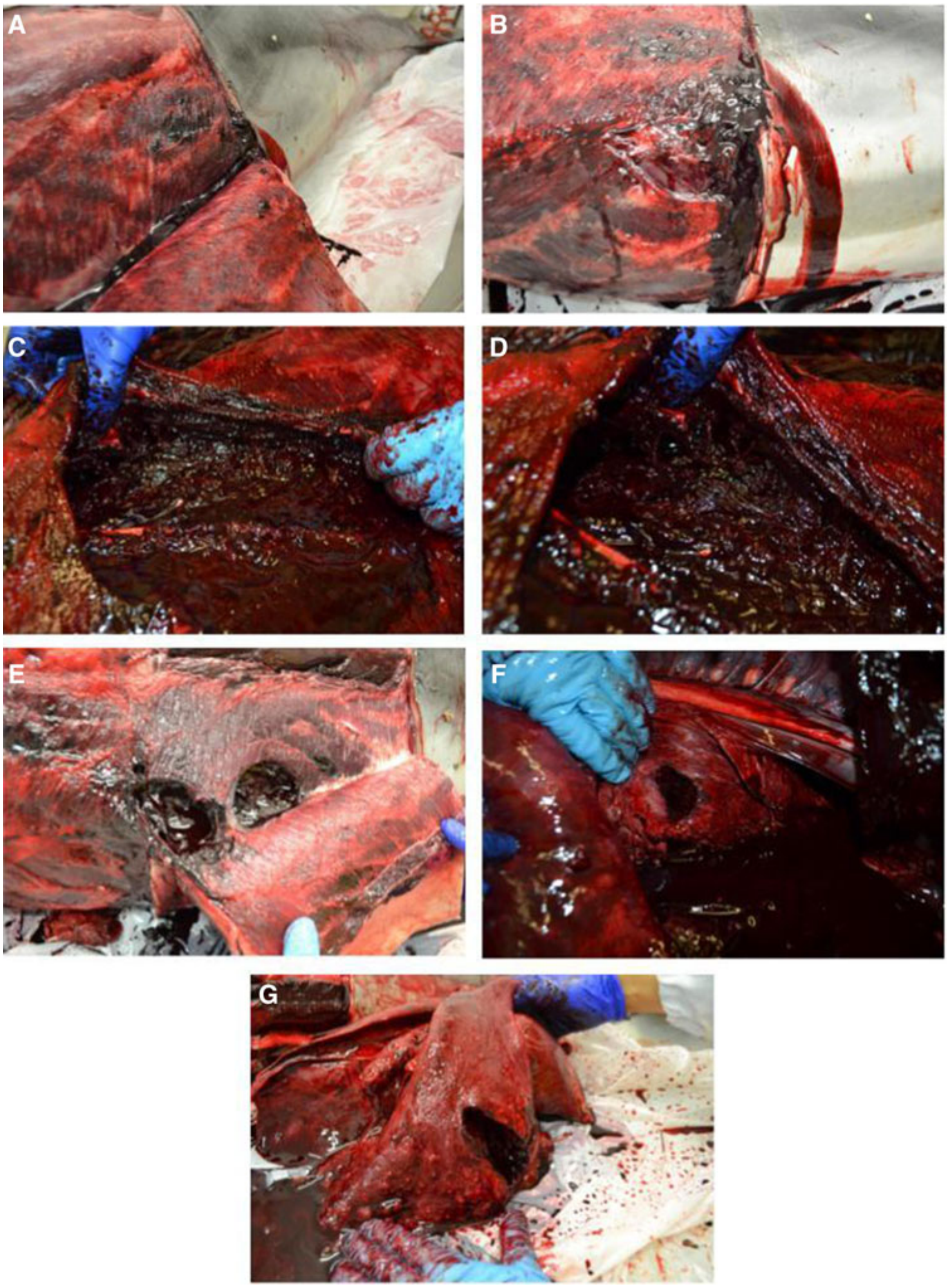

Figure 2. Representative internal macroscopic findings in a mature female striped dolphin. (A, B) Left thoracic region. Subcutaneous and muscular haematoma and haemorrhages; (C, D) thorax, medial-caudal side, rib fractures arrowheads; (E) left thoracic region, multiple intercostal muscle tearing, haematoma and haemorrhage; (G, F) thoracic cavity. Haemothorax and lung perforations; particular of right lung.

consisting of a series of parallel lacerations spaced 1–1.2 cm apart (acute tooth rake marks) and of subcutaneous and muscular haematomas and haemorrhages, multiple vertebral and rib fractures, haemothorax and lung laceration. All main lesions detected were consistent with the occurrence of a traumatic event, compatible with social interspecific behaviour, considered to be the leading cause of death due to the severity of internal injuries.

Spacing between the lacerations was compatible with the interdental space for a single odontocete species in the western Mediterranean Sea, the bottlenose dolphin, the same attacker species reported by Barnett *et al.* (2009) in UK waters and Puig-Lozano *et al.* (2020) in Spain. Some of these scars showed an acute change in directionality, typical of aggressive interactions of bottlenose dolphins on harbour porpoises, as reported by Ross and Wilson, (1996). Most importantly, tooth rakes were present on less than 50% of the body and prevalently on the back of the animal. The posterior position of most of these rakes can be likely explained by chasing behaviours carried out by bottlenose dolphins during the attack.

Furthermore, all the major internal traumatic findings may be attributable to a fatal interspecific aggression**.** The thoracic region, the most affected portion of the body, presented several subcutaneous focal/multifocal haemorrhages and haematomas, consistent with blunt traumas (multidirectional), several vertebral and rib fractures and pulmonary perforation, as in Ross and Wilson, (1996), Barnett *et al.* (2009) and Puig-Lozano *et al.* (2020).

In addition, microscopic findings of mild corticomedullary adrenal haemorrhages and a moderate bronchiolar sphincter contraction, previously reported in association with stressful agonal events or in severely polytraumatized animals (Puig-Lozano *et al.*, 2020), were also evident.

The significance of the systemic infection by *Empedobacter brevis*, an environmental Gram-negative bacillus included within the family *Flavobacteriaceae*, occasionally involved in nosocomial

https://doi.org/10.1017/S0025315423000942 Published online by Cambridge University Press

infections in debilitated and immune-compromised humans (Bokhari *et al*., 2015; Basani and Aepala, 2018) and never reported in marine mammals (Bogomolni *et al*., 2008), is unclear. No associated signs of bacterial disease were indeed observed at macroscopic and microscopic levels. The multiorgan pattern of infection, including the mammary gland, suggests a possible ascendent route of infection, likely representing a secondary/opportunistic infection.

Stomach content was present only in the pyloric part, indicating a non-recent meal, but no additional findings suggested a compromised health status of the animal.

As evidenced by several sources (Grattarola *et al*., 2016, 2019; Giorda *et al*., 2017, 2021; C.Re.Di.Ma., 2016, 2017, 2018, 2019, 2020, 2021, accessed online 17 December 2022) cetaceans in the Pelagos Sanctuary frequently deal with pathogens, which may adversely affect their individual health, sometimes experiencing toxicological stress related to immunotoxic levels of OC pollutants. This individual, instead, did not reveal a compromised health status, although the unhazardous levels of OC pollutants detected could have been influenced by the detoxification mechanism that occurred during the lactation (Aguilar *et al*., 1999; Genov *et al*., 2019).

Because of the entity, positions and numbers of the severe traumatic findings found, we excluded other possible traumatic events involved, such as vessel collision, other predator attacks and fishery interaction (Puig-Lozano *et al*., 2020; Crespo-Picazo *et al*., 2021). Collision, in fact, usually produces unidirectional contusions, haemorrhages and haematomas, with the possibility of multiple, evenly spaced, parallel cuts caused by propellers, located mostly on the dorsal parts of the body (Townsend and Staggs, 2020). In the event of shark attacks, the external wounds are usually deeper, spaced and with a circular shape corresponding to the shark's jaws (Townsend and Staggs, 2020). In case of interaction with fisheries, acute linear marks and wounds are found together with bone fractures but are always associated with direct certain and pathognomonic evidence (i.e. presence of fishing gears on the body, capture myopathy, mutilation) and other consistent findings (i.e. gas bubbles in main vessels, good nutritional status, presence of fresh stomach content) (Ijsseldijk *et al*., 2019; Life DELFI, 2020, accessed online 31 August 2023).

These findings, both external and internal, were consistent with fatal inter-specific aggressive behaviours by bottlenose dolphins, with no obvious concurrence of synergistic agents, representing, to the authors' knowledge, the first case reported in literature within Italian waters, specifically the Ligurian Sea.

Similar aggressive behaviours of bottlenose dolphins towards other cetacean species have been previously reported in other regions, particularly in the UK (Ross and Wilson, 1996; Jepson and Baker, 1998), the Western US waters (Cotter *et al*., 2012) and Northwestern Spain (Methion and Díaz López, 2021). In the Mediterranean Sea, attacks directed on striped dolphins were reported by necropsies in Spain and France (Dhermain, 2020; Crespo-Picazo *et al*., 2021; Gannier, 2021).

In the Ligurian Sea, striped dolphins and bottlenose dolphins are regularly present all year round (Azzellino *et al*., 2008; Notarbartolo-Di-Sciara *et al*., 2008). Moreover, in the western part of the Ligurian Sea, where the necropsied animal was found stranded, a recent increase in the presence of bottlenose dolphins was observed (Ascheri *et al*., 2022). Usually, the two species are found in different habitats in relation to their depths: striped dolphins show a habitat distribution range that includes the continental slope and the pelagic area (Gannier, 2005; Moulins *et al*., 2008; Panigada *et al*., 2008), while bottlenose dolphins are mostly found in coastal waters on the continental platform (Bearzi *et al*., 2009; Gnone *et al*., 2011). Nevertheless, striped dolphins can also be found on the continental platform or in the deep-water canyons close to the shore to feed on neritic preys (Aznar *et al*., 2017). The Ligurian Sea, and more importantly its Western part, presents a particular geomorphology, with a very restricted and narrow continental platform crossed by several submarine canyons (Migeon *et al*., 2011), allowing for the two species' ranges to occasionally overlap.

Both in Spain (Crespo-Picazo *et al*., 2021) and France (Dhermain, 2020; Gannier, 2021), it has been noted that strandings of striped dolphins with injuries referable to bottlenose dolphins' aggressions were mostly located in areas with a very narrow continental platform, similar to the area of this case study, rather than in areas with a larger continental platform, presenting scarcer possibilities of encounter between the two species. The partial overlap in habitats could, in fact, result in territory defence behaviours by bottlenose dolphins and/or the competition for available resources between sympatric species as reported in other cases worldwide (Cotter *et al*., 2012; Methion and Díaz López, 2021).

These episodes are scarcely observed in the wild and rarely evidenced on carcasses during necropsies (e.g. in the Canary Islands 24 out of 540 stranded cetaceans examined in 17 years showed intra-interspecific traumatic lesions (Puig-Lozano *et al*., 2020); in Valencian Community shoreline, Spain 16 cases out of 136 examined in 11 years (Crespo-Picazo *et al*., 2021); in Italy 1 case (the one here reported), out of 394 cetaceans necropsied in 6 years (C.Re.Di.Ma, accessed online 17 December 2022)). As evidenced by Puig-Lozano *et al*. (2020), detailed pathological studies regarding aggressive encounters are lacking, making these reporting even more relevant to create a conventional intra-interspecific interaction diagnosis different from other possible traumatic causes.

The reported case is therefore of great interest as it highlights the existence of these interspecific aggressive interactions in an area where they have not been described before, allowing the tracking of a very peculiar phenomenon and its potential recurrence across time. Consequently, our results expand the known geographic range of interspecific attacks by bottlenose dolphins in the western Mediterranean Sea.

Data availability. The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgments. The authors are grateful to the Department of Prevention, Local Veterinary Services (ASL 1 Imperiese) for their assistance. A big thanks to Caroline O'Connor and Katerina Conrad for the constructive comments to enhance the article.

Author contributions. D. A., E. F. and C. G. coordinated the paper and were responsible of writing the original draft. D. A., C. G., E. d. F. and E. B. participated in the necropsy. L. M. performed the toxicological analysis, A. D. performed the bacteriological analysis, M. G. and L. S. performed the biomolecular analysis, C. F. performed the serological analysis, K. V. performed the histopathological analysis, F. G. performed the neuropathological analysis and F. Ga. analysed the stomach contents, C. G., F. G. and C. C. were responsible of the review and editing. All the authors reviewed and approved the final manuscript.

Financial support. This research was funded by the Italian Ministry of Health (Ricerca Corrente 2019 IZS PLV 05/19).

Competing interest. None.

Ethical standards. Not applicable

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