



Pathological Findings in Cetaceans Sporadically Stranded Along the Chilean Coast

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Chile has one of the largest coastlines in the world with at least 50% of the world cetacean species occurring within its jurisdictional waters. However, little is known regarding the health status and main causes of death in cetaceans off continental Chile. In this report, we summarize the major pathological findings and most likely causes of death of 15 cetaceans stranded along the Chilean coast between 2010 and 2019. Drowning, due to fishing gear entanglement, was the most likely cause of death in 3 Burmeister's porpoises (*Phocoena spinipinnis*), a Risso's dolphin (*Grampus griseus*) and a short-beaked common dolphin (*Delphinus delphis*). Additionally, the 3 Burmeister's porpoises had mild to moderate eosinophilic and histiocytic pneumonia with pulmonary vasculitis associated with the nematode *Pseudalius inflexus*. A fourth Burmeister's porpoise died of drowning after stranding alive at a sandy beach. Two fin whales (*Balaenoptera physalus*) died most likely of trauma associated with large vessel collision. A long-finned pilot whale (*Globicephala melas*) and an Orca (*Orcinus orca*) stranded most likely due to traumatic intra/interspecific interaction with other odontocete although for the pilot whale, osteoporosis with loss of alveolar bone and all teeth could have played a role. For a Strap-toothed beaked whale (*Mesoplodon layardii*), Dwarf sperm whale (*Kogia sima*), Southern right-whale dolphin (*Lissodelphis peronii*), Peale's dolphin (*Lagenorhynchus australis*) and a dusky dolphin (*Lagenorhynchus obscurus*), the cause of stranding could not be determined. This study shows, despite the small number of examined carcasses that in Chile, human related trauma is an important cause of single cetacean stranding events.

Keywords: Burmeister's porpoise, cetaceans, Chile, mortality, pathology, stranding

INTRODUCTION

Marine mammals are sentinels of ocean ecosystems health; therefore, it is crucial to understand and monitor how environmental and human factors impact their well-being (Bossart, 2011; Hazen et al., 2019). However, for most marine mammal species and especially for cetaceans, there is comparatively little knowledge regarding their basic physiological traits, and the response of their

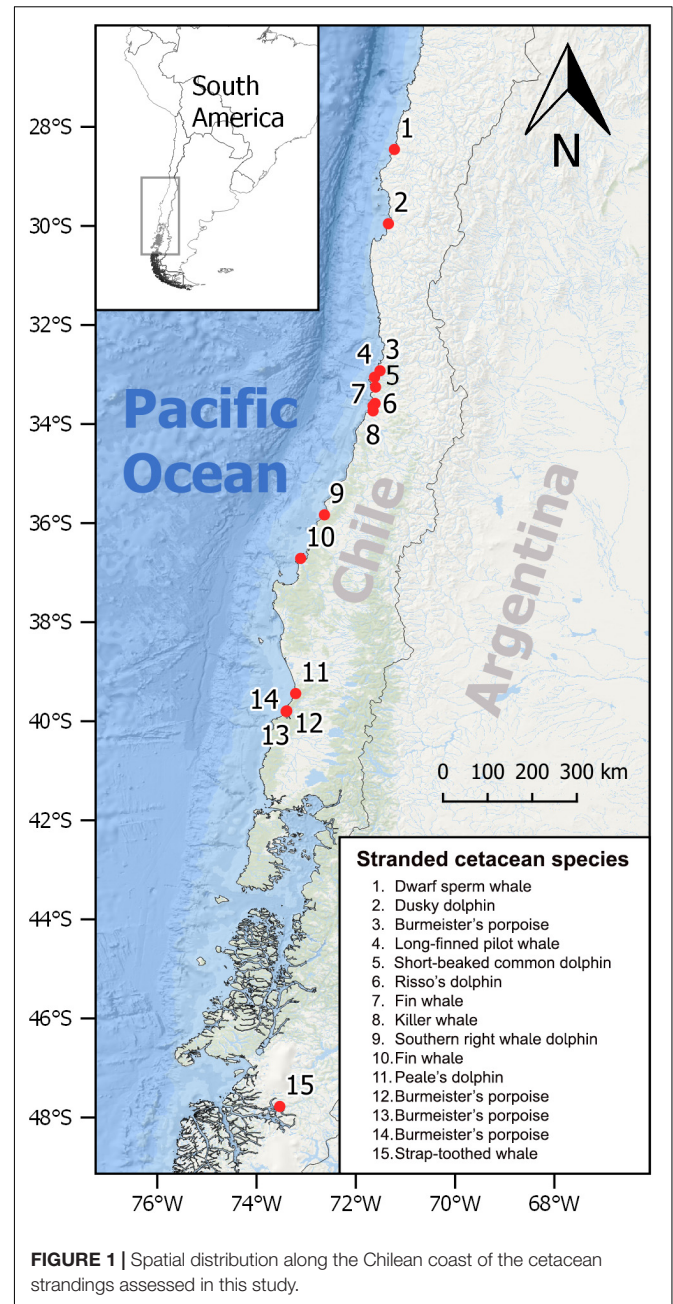
organs and tissues to disease processes. For most cetacean species, single and mass stranding events are one of the few opportunities to examine and collect tissues and isolate disease agents. However, the extraction of relevant biological data from stranded cetaceans is complicated by the deterioration of carcasses with time and the prompt detection, and accessibility to cetaceans when the strandings occur in remote locations (Fretwell et al., 2019; Gómez-Hernández et al., 2020). Overcoming these limitations requires substantial material and human resources that are usually not available in most parts of the world (Alvarado-Rybak et al., 2019; Fretwell et al., 2019).

Chile is one of the countries with the largest coastline in the world (World Resources Institute WRI, 2020), yet it has a small human population disproportionally distributed around its capital (~17.5 million; Instituto Nacional de Estadísticas de Chile INE, 2018). This means that most of its coastline is uninhabited and far from populated areas. Furthermore, in the Austral region, the rugged geography and harsh climate make most of the coastline inaccessible by land and difficult to monitor from sea and air (Häussermann et al., 2017; Fretwell et al., 2019). Through most of Chile's coastline, several cold-water currents and fresh water sources mix in fjords and bays creating some of the most productive marine ecosystems in the world (Buchan and Quiñones, 2016; Viddi et al., 2016). This high marine productivity favors a high abundance of marine vertebrates, including marine mammals. In the territorial waters of Chile, approximately half of the known cetacean species of the world have been identified, and many of them occupy specific areas for breeding, nursing and foraging (Galletti-Vernazzani et al., 2016; Viddi et al., 2016; Seguel and Pavés, 2018). Despite this high abundance of cetacean species, there is limited knowledge about the biology of these populations, the disease processes that affect them and the causes of single stranding events. For instance, two recent cetacean mass stranding events in Chilean Patagonia, were discovered in regions where the presence of these species was undocumented or considered anecdotal (Häussermann et al., 2017; Alvarado-Rybak et al., 2019). These events highlighted the limited baseline pathological knowledge in these populations, complicating a comprehensive investigation on the causes of the mass stranding events (Häussermann et al., 2017; Alvarado-Rybak et al., 2019). In this report, we attempted to partially fill in this knowledge gap and compiled stranding and postmortem examination data of single sporadic stranding events of cetaceans along the Chilean coast. We present the major gross and histologic findings in these animals and discuss the limitations faced to obtain good quality diagnostic samples and the recommended alternatives to improve these outcomes in the future.

MATERIALS AND METHODS

Animals

The cetaceans included in the study were found stranded on beaches of Chile between May 2010 and April 2019 (Figure 1). The animals were found along most of the central-south coast of Chile (from 28° to 48° S, approximately 2,159 km). Some animals stranded alive and subsequently died, while others washed ashore



dead. Only carcasses with decomposition codes 2 to 4 were included in this study (Pugliares-Bonner et al., 2007). Civilian reports and regular monitoring of the coast by collaborating institutions (e.g., National Fisheries Service or Universities) typically initiated veterinary medical deployment followed by field necropsies. Two different age classes (adults and subadults) were distinguished based on maximum length size and life history for each species (Taylor et al., 2007). In addition, in the case of the Risso's dolphin (*Grampus griseus*), the intensity of skin scars was an indicator that it was an adult animal (Hartman et al., 2016). Body condition was assessed based on the level of skeletal muscle and blubber development (Pugliares-Bonner et al., 2007).

Necropsies were performed in all cases following a standard protocol (Pugliares-Bonner et al., 2007). However, gross examination and tissue collection varied depending on degree of autolysis and accessibility to the carcass (**Supplementary Table S1**). In two cases [a fin whale (*Balaenoptera physalus*) (case No 7) and an orca (*Orcinus orca*) (case No 8)] partial necropsies were performed because severe weather and tide conditions precluded complete examination. During complete necropsies, tissue sections were routinely collected from any organ displaying gross lesions, and from lung, respiratory lymph nodes, liver, spleen, kidney, adrenal, thyroid, gonads (testis, ovaries), skeletal muscle, heart, skin, stomach, small and large intestines and mesenteric lymph nodes. Brain was collected in 5 cases. Tissues were fixed in 10% buffered formalin, embedded in paraffin-wax, sectioned and stained with hematoxylin and eosin. The skull of a long-finned pilot whale (*Globicephala melas*) was prepared for osteological examination according to the protocol described by Riquelme et al. (2018).

Ancillary Testing

Sections of lung, bronchial lymph nodes, and spleen from 3 Burmeister's porpoises (*Phocoena spinipinnis*) underwent immunohistochemistry for morbillivirus. Briefly, in deparaffinized tissue sections, antigen retrieval was achieved with citrate treatment for 5 min at 120°C degrees. After hydrogen peroxide treatment, slides were incubated for 1 h at room temperature with a mouse primary antibody against canine morbillivirus, known to cross react with cetacean morbilliviruses (Stone et al., 2011). After incubation with secondary biotinylated antibody (Invitrogen, Carlsbad, CA, United States), antigen-antibody complexes were visualized using horseradish

peroxidase labeled with diaminobenzidine chromogen (DAB, Vector Laboratories, Burlingame, CA, United States). For all assays, negative controls consisted of the same tissue sections undergoing the same immunohistochemistry protocol but the incubation with saline solution instead of primary antibody. Positive controls always yielded marked cytoplasmic positive staining and consisted of lung, spleen and bronchial lymph nodes from bottlenose dolphins (*Tursiops truncatus*) that died due to disseminated morbillivirus infection (University of Georgia, Veterinary Diagnostic Lab, Athens, GA, United States).

In 3 Burmeister's porpoises, metazoan parasites collected during necropsy were placed in 70% ethanol. Nematodes were cleared in lactophenol and mounted on a glass slide for morphological identification to the genus or species level following standard parasitological keys (Delyamure, 1955; Hartwich, 1974; Anderson, 1978). In these animals, fresh tissues or swabs aseptically collected at necropsy underwent aerobic bacteriological culture (37°C, 48 h) using MacConkey and blood agars. Isolated bacterial colonies were identified to the genus or species level by Gram stain and biochemical reactions using a BBL Crystal ID System for enteric/non-fermenting and gram-positive bacteria (BD, Sparks, MD, United States).

RESULTS

In total, we examined 15 stranded cetaceans of 11 different species along the Chilean Coast (**Figure 1**). The 15 animals consisted of 7 males and 6 females, whereas in 2 cases the sex was not recorded. Seven animals were categorized as subadults and 8 as adults (**Table 1**). In 12 cases, the animals were found dead with variable degrees of postmortem autolysis, whereas in 3 cases the

TABLE 1 | Overview of cetaceans sporadically stranded along the Chilean coast and necropsied between May 2010 and April 2019.

ID	Species common name	Scientific name	Date	Loc	Type	Code	NS	Age	Sex	FS	Most likely Cause of Stranding or Death	Lat	Long
1	Dwarf sperm whale	<i>Kogia sima</i>	28/4/2017	Huasco	D	3	4	S	M	N	Undetermined	-28.4	-71.2
2	Dusky dolphin	<i>Lagenorhynchus obscurus</i>	13/7/2017	Coquimbo	D	3	3	A	U	N	Undetermined	-29.9	-71.3
3	Burmeister's porpoise	<i>Phocoena spinipinnis</i>	12/1/2019	Concón	A	2	4	S	F	Y	Drowning	-32.9	-71.5
4	Long-finned pilot whale	<i>Globicephala melas</i>	26/8/2015	Valparaíso	D	3	2	A	F	N	Undetermined	-33.1	-71.6
5	Short-beaked common dolphin	<i>Delphinus delphis</i>	27/1/2015	Quintero	D	2	4	A	F	N	Drowning due to bycatch	-33.2	-71.6
6	Risso's dolphin	<i>Grampus griseus</i>	24/4/2019	San Antonio	D	3	4	S	M	Y	Drowning due to bycatch	-33.5	-71.6
7	Fin whale	<i>Balaenoptera physalus</i>	25/5/2018	Santo Domingo	D	3	4	S	F	U	Trauma (vessel collision)	-33.6	-71.6
8	Orca	<i>Orcinus orca</i>	13/7/2017	El Yali	D	4	3	S	M	Y	Inter/intra specific trauma	-33.7	-71.6
9	Southern right whale dolphin	<i>Lissodelphis peronii</i>	8/6/2015	Pelluhue	A	2	3	A	F	N	Stranding stress	-35.8	-72.6
10	Fin whale	<i>Balaenoptera physalus</i>	25/5/2018	Talcahuano	D	3	4	S	M	U	Trauma (vessel collision)	-36.7	-73.1
11	Peale's dolphin	<i>Lagenorhynchus australis</i>	5/6/2017	Aldachildo	D	3	3	A	U	U	Undetermined	-39.4	-73.2
12	Burmeister's porpoise	<i>Phocoena spinipinnis</i>	5/08/2012	Valdivia	D	3	4	A	F	Y	Drowning due to bycatch	-39.7	-73.3
13	Burmeister's porpoise	<i>Phocoena spinipinnis</i>	5/15/2010	Valdivia	D	3	4	A	M	Y	Drowning due to bycatch	-39.8	-73.4
14	Burmeister's porpoise	<i>Phocoena spinipinnis</i>	5/15/2010	Valdivia	D	3	4	S	M	Y	Drowning due to bycatch	-39.8	-73.4
15	Strap-toothed beaked whale	<i>Mesoplodon layardii</i>	30/3/2019	Tortel cove	A	4	4	A	M	Y	Undetermined	-47.7	-73.5

Provided are the species common name, scientific name, date and location of stranding, type of stranding (D = dead vs. A = alive animal), decomposition stage of the necropsied carcass (Code), age, sex, food in stomach (FS), most likely cause of death, latitude (Lat) and longitude (Long). Strandings are ordered according to latitude, from north to south. For age class and sex, A: adult, S: subadult, M: male, F: female and U: unknown. Decomposition code; 2 = minimal, 3 = mild, 4 = moderate. Nutritional status scale (NS): 1 = emaciated, 2 = poor, 3 = fair, 4 = good, 5 = very good. Food in stomach: Y = yes, N = No, unknown = U.

animals stranded alive and died on the beach during attempts for refloating (Table 1).

Pathological Findings Per Cetacean Species:

Burmeister's Porpoises (*Phocoena spinipinnis*)

One subadult female, Burmeister's porpoise in good body condition died moments after live stranding on a sandy beach in central Chile on January 2019. This animal had hepatomegaly and dark-red, uncollapsed lungs with dark-pink foam admixed with scant amount of sand in the trachea and major bronchi. The stomach contained a few whole small fishes. Microscopically, in the lung, abundant edema admixed with scant cellular debris and hemorrhage occupied bronchioles and alveoli, whereas in the liver, moderate congestion expanded sinusoids and in the skeletal muscle there were hypereosinophilic contraction bands and edema. Throughout 60% of the body surface, we observed multiple 1 to 2 cm in diameter, coalescing, circular, silver lesions with a thin dark-gray rim (tattoo-like lesions) (Figure 2A). Histologically, these lesions were categorized as mild, hyperplastic, lymphoplasmacytic dermatitis with hyperpigmentation and amphophilic intracytoplasmic inclusion bodies (Figure 2B). In the pyloric stomach, there were two small ulcers (<1 cm diameter) and small numbers of nematodes (*Anisakis* spp.) in the lumen. We considered drowning the most likely cause of death, although it is likely this occurred on the beach while the animal was being washed by waves.

Three, Burmeister's porpoises (1 female and 2 males, 2 adults and 1 subadult) were found dead on the Valdivia coast, Southern Chile on May 2010 and May 2012. These animals were good body condition and had fishing line marks in the fluke, dorsal and pectoral fins, and uncollapsed and heavy lungs with abundant pink froth in airways (edema) (Figure 2C). Their stomachs contained a few whole fishes (sardines). Microscopically, in all these animals, abundant edema admixed with scant cellular debris and hemorrhage occupied bronchiole and alveoli (Figure 2D). In the 3 porpoises, in multifocal areas of the lung and affecting up to 30% of the sections examined, there were numerous intrabronchial and intravascular, large (900 μ m diameter) nematodes. Morphological features of these nematodes included a thick cuticle, prominent coelomyarian musculature, prominent lateral bands and gastrointestinal tract with a few multinucleated epithelial cells (Figure 2E). *Pseudalius inflexus* was the only nematode within this size range recovered from the lung of these animals. These nematodes were associated with substantial alteration of the bronchial and vascular morphology, including erosion and necrosis of the epithelium and intima, respectively, with lymphocytes, eosinophils and plasma cells obscuring significant portions of the vascular and bronchial walls (Figure 2E inset). In some areas, chronic, partially recanalized thrombi admixed with numerous eosinophils obliterated the arterial lumen. Additionally, in a few, small, multifocal areas of the lung of two of these porpoises, there were a few cross sections of small (100 μ m in diameter) nematodes. The parasites were characterized by thin cuticle, delicate coelomyarian musculature,

small lateral cords, gastrointestinal tract with few multinucleated epithelial cells with small brush border and reproductive tract with developing larvated eggs (Figure 2F). Surrounding these nematodes, there were variable numbers of macrophages, few eosinophils and cellular debris. Adjacent areas usually contained nodular aggregates of lymphocytes and fewer plasma cells (Figure 2C). The only parasite recovered from lung matching this size and description was *Stenurus australis*. Additional metazoan parasites recovered from these 3 porpoises included *Polymorphus cetaceum*, which was present in large numbers in the third stomach and duodenal ampulla and *Stenurus australis*, which was in high numbers in the periotic sinuses and middle ear. No histological changes were associated with these infections, although the tympanoperiotic complexes were not processed for histopathology. No morbillivirus antigen was detected in the lung and spleen of the 3 porpoises assessed. Given the fresh fishing line marks, pulmonary edema, fresh fish in stomachs and lack of major health issues, we considered drowning due to entanglement the most likely cause of death of these 3 porpoises.

Fin Whales

Two subadult fin whales (one male and one female), in good body condition stranded dead on the central-south coast of Chile in 2018 and 2019 at Talcahuano and Santo Domingo, respectively. One whale (case No 10) had blunt trauma to the left flipper and thorax with fracture of the radius and ulna. This animal also had multiple, parallel, skin, blubber and muscle lacerations on the right abdominal, pelvic and peduncle regions associated with hemorrhage in the surrounding tissues (Supplementary Figure S1). The other whale (case No 7), had significant blunt trauma over the left flipper (1.5 \times 1.5 m) with hemorrhage and loss of the subcutaneous and muscle tissue, and abundant hemorrhage in the subcutis of the left thoracic region. Additionally, there was a 2 m laceration in the right lumbar area with abundant hemorrhage in the surrounding blubber and muscle. Histological examination of both whales tissues was unremarkable although most tissues had moderate to advanced autolysis. We considered these findings consistent with large vessel collision.

Southern Right Whale Dolphin (*Lissodelphis peronii*)

An adult, female Southern right whale dolphin in good body condition was found stranded alive on June 2015 in Pelluhue, central coast of Chile, after a severe storm. The individual was transported to a rehabilitation center, where it died 27 h after being admitted. Externally, there were multiple lacerations (approximately 2 \times 6 cm and 0.5 cm deep) between the caudal peduncle, the abdominal wall, and the left pectoral fin. The lacerations penetrated into the subcutis and blubber and were accompanied by hemorrhage. In the lung, abundant edema fluid filled alveoli and small numbers of lymphocytes, macrophages and neutrophils slightly expanded the alveolar septa. In the *longissimus dorsi* muscle, random myocytes were hypereosinophilic, with loss of cross striations and rare hypereosinophilic contraction bands. Similar changes were observed in the myocardium. Additionally, in the skeletal muscle there were occasional, 60 μ m

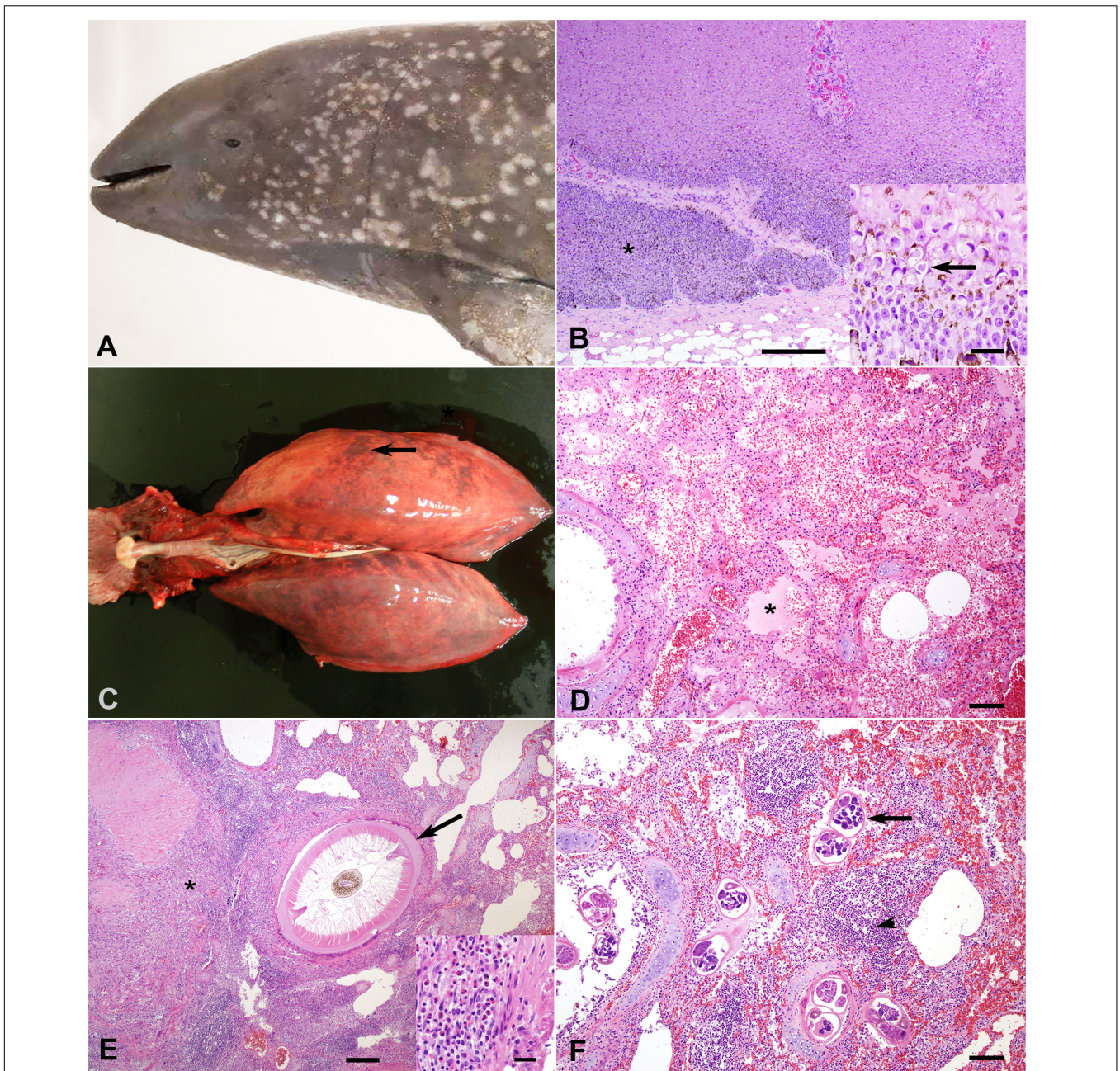


FIGURE 2 | Postmortem findings in Burmeister's porpoises (*Phocoena spinnipinis*). **(A)** Multifocal to coalescing tan, circular lesions throughout the skin (tattoo skin lesions) of a subadult porpoise. **(B)** Histologically, the skin lesions showed in **(A)** were characterized by stratum basale hyperplasia with hyperpigmentation (asterisk) and vacuolar degeneration of lipokeratinocytes with rare amphophilic intracytoplasmic inclusion bodies (arrow inset). H&E, scale bar = 200 μ m, inset scale bar = 25 μ m. **(C)** Lung of an adult Burmeister's porpoise that died most likely due to drowning. Note the uncollapsed lung with mild rib impressions (edema) and multifocal areas of hemorrhage (arrow). **(D)** Histological section of the lung showed in **(C)**. Alveoli contain abundant eosinophilic homogeneous material (edema, asterisk) and numerous erythrocytes. H&E, scale bar = 150 μ m. **(E)** Verminous, eosinophilic bronchopneumonia and vasculitis. A large nematode (most likely *Pseudalius inflexus*) (arrow) obliterates a bronchus and numerous eosinophils (inset) obscure peribronchiolar tissues. Adjacent to the nematode section, eosinophilic inflammatory infiltrate obscure a thrombosed large artery (asterisk). H&E, scale bar = 200 μ m, inset scale bar = 30 μ m. **(F)** Chronic verminous pneumonia. There are several sections of small metastrongyle nematodes (arrow) associated with lymphoplasmacytic inflammatory infiltrates (arrow head). H&E, scale bar = 100 μ m.

in diameter, *Sarcocystis* spp. -like cysts filled with 3 μ m long bradyzoites, and small numbers of lymphocytes and plasma cells surrounded blood vessels. In the kidney proximal tubules, moderate amount of eosinophilic, homogeneous material

occupied the lumen and small, discrete vacuoles enlarged the cytoplasm of the tubuloepithelial cells. We considered stranding stress (capture myopathy) the most likely cause of death of this animal.

Long-Finned Pilot Whale (*Globicephala melas*)

An adult female, long-finned pilot whale was found dead on June 2015 near Los Molles on the central coast of Chile. The animal was in poor body condition and had multiple parallel lacerations (rake marks, 10 mm apart) throughout 70% of the body surface with mild to moderate hemorrhage in the surrounding blubber. In the lung, there was abundant edema, and in the oral cavity there was loss of all teeth (**Figure 3A**). Subsequently, the animal's skeleton was analyzed. The skull was light, and in the maxillary and mandibular area of the skull, and in ribs and vertebrae, bone trabeculae were thin, increasing the porosity of cancellous bone (osteoporosis) (**Figure 3B**). This was particularly severe in the alveolar bone with almost complete loss of some alveolar processes (**Figure 3C**). Marked postmortem autolysis (due to inadequate sample fixation) precluded detailed histological assessment. We considered negative inter/intraspecific interactions as the most likely cause of stranding, although osteoporosis, dental loss and poor body condition could have played a significant role.

Short-Beaked Common Dolphin (*Delphinus delphis*)

An adult, female short-beaked common dolphin, stranded dead on January 2015 in Quintero, central Chile. Externally, the animal had a good body condition and surrounding the dorsal fin, there were several, linear, 3 mm width, skin depressions and lacerations associated with mild hemorrhage (fishing line marks). Internally, the animal had uncollapsed, heavy lungs with abundant pink froth in airways and small foci of ecchymosis in the right anterior pulmonary lobe. The animal also had a pale liver, splenomegaly, and sand and anisakis nematodes in the stomach. We considered drowning due to entanglement the most likely cause of death of this animal.

Risso's Dolphin (*Grampus griseus*)

A subadult, male Risso's dolphin, stranded dead in San Antonio, central Chile on April 2019. The animal was in good body condition and externally, over the abdominal wall, there was

one skin laceration (6 cm long) associated with hemorrhage, and a subcutaneous hematoma (8 cm in diameter). Additionally, there were several 5 mm linear (net) marks on flippers, fluke and dorsal fin. Internally, the animal had hepatomegaly and uncollapsed, heavy lungs with abundant pink froth in airways. The stomach contained several squid beaks and partially digested squid flesh. Microscopically, abundant edema fluid admixed with rare erythrocytes, neutrophils, lymphocytes and plasma cells filled pulmonary alveoli. In the liver, numerous erythrocytes expanded the sinusoids. In the myocardium and *longissimus dorsi* muscles, there were occasional *Sarcocystis* spp.-like cysts. We considered drowning due to entanglement the most likely cause of death of this animal.

Orca

A subadult, male orca stranded dead near to San Antonio port, central Chile on July 2017. The animal was in fair body condition and had multiple parallel lacerations (rake marks, ~20 mm apart) associated with mild hemorrhage of the subcutis (**Supplementary Figure S2**). The stomach contained scant amount of partially digested fish and some otoliths. Histological evaluation was unremarkable although all tissues had moderate to advanced autolysis. We considered traumatic intra/interspecific interaction the most likely cause of stranding.

Dusky Dolphin (*Lagenorhynchus obscurus*)

An adult dusky dolphin was found dead on July 2017 in Tongoy, south of Coquimbo. In the lung, a few neutrophils, lymphocytes and plasma cells admixed with homogeneous eosinophilic material occupied alveolar sacs. In the subcutis, numerous neutrophils, histiocytes, lymphocytes and multinucleated giant cells, admixed with occasional gram-positive cocci surrounded a *Phyllobotrium* spp. parasitic cyst. In the kidney, occasional glomerular tufts were sclerotic and in proximal tubules, small and discrete vacuoles enlarged the cytoplasm of the epithelial cells. In the heart, there were occasional *Sarcocystis* spp.-like cysts (**Supplementary Figure S3**). We did not identify a most likely cause of death for this animal.

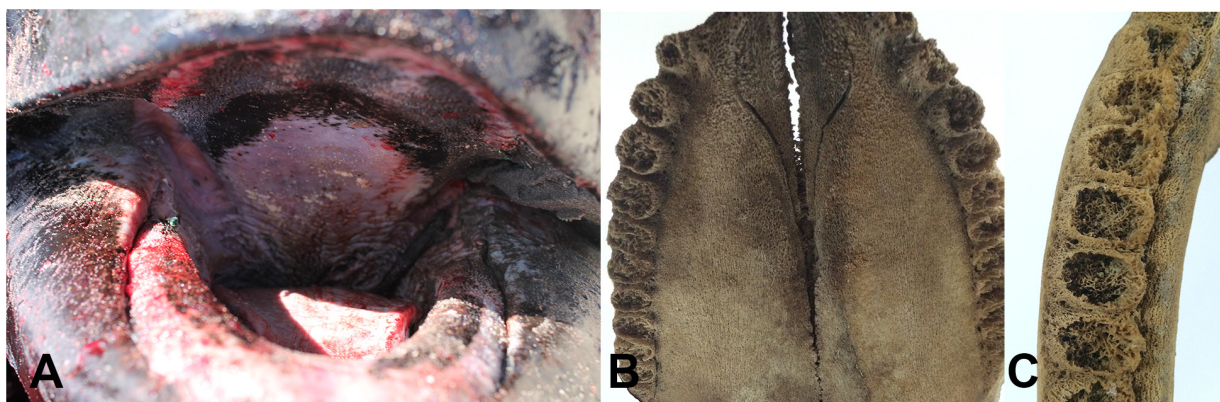


FIGURE 3 | Adult long-finned pilot whale (*Globicephala melas*) with complete edentulism and osteoporosis. **(A)** Oral cavity with lack of mandibular and maxillary teeth. **(B)** Dorsal view of the mandibular branch. **(C)** Closer view of the maxillary alveolar processes. Note the increased bone porosity with thin and irregular bone trabecules.

Peale's Dolphin (*Lagenorhynchus australis*)

An adult Peale's dolphin in good body condition was found dead on June 2017 in Aldachildo, Los Lagos. There were no major gross or histological changes in the examined tissues although advanced postmortem autolysis precluded detailed assessment.

Dwarf Sperm Whale (*Kogia sima*)

A subadult, male, dwarf sperm whale in good body condition stranded dead on April 2017 in Huasco, northern Chile. Over the thorax, there was one, 4 cm in diameter, circular laceration. In the subcutis of the caudal peduncle, there were several *Phyllobothrium* spp. cysts. There were numerous anisakis nematodes in the stomach lumen and metastrongyle nematodes in the lungs. Microscopically, abundant edema fluid filled the lung alveoli. In the kidney, there was a small focus of lymphoplasmacytic inflammation in the renal cortex. We did not identify a most likely cause of death in this animal.

Strap-Toothed Beaked Whale (*Mesoplodon layardii*)

An adult, male Strap-toothed beaked whale stranded alive on March 2019 near Tortel Cove, southern Chile. The distressed individual was transported to deeper waters, but the animal repeatedly returned to the beach until finally stranded dead (Español-Jiménez et al. unpublished data). The animal was transported (almost 2,300 km) to a location suitable to perform a necropsy. On external examination, there were several 20 cm to 45 cm lacerations in middle-caudal area of the body, ventral to left flipper and in the cervical area. The stomach contained several squid beaks and small amount of partially digested fish. We found a subcutaneous hematoma (20 x 5 cm) on the dorsum and *Phyllobothrium* spp. cysts. in the caudal peduncle. Around the left eye, we observed four tooth scars possibly associated with cookiecutter shark (*Isistius* sp.) bites. Internally, next to the bladder, there were two, small (2 cm in diameter), abscesses (likely inflamed *Monorygma* spp. cysts). Histological examination was unremarkable although most tissues had advanced autolysis. A definitive cause of death could not be determined.

DISCUSSION

This report is the first attempt to compile the main postmortem findings of single cetacean stranding events in Chile. However, the number of cases reported, correspond to only a small portion of the cetacean strandings registered in Chile during the study period. Between January 2010 and December 2019, a total of 291 cetacean stranding events, affecting 984 individuals, were recorded along the Chilean coast (Alvarado-Rybak et al., 2020). Ninety four percent ($n = 275$) of these corresponded to single stranding events (\leq two individuals), ten (3.4%) were mass stranding events (three to 24 individuals) and six (2%) corresponded to unusual large mass stranding event (>25 individuals) (Alvarado-Rybak et al., 2020). The reasons for the small number of cases included in this compilation include a lack of prompt detection of fresh carcasses, and in several cases, the lack of a coordinated response to perform necropsies. Additionally, the necropsies and diagnostics performed in

each case were complicated by several logistical challenges, including access to the large and rugged Chilean coast and transportation of supplies and personnel. This, in several cases, undermined a thorough examination and compromised the quality of the diagnostic material obtained. Despite these difficulties, we determined that an important proportion of the examined cetaceans (7/15, 47%) died most likely due to anthropogenic trauma.

The most common form of anthropogenic trauma in small cetaceans is drowning due to bycatch (Reeves et al., 2013). We found something similar in this study with most small and medium size cetacean species affected by fishing net entanglement and drowning. The diagnosis of this condition is difficult in carcasses with moderate to advanced autolysis, therefore, the real magnitude of this problem is hard to assess when access to fresh stranded carcasses is limited. However, an on-site study performed in the south-central and southern Chile highlighted that the species most commonly bycaught are Commerson's dolphin (*Cephalorhynchus commersonii*), Chilean dolphin (*C. eutropia*), bottlenose dolphin and Burmeister's porpoise (González-But and Sepúlveda, 2016).

We observed two subadult fin whales with severe blunt trauma, most likely due to collision with a large vessel. These animals were found close to two ports with high maritime traffic, which could have increased the probabilities of ship strike. Fin whales are among the species most commonly affected by ship strikes (Laist et al., 2001; Panigada et al., 2006; Redfern et al., 2013). Although the reasons for this higher susceptibility to ship strikes is unknown, a possible contributing factor is their surface foraging behavior with short feeding dives and long recovery periods on the surface (Goldbogen et al., 2006). Additionally, younger fin whales tend to spend more time on the surface, which could make them more susceptible to ship(s) encounters (Laist et al., 2001). Additionally, the impact of environmental sounds produced by vessels can induce behavioral changes in large whales making them more susceptible to boat strikes (Blair et al., 2016).

We observed a few important disease processes in some odontocetes, including verminous pneumonia and vasculitis due to *P. inflexus* in Burmeister's porpoises. This nematode is a well-known parasite of porpoises worldwide, and it can cause direct mortality or lead to secondary bacterial pneumonia and death (Siebert et al., 2001). In our case series, although the lesions observed were significant, is hard to determine if they could have complicated escape from entanglement or increase entanglement risk in these animals. The pathogenic potential of the other metazoan parasites found on Burmeister's porpoises is unknown. However, respiratory and periotic sinuses nematodes within the *Stenurus* genus have been suggested to potentially impair earing and echolocation (discussed in Geraci and St. Aubin, 1987), although the evidence is mostly circumstantial and in most cases these can be found in large numbers without major evidence of tissue damage (Geraci and St. Aubin, 1987; Wohlsein et al., 2019). In our cases, because of the lack of histopathologic examination of the periotic complex we could not assess the real impact of *Stenurus* sp. parasites on Burmeister's porpoises.

The macroscopic aspect of the skin lesions in one Burmestair's porpoise, characterized by clear internal area, and stippled aspect surrounded by dark margins, is compatible with an advanced tattoo lesion (Geraci et al., 1979). Histopathological findings were consistent with previous description of Cetacean poxvirus-like lesions (CePV; Sacristán et al., 2018) or tattoo-skin disease (Van Bressem et al., 2009), although the role of other viral pathogens such as herpesvirus and papillomavirus cannot be ruled out. Unfortunately, we could not assess the etiology of these tattoo lesions through molecular analyses, but regardless of their origin, it is known that these type of skin lesions can be an indicator of poor health status. Tattoo-skin lesions have been associated with poor health in adult short-beaked common dolphins, harbor porpoises (*Phocoena phocoena*), and Burmeister's porpoise juveniles (Van Bressem et al., 2009). Additionally, in the Pacific coast of South America, cases of tattoo-skin lesions have been described based on visual assessment in long-beaked common dolphin (*Delphinus capensis*), dusky dolphin, Chilean dolphin, Peale's dolphin and bottlenose dolphin, highlighting the wide range of small cetacean species affected by this condition in the South Pacific (Van Bressem et al., 1993, 2007).

The lesions observed in the skeletal muscle of the Southern right whale dolphin have been described in cetaceans with stranding myopathy ("capture myopathy") (Sierra et al., 2017). The pathophysiology of this stress myopathy in cetaceans is thought to be similar to the capture myopathy syndrome described in terrestrial wildlife species and is one of the main causes of the poor rehabilitation outcome of cetaceans receiving veterinary care after a live stranding event (Herráez et al., 2013). Another interesting finding in striated muscles was the presence of *Sarcocystis* spp. -like cysts in 3 different dolphin species, which indicates that some odontocetes could act as intermediate host for these protozoa (Ewing et al., 2002). The parasites within the genus *Sarcocystis* require at least two hosts for their life cycle. It is possible that the definitive host could be a species that prey on dolphins (De Guise et al., 1993), such as the sleepy shark (*Somniosus pacificus*) (Heithaus, 2001) or the orca (Pitman and Ensor, 2003). The intermediate stages (plerocercoids) of two cestode groups, *Phyllobothrium* sp. found in the subcutis in *K. sima* and *L. obscurus* and *Monorygma* sp. next to the urinary bladder in *M. layardii*, were identified in this study. Both cestodes have been commonly reported in dolphins and beaked whales (Gibson et al., 1998; Fernández et al., 2018), while large sharks are the likely definitive hosts (Aznar et al., 2007).

Bone lesions such as osteolysis, osteomyelitis and periostitis commonly affect the skull (Pascual et al., 2000), vertebral bones and ribs of cetaceans (San Martín et al., 2016). However, skull osteoporosis in odontocetes, as what we described in the long-finned pilot whale is rare. In this species, there is only one record of ankylosing spondylitis and osteoporosis of the vertebral bodies in individuals of three mass stranding events (Sweeny et al., 2005). In humans and domestic animals, metabolic bone disease can lead to osteoporosis and predispose individuals to alveolar bone loss and subsequent loss of teeth (edentulism) (Streckfus et al., 1997; Johnson et al., 2002). An alternative explanation for the lack of teeth in this animal is congenital anodontia, however, the level of development of some alveolar processes

and alveoli, suggest that the animal had teeth during its life. Additionally, the poor body condition of the animal suggests the edentulism could have led to problems capturing and/or ingesting prey. Finally, although we cannot directly associate osteoporosis and edentulism with the stranding, these could have acted as predisposing factors, since the animal appeared to have had negative interactions with other odontocetes.

This study provides insights into the pathological findings and causes of death of a small subset of cetaceans stranded along the Chilean coast. Long-term health monitoring of cetaceans includes difficult, complex and demanding research and logistics, especially in remote areas (e.g., fjords and islands). These factors influenced the low number of cases compiled in this report, despite the fact that cetacean strandings in Chile have been steadily increasing over the last two decades (Alvarado-Rybak et al., 2020). However, we believe the acquisition of more and better diagnostic material from stranding events is possible if the current stranding response policies are improved. For instance, given the particular Chilean geography, the building of technical and human resources to respond to cetacean stranding events in the different provinces will improve the time to response and the quality of the postmortem assessments. Under this scenario, the role of a centralized unit dealing with marine mammal stranding should be to standardize protocols, coordinate response and curate data.

Given the large biodiversity of cetacean species in Chile, and the endangered or unknown conservation status of many of these species, is urgent to improve and standardize the response to stranding events in this territory. This is critical, considering that most of the cetacean deaths in this study were associated to anthropogenic activities. Although stranding investigations have biases and limitations, they are a first approximation on the challenges faced by different cetacean species. Our work here stresses the need for additional efforts to examine the potential impact of disease processes on the health of these cetacean populations and highlights anthropogenic trauma as an important cause of cetacean mortality in Chile.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for this animal study, in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

MA-R and MS conceived the research idea, collected and analyzed data, and wrote the manuscript. FT, PA, EP, and SE-J

collected data. All authors edited several manuscript drafts and gave approval to the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.00684/full#supplementary-material>

FIGURE S1 | Lesions in Fin whale (*Balaenoptera physalus*) stranded in Santo Domingo due to large vessel trauma. There are several transversal hematomas in the peduncle, most likely caused by a large vessel propellers.

FIGURE S2 | A stranded Orca (*Orcinus orca*). (A) Multiple parallel skin lacerations (rake marks) throughout the body. (B) Details of rake marks (~20 mm apart) over the melon.

FIGURE S3 | Micrograph of the heart of a Dusky dolphin (*Lagenorhynchus obscurus*). There is *Sarcocystis* spp-like cyst within a cardiomyocyte.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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