

TESIS DOCTORAL

**TRAUMATIC PATHOLOGIES IN
STRANDED CETACEANS,
CANARY ISLANDS**



RAQUEL PATRICIA PUIG LOZANO

DOCTORADO EN SANIDAD ANIMAL Y SEGURIDAD
ALIMENTARIA

LAS PALMAS DE GRAN CANARIA

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INFORMA,

Que la Comisión Académica del Programa de Doctorado, en su sesión de fecha / / tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada **“Traumatic pathologies in stranded cetaceans, Canary Islands”** presentada por la doctoranda **D^a Raquel Patricia Puig Lozano** y dirigida por el **Doctor Miguel Antonio Rivero Santana y el Doctor Manuel Antonio Arbelo Hernández.**

Y para que así conste, y a efectos de lo previsto en el Art^o 11 del Reglamento de Estudios de Doctorado (BOULPGC 7/10/2016) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 25 de febrero de dos mil veintiuno.

Acaba de terminar una etapa de mi vida
y ha llegado el momento de hacer balance . . .
Lo cierto es que tú nunca me has fallado, jamás.
No has dejado que me rindiera, que abandonara mi sueño,
y me has visto luchar por él con sudor y lágrimas.
Siempre me has regalado tu mejor versión.
Me has hecho sentir como en casa teniendo a mi familia lejos.
Me has puesto en el camino de personas maravillosas con las que crecer.
Me has consentido explorarte sin reparos y rebosante de vida,
desde las profundidades del océano hasta tu punto más alto.
Me has permitido perderme en tu naturaleza salvaje
de la manera más intensa posible,
y he sido tremendamente feliz.
Por eso te mereces, mi querida amiga
ser la única protagonista en recibir el fruto de mi esfuerzo.
Porque a la vez que buscaba en ti respuestas,
me encontraba a mí misma.

A mi paraíso,

Gran Canaria

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Introduction



Introduction

1. Canary Islands

1.1. Oceanographic features

The Canary Islands are located in the Macaronesia geographical area, which also includes Azores, Madeira, Savage Islands, and Cape Verde. This archipelago is located between the parallels 27°37'-29°25' N and the meridians 13°20'-18°10' W, 55 miles away from the north-west coast of Africa. These islands extend over more than 500 km and include eight main islands, from East to West: La Graciosa, Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma, and El Hierro.

This archipelago has a volcanic origin and scarce continental platform, presenting great depths close to the coasts, increasing from 1,200 m to the west of Lanzarote and Fuerteventura to 4,000 m in La Palma and El Hierro. Some islands presented periods of volcanic activity alternated with phases of rest and intense erosion, while others, for example, La Palma and El Hierro remained in almost continuous activity (Agudo-Bravo & Mangas 2015).

Four uninhabited islets, Montaña Clara, Alegranza, Roque del Este, and Roque del Oeste, with the island of La Graciosa, constitute the Chinijo archipelago, and, together with the islet of Lobos, and other small rocks are used to delimit the internal waters of the archipelago (Figure 1).

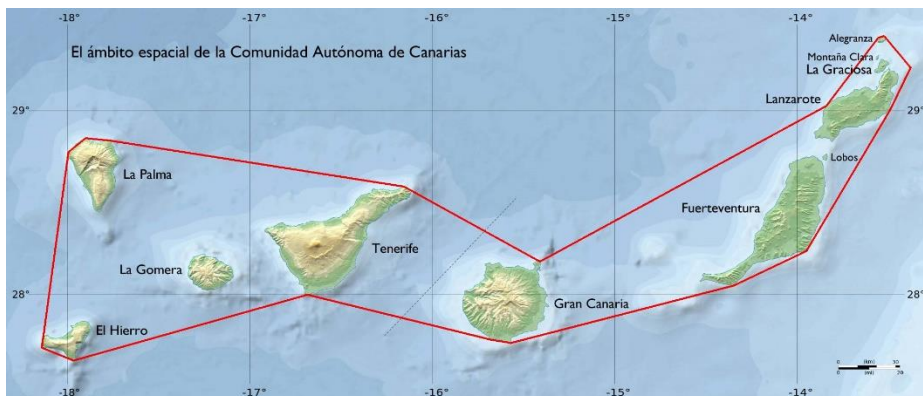


Figure 1. Blank topographic map of the Canary Islands (De Koppchen 2018).

The Canary Islands are a fragmented and high populated archipelago (2,175,952 inhabitants)¹, being the largest and most populated archipelago of Macaronesia. Most population is concentrated in the two capital islands, Tenerife and Gran Canaria. This archipelago is a tourist destination for over 13 million visitors per year², especially Tenerife, Gran Canaria, Fuerteventura, and Lanzarote, attracted by their subtropical climate all over the year. In recent years, more eco-sustainable tourism is being developed, due to the high biodiversity of the archipelago. These islands hold four National Parks, two of them declared Human Patrimony by the UNESCO and three, Biosphere Global Reserves. In the canary waters, whale and dolphin watching is a well-developed touristic activity, with local authorized businesses on every island and specific legislation [Decreto 178/2000, de 6 de septiembre]. Recently, Tenerife-La Gomera Marine Area became a Certified Whale Heritage Site. Tenerife has the largest and most famous whale watching industry in the world, with €42 million revenue annually from 1.4 million tourists. Whale Heritage Site is a global accreditation that recognises outstanding destinations for responsible and sustainable activity³.

1.2. *Marine biodiversity and preservation of the Canary waters*

The Canary waters are a hotspot for biodiversity due to its oceanographic features. The high productivity in this archipelago is explained by constant warm temperatures, the trade winds, and the wind-driven surface current called the Canary Current, which comes from the North Atlantic Gyre. Furthermore, the Canary Islands are involved in one of the four major upwelling systems of the world, the Canary Current Large Marine Ecosystem (CCLME), which extends from the Strait of Gibraltar (around 36°N 5°W) to the Bissagos Islands in the South of Guinea-Bissau (around 11°N 16°W). CCLME is related to the abundance of cephalopods on Africa's West coast (Rocha & Cheikh 2015). Other factors that contribute to the abundance of cetaceans are the calm waters of the southwestern parts of the islands, due to the island-mass effect, and the narrow oceanic platform of the islands that implies the presence of deep waters close to the coast.

The Canary Islands were declared World Biosphere Reserve by the UNESCO and have several protected areas⁴, both terrestrial and maritime. Additionally, the Canary Islands were recognized as a Particularly Sensitive Sea Area (PSSA) by the International Maritime Organization (IMO) in 2005, as their biodiversity requires special protection against international maritime activities.

A framework for community action in marine environmental policy was established by the Directive 2008/56/EC of June 17th of the European Parliament and the Council. In this Directive, the Canary waters were included under the sovereignty and jurisdiction of the

Member States of the European Union. For that reason, Spain is responsible for applying coordinated programs to monitor the conservation status of the Canary waters. Some indicators were designated to reach or maintain a good environmental status. One of them is the study of cetacean's species and its threats establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive)⁵. Finally, due to the presence of the Atlantic bottlenose dolphin (*Tursiops truncatus*) and the loggerhead turtle (*Caretta caretta*), species listed in Annexes II and IV of the European Commission Habitats Directive (Council Directive 92/43/EEC), 24 Special Areas of Conservation (SACs) have been designated in the Canary Islands [Órden ARM/2417/2011 de 30 de agosto]⁶.

Regarding this, broad protective fishing legislation of the waters exists in this archipelago. Fisheries are mostly artisanal and multi-specific, characterized by be multispecific and using small vessels (less than or equal to 15 meters in total length). Also, there is a small fleet formed by larger vessels with tuna as target species⁷. Trawls are forbidden; meanwhile, gillnets are allowed in specific periods in some designated areas. In marine reserves and the islands of El Hierro, Lanzarote, and Fuerteventura, some fishing gears as longline and traps are forbidden [Decreto 182/2004, de 21 de diciembre. Anexo I]. The competence for the regulation of fishing activities in internal waters falls on the Government of the Canary Islands [Ley 11/2003, de 10 de abril]. When the activity is carried out in territorial waters, the competence falls on the Government of Spain [Ley 3/2001, de 26 de marzo]. With the aim of reduce cetacean's bycatch, the Spanish Government has recently approved specific legislation for fisheries [Orden APA/1200/2020, de 16 de diciembre].

2. Cetaceans species in the Canary waters

Concerning cetaceans species, the Canary waters holds the greatest cetacean biodiversity in Europe with up to 30 species described (Biocan - Banco del Inventario Natural de Canarias⁸), 7 mysticetes and 23 odontocetes: Atlantic bottlenose dolphin, Atlantic spotted dolphin (*Stenella frontalis*), Blainville's beaked whale (*Mesoplodon densirostris*), blue whale (*Balaenoptera musculus*), Bryde's whale (*Balaenoptera edeni*), Cuvier's beaked whale (*Ziphius cavirostris*), dwarf sperm whale (*Kogia sima*), false killer whale (*Pseudorca crassidens*), fin whale (*Balaenoptera physalus*), Fraser's dolphin (*Lagenodelphis hosei*), Gervais' beaked whale (*Mesoplodon europaeus*), harbor porpoise (*Phocoena phocoena*), humpback whale (*Megaptera novaeangliae*), killer whale (*Orcinus orca*), long-finned pilot whale (*Globicephala melas*), minke whale (*Balaenoptera acutorostrata*), North Atlantic right whale (*Eubalaena glacialis*), northern bottlenose whale

(*Hyperoodon ampullatus*), pygmy killer whale (*Feresa attenuata*), pygmy sperm whale (*Kogia breviceps*), Risso's dolphin (*Grampus griseus*), rough-toothed dolphin (*Steno bredanensis*), sei whale (*Balaenoptera borealis*), short-beaked common dolphin (*Delphinus delphis*), short-finned pilot whale (*Globicephala macrorhynchus*), Sowerby's beaked whale (*Mesoplodon bidens*), sperm whale (*Physeter macrocephalus*), spinner dolphin (*Stenella longirostris*), striped dolphin (*Stenella coeruleoalba*), and True's beaked whale (*Mesoplodon mirus*).

With different assiduity, some species are sighted all year round: Atlantic bottlenose dolphin, Atlantic spotted dolphin, Blainville's beaked whale, Bryde's whale, Cuvier's beaked whale, Gervais' beaked whale, pygmy sperm whale, Risso's dolphin, rough-toothed dolphin short-finned pilot whale, sperm whale, and striped dolphin. Of them, some populations of Atlantic bottlenose dolphins and short-finned pilot whales are considered residents. Other species are sighted seasonally, like the short-beaked common dolphin (personal communication, Canary Islands Stranding Network).

The International Union for Conservation of Nature (IUCN) classified the blue whale, the fin whale, the North Atlantic right whale, and the sei whale as endangered. The Habitats Directive, Annexes II and IV recognized every cetacean species as "species with community interest". Specifically, the Atlantic bottlenose dolphin and the harbor porpoise were declared as special interest species. Furthermore, cetaceans are protected by the Spanish Government (Real Decreto 556/2011, de 20 de abril; Real Decreto 1727/2007, de 21 de diciembre; Ley 42/2007, de 13 de diciembre) and specifically by the Canary Islands Regional Government (Decreto 20/2014, de 20 de marzo).

3. Pathological studies on stranded cetaceans

Cetaceans have long life spans, feed at a high trophic level, and depots anthropogenic pollutants in their fat stores. In addition, some species have long-term resident habits. Thus, they are exposed to the same environmental stressors that human populations (chemical pollutants, harmful algal biotoxins, and emerging or resurging pathogens). For these reasons, cetaceans – as other marine mammals- are considered sentinel species for oceans' health. In this way, "one health, one medicine" means that cetaceans' health directly correlated with human and global health (Bossart 2011).

Fishing interactions, ship strikes, foreign body ingestion, and military maneuvers, among others, are direct anthropic causes of cetacean strandings and death. At the same time, other anthropic threatens such as chemical pollution, noise, and disruption of the habitat due to human activities (ocean natural resources exploration

and exploitation, maritime traffic, etc.), the exploitation of living marine resources (overfishing), and other environmental stressors consequence of global warming contribute to decreasing cetacean welfare and health. Long-term effects of the detrimental health of aquatic ecosystems concern and its consequences in animal health are undetermined. Chronic stressing consequences of anthropic threats may affect the immune response of individuals versus different natural pathogens. In recent years, Morbillivirus epizootics caused high mortality rates in cetaceans. In some affected cetacean populations, high contaminant burdens, such as PCBs, were detected (Aguilar & Borrell 1994). In addition, direct contact with wildlife population may contribute to the spread of some infectious agents, some of them zoonotic (Bossart 2011). Emerging diseases in cetaceans are remarkable and concerning due to their epizootic potential, zoonotic implications and complex pathogenesis (Bossart 2011). Marine pollution has been related to neoplasms and detrimental reproductive rates in cetaceans worldwide, especially in Saint Lawrence Estuary (Martineau et al. 1999). Cetaceans populations not only face natural infectious and parasitic agents present currently in their environment. They must face other pathogens that come from land (such as *Toxoplasma gondii*) (from industrial, urban or agricultural sources) or from illegal dumping into the ocean (commercial, fishing, and otherwise ships). Furthermore, some pathogens can be transported by marine currents on anthropic floating items/marine debris (such as microplastics) or from other aquatic species. Thus, the barrier between anthropic and natural disease in free-ranging cetaceans is poorly delimited.

In the Canary coasts, the three most stranded species are the Atlantic spotted dolphin, the short-beaked common dolphin, and the striped dolphin (Arbelo et al. 2013, Díaz-Delgado et al. 2018). These species are regularly sighted in the archipelago. Recently, the number of cetaceans stranded has increased from 39-45.7 year⁻¹ to 50-60 year⁻¹. In 2018 this number raised to 68, reaching 104 animals in 2019, although no explanation has been found for that increase yet.

The Atlantic Center for Cetaceans Research (ACCR) has been monitoring the health of free-ranging cetaceans stranded in the Canaries for more than twenty years. The first necropsy was carried out in 1992. The development of a stranding network in the archipelago allowed systematic studies on stranded cetaceans from 1999 in advance. Although, advanced decomposition code or logistic impairments impede the necropsy of every stranded cetacean, almost eight hundred cetaceans have been studied to date. As in many free-ranging animals, clinic history is absent, the ACCR use the term pathological entity (Arbelo et al. 2013) to refer to the most probable cause of death, englobed in those of direct anthropic origin or those of natural origin and/or not directly related to anthropogenic activities (Figure 2).

Pathological entities of stranded cetaceans in the Canary

Anthropic	Ship strikes	
	Fishing interaction	
	Foreign body pathology	
	Military manoeuvres	
Natural	Natural pathology associated with	<u>Poor-very poor body condition</u>
	Intra-interspecific interaction	<u>Fair-good body condition</u>
	Neonatal/perinatal pathology	
	Live stranding	

Figure 2. Causes of death (pathological entities) of stranded cetaceans in the Canary Islands.

Retrospective studies on stranded cetaceans in this archipelago confirmed that most of the individuals died due to natural etiologies. There is a lower percentage of deaths caused by human interactions, as fishing activities, ship strikes and interaction with marine debris. Since sonar moratory in 2004, no more cetaceans appeared stranded with lesions compatible with gas or fat embolism related to military maneuvers (Fernández et al. 2013). However the implication of other indirect effects of environmental factors that affect cetacean's health should not be ruled out (Arbelo 2007, Díaz-Delgado et al. 2015).

Annually, some stranded cetaceans appear with traumatic injuries and other related pathologies whose origin is difficult to determine. This could be indicating an underestimate interaction of anthropic or natural origin. This thesis project aims to determine the origin of traumatic injuries and, therefore, to improve the diagnosis of the causes of death in stranded cetaceans. For this purpose, the following objectives were proposed.

Objectives



Objectives

The main general objective of this thesis is:

To identify pathological findings (lesions) associated with traumatic cause/s of death in cetaceans stranded in Canary Islands.

To achieve this general objective, three specific objectives were proposed:

1. To identify pathological findings associated with foreign body ingestion and its prevalence in different cetaceans' species stranded in Canary Islands (first publication).
2. To identify pathological findings associated with lethal intra-interspecific interactions and its prevalence in different cetaceans' species stranded in Canary Islands (second publication).
3. To identify pathologic findings associated with lethal fishery interactions and its prevalence in different cetaceans' species stranded in Canary Islands (third publication).

Literature review



Literature review

1. Definition of physical trauma

In terrestrial mammals, the body surface resists a wide variety of forces during the ordinary course of life, ranging from gravitational forces to surface contact when walking or sudden impacts to the body against animate and inanimate objects. When the energy of the impact exceeds the resistance and compliance of the tissues, it produces damage. A wound develops when the applied force's intensity exceeds tissue's adaptability (Saukko & Knight 2004, Ressel et al. 2016).

The force's intensity obeys this formula: kinetic energy = $\frac{1}{2}$ mass x velocity², which means that a force varies directly with the weapon's mass and with the square of the velocity of impact. The area over the force acts is essential. For example, if the skin is struck with a plank of wood, the tissues' damage will be different if the narrow edge or the flat surface is used. The same occurs in stab wounds, in which all the kinetic energy is concentrated in the tiny area of the tip of the blade (Saukko & Knight 2004).

According to Cooper & Cooper (2008), different traumatic injuries result in compression, stretching, torsion, or penetration of the tissues. Besides, the resultant damage also depends on the tissues (Saukko & Knight 2004). Depending on the force involved and tissues affected, a trauma can damage internal organs. In mild superficial traumas, only the skin tissue is affected, and lesions can be observed in the epidermis and dermis. However, in more profound injuries, subcutaneous, muscular, and nervous tissues may be damaged. Severe traumas produce bone fractures, large blood vessels' ruptures, or tears in internal organs with associated hemorrhages. It is also linked with secondary effects, such as anemia caused by blood loss and local infections or septicemia, as injuries are portals of entry for pathogens (Cooper & Cooper 2008).

2. Types of trauma in domestic animals

The figure of a veterinary pathologist is essential when there is suspicion of animal cruelty or iatrogenic injury. In addition to the necropsy or alive examination, blood analysis and other complementary techniques are considered. For example, the radiographic study is employed in cases with bone fractures or internal foreign bodies such as blade fragments or surgical material. Radiographs indicate distinct tool marks, which may match the crime weapon (Cooper & Cooper 2008).

Unlike in human where skin wounds are typically apparent, hair coat in animals difficult its visibility (Cooper & Cooper 2008). The most common trauma is blunt-force injuries (Figure 3). Typically, these traumas present soft tissue lacerations between wound's edges, which appear irregular, torn, and ragged (de Siqueira et al., 2016). Examples of blunt-force traumas are contusions, abrasions, lacerations, and bony fractures. Another type of traumatism, sharp-force injuries, are caused by sharp objects using mechanical force against the skin. Some examples of instruments used in sharp-force are knives, axes, machetes, scissors, screwdrivers, needles, barbecue forks, broken glass, and arrows (De Siqueira et al. 2016). In domestic animals, these lesions are less frequent than blunt lesions and are even less frequent than poisoning.

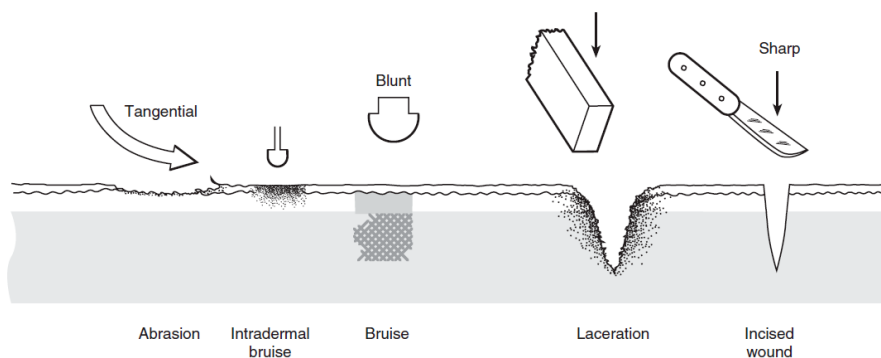


Figure 3. Types of injury to the skin (Saukko and Knight 2004).

De Siqueira *et al.* (2016) classified sharp injuries in animals as follows:

- Stab wounds: the depth of the wounds exceeds its length and results from the movement of the long axis of the blade in the plane approximately perpendicular to the body's surface.
- Incised wounds: these wounds are slashes and cuts where the length exceeds the depth.
- Chop wounds: produced by heavy instruments (i.e., cleavers, axes, and machetes), incising wound on the skin and bony fractures and/or a deep groove in the bone.
- Therapeutic/diagnostic wounds: which result from a veterinary intervention.

The differentiation between sharp and blunt-force traumas are crucial. In sharp injuries, the edges of the wound are linear or angular, and contusions are rare. For example, when it appears in stabs, it presents a square shape due to the dull part of the knife (De Siqueira et al. 2016).

3. Stranding events and necropsies of cetaceans in the Canary Islands

Full anatomopathological studies carried on stranded cetaceans revealed the presence of various traumatic injuries that correspond to pathological entities of different etiology (Arbelo et al. 2013, Díaz-Delgado et al. 2018):

- Non-anthropogenic trauma: live stranding and traumatic intra-interspecific interaction.
- Anthropogenic trauma: ship strike, fishery interaction, foreign body associated pathologies.

3.1. Non-anthropogenic trauma

a) Live stranding

A live stranding situation is when a cetacean strand alive on the beach or in shallow water, unable to free itself (Geraci & Lounsbury 2005). It is an anomalous distress situation for an animal adapted to the aquatic environment (Gulland et al. 2018). When two or more individuals (other than a female and her calf) coming ashore alive at the same time and place, it is called mass stranding (Geraci & Lounsbury 2005).

There are multiple causes for live strandings, and some coastlines seem particularly prone to them. Natural pathologies that severely affect cetaceans' welfare and health can explain live strandings (i.e., Morimitsu et al. 1987, Domingo et al. 1995, Arbelo et al. 2013, Díaz-Delgado et al. 2018). For example, infectious agents, such as Morbillivirus, produce epizootics with mass mortalities, and some individuals may strand alive (i.e., Simmonds & Symoens 1991, Domingo et al. 1995, Jepson et al. 2013). Apart from illness or injury, live strandings may be explained by predation close inshore, shallow sloping shores, confusions by coastal topography, and natural disturbances on the magnetic field within others (i.e., Klinowska 1988, Simmonds 1997). Healthy animals follow an ill or injured lead in some species produce mass strandings (i.e., Simmonds 1997). Besides, anthropogenic threats may lead to live strandings. Cetaceans that suffer from fatal interaction with human activities or marine debris may

strand alive. However, in some cases, the leading cause of live stranding remains unknown (Geraci & Lounsbury 2005, Mazzariol et al. 2011).

Grossly, skin damages (cutaneous erosions/lacerations/abrasions affecting rostrum, the cranial edge of pectoral and dorsal fins, tail, and ventral parts of the body) and fractures are common findings in live stranded cetaceans (Díaz-Delgado et al. 2018, Câmara et al. 2020). In stranded or floating dead cetaceans, that appear with these external lesions, live stranding should not be ruled out, as they may strand alive before been found.

In addition to that, the fact of being onshore is a stressful situation for a mammal adapted to an aquatic environment. Thus, besides the primary cause of stranding and the traumatic lesions due to live stranding, the stressful situation of stranding itself and its pathophysiological response can lead directly to the animal's death or difficult its release back into the ocean (Simmonds 1997, Gulland et al. 2018).

In cetaceans, as in other wild animals, capture, handling, and transportation produce a pathological state of acute stress due to an extreme or prolonged response with the release of catecholamines (Cowan & Curry 2002, 2008). This situation produces what is called "capture myopathy syndrome" in wild animals, joining multisystemic pathophysiology and clinical changes that lead stressed animals to death (Geraci & Lounsbury 2005, Herráez et al. 2007, 2013, Gulland et al. 2018). In addition to catecholamine surge, neurogenic shock and body compression (that impede venous flow return) produces local to generalized vasospasm and vasodilatation. This situation of ischemia-reperfusion severely damages multiple organs:

- Skeletal muscle: Usual absence of gross muscle pathologic changes. Histologically, acute to subacute muscle degeneration (rhabdomyolysis) consisted of segmental hypercontraction, contraction band necrosis, and segmental myonecrosis with myoglobin depletion preferentially in type I fibers. Interstitial edema with fibrinogen deposition and mild infiltration of phagocytic cells were detected. Occasionally, the concomitant presence of segmental myodegeneration and myofiber regeneration may indicate re-stranding (Herráez et al. 2007, 2013).
- Cardiac muscle: Excess catecholamines released are believed to produce myocardial dysfunction in sarcoplasmic reticulum calcium channels, producing a leakage of calcium into the sarcoplasm, which leads to contraction impairment and myocardial necrosis (Ellison et al. 2007). Acute multifocal degenerative lesions in the myocardium and

subendocardial areas (contraction band necrosis, wavy fibers, hypereosinophilia, and vacuolar degeneration), associated with the presence of vascular changes and inflammatory infiltration are described (Câmara et al. 2019). Besides, immunohistochemically, affected cardiomyocytes showed depletion of myoglobin and intracytoplasmic staining for fibrinogen. Positive immunoreaction with anti-myoglobin antibody was detected intravascularly in the affected areas (Herráez et al. 2013, Câmara et al. 2019). These findings are also found in skeletal muscle and prove that cetaceans experience stress cardiomyopathy syndrome, similar to human beings. Thus, these lesions compromise rehabilitation or therapy effectiveness in live stranding cetaceans and might contribute to their mortality (Câmara et al. 2019).

- Kidneys: Grossly, they are intensely dark secondary to vascular compromise. Massive muscle damage produces acute renal failure. It is histologically represented by associated renal tubular necrosis and myoglobinuric nephrosis. Immunohistochemically, kidneys are positive for anti-myoglobin antibody, and some degenerated tubular cells have a granular cytoplasmic immunoreaction for HSP70 (Herráez et al. 2007, 2013).
- Multifocal areas of necrosis in other viscera could also be observed, for example, in the Central Nervous System (CNS), lung, liver, intestine, pancreas, and lymph nodes (Herráez et al. 2007, 2013, Seguel et al. 2014).

In cases in which a stranding event produces severe traumatism, fat embolism could be detected using appropriate laboratory techniques (Bernaldo de Quirós et al. 2019).

Although the pathological findings vary amongst individuals, laboratory abnormalities are usually detected (Spraker 1993). Hypovolemia, hypocalcemia, hyperphosphatemia, metabolic acidosis, and disseminated intravascular coagulation have been described in cases with massive rhabdomyolysis. Regarding biochemical results, apart from catecholamines, the release of ACTH increases cortisol and aldosterone concentrations (St Aubin & Geraci 1990, Fair & Becker 2000). Also, increased creatinine kinase, cardiac troponin, aspartate aminotransferase, alanine aminotransferase, and blood urea nitrogen have been reported (Colgrove 1978, Câmara et al. 2019, 2020).

b) *Traumatic intra-interspecific interaction*

i. *Social traumatic interaction between cetaceans*

An ecosystem is formed by the interaction of a community of organisms within them and their environment. Among themselves, different organisms interact through processes such as competition, predation, parasitism, and symbiosis. Sometimes social interactions become aggressive and produce traumatism that may threaten life. In general, when interactions are produced within individuals of the same species are called intraspecific. Meanwhile, it is called interspecific if they refer to different species.

The motivations of these attacks are multiple and even misunderstood. The most reported is copulation motivation. In the case of dolphins, violent kidnappings (“herding events”) of non-pregnant females are described in male alliances, especially in bottlenose dolphins (i.e., Connor et al. 1992, Wiszniewski et al. 2012). Regarding sex competition, male alliances have been reported attacking other single adult male dolphins (Parsons et al. 2003). When observed directly, these aggressions include head-to-head posturing, acoustic threats, and physical violence (i.e., Herzing 1996, Blomqvist & Amundin 2004, Scott et al. 2005). In the case of bottlenose dolphins, multiple aggressive behaviours on smaller cetaceans, such as harbor porpoises (i.e., Ross & Wilson 1996, Dunn et al. 2002, Spitz et al. 2006, Barnett et al. 2009, Coscarella & Crespo 2010, Cotter et al. 2012, Gross et al. 2020) or individual of other species (i.e., Herzing & Johnson 1997) have been reported.

With the same intention of copulation with non-pregnant females, nursing calves are victims of infanticides in Amazon river dolphins (*Inia geoffrensis*) (Bowler et al. 2018), Indo-Pacific humpback dolphins (*Sousa chinensis*) (Zheng et al. 2016), killer whales (Towers et al. 2018), tucuxi dolphins (*Sotalia guianensis*) (Nery & Simão 2009), and bottlenose dolphins (i.e., Patterson et al. 1998, Dunn et al. 2002, Kaplan et al. 2009, Díaz López et al. 2018). In mysticetes, male humpback whales have been reported escorting receptive females, threatening other males by thrashing their flukes (Würsig et al. 2009). Male alliances have also been reported in whales. However, aggressive reactions are not usual and rarely lethal (Clapham 1996).

Violent interspecific interactions may occur for reasons other than sexual competition. Other motivations for these fatal encounters are prey competition (Spitz et al. 2006), fight practice (Jepson & Baker 1998) nursing failure, or predation. In the last case, killer whales are usually feared by other marine mammals, including cetaceans. This species has been observed attacking or harassing about 20 different cetaceans species (odontocetes and mysticetes) (Baldrige 1972, Silber

et al. 1990, Jefferson et al. 1991, Ott & Danilewicz 1998, Visser et al. 2010, Saulitis et al. 2015). Also, false killer whales have been reported on species of the genus *Stenella* spp. and short-beaked common dolphins (Perryman & Foster 1980). Among other marine species, other marine mammals also predate on cetaceans, for example, gray seals (*Halichoerus grypus*) on harbor porpoises (Haelters et al. 2012, Bouveroux et al. 2014, Leopold et al. 2014) and polar bears (*Ursus maritimus*) on belugas (*Delphinapterus leucas*) (Lowry et al. 1987).

In canary waters, most cetacean species coexist. However, there is evidence of habitat partitioning in La Gomera (Smit et al. 2010). Of the species described above, Atlantic bottlenose dolphins are considered resident populations in some locations, seasonal sightings of killer whales are annually reported in the archipelago, and occasional strandings and sightings of false killer whales are reported. Thus, numerous social intra-interspecific interactions between different cetacean species are expected.

In cetaceans, social interactions within members of the same or different species produce “tooth-rake marks” on the skin. They are defined as external linear parallel erosions inflicted by teeth, frequently observed healed. In aggressive encounters, tooth-rake marks are severe. They can ulcerate the skin, even producing tearing of the blubber and severe internal damages. Distance between teeth in tooth-rake marks are useful to determine the aggressor species. However, the absence of them cannot rule out an intra-interspecific interaction. For example, killer whales striking with their snouts produced internal injuries in other cetaceans without external wounds (Jefferson et al. 1991). In blunt traumas producing during aggressive encounters between cetaceans, extensive vascular damage (i.e., subcutaneous hematomas, hemorrhages in skeletal muscles, hemothorax, retroperitoneal hemorrhages), as well as myonecrosis; cranial, vertebral and/or ribs fractures; tearing of the parietal pleura and lung perforations; perforation of the abdominal wall; and liver lacerations among others are described (Ross & Wilson 1996, Jepson & Baker 1998, Arbelo et al. 2013, Díaz-Delgado et al. 2018).

Histologically, acute monophasic degeneration and hemorrhages on skeletal muscle are common findings (Sierra et al. 2017, Díaz-Delgado et al. 2018). In severe polytraumatized cases, the release of fat into the bloodstream (from bone marrow in bone fractures and skin fat) could be appreciated as pulmonary fat emboli (Arregui et al. 2019). Also, myoglobinuric and/or hemoglobinuric nephrosis can be observed by specific staining (Díaz-Delgado et al. 2018).

ii. Traumatic death due to an accident during predation

Cetacean species feed on a wide variety of marine species, from zooplankton to other cetaceans. Like other marine mammals, cetaceans are considered opportunistic foragers based on the relatively large number of different species that have been reported in the stomachs and feces. However, their diets are typically dominated by fewer species. The complexity of biological interaction within cetaceans and other marine organisms remains poorly understood (Würsig et al. 2009).

Fatal encounters with the prey have been reported. For example, laryngeal luxation in bottlenose dolphins was reported by the ingestion of black margate (*Anisotremus surinamensis*) (Mignucci-Giannoni et al. 2009) and beheaded sheepshead (*Archosargus probatocephalus*) (Watson & Gee 2005). In long-finned pilot whales (*Globicephala melas*), two cases of asphyxia due to obstruction of the airway was reported with a common sole (*Solea solea*) (IJsseldijk et al. 2015) and another by a European eel (*Anguilla anguilla*) (Fernández-Maldonado 2016). Also, the ingestion of fish species with strong dorsal spines is known to produce severe inflammation of the throat, and dolphins may face death due to asphyxiation or aerodigestive tract obstruction (Byard et al. 2010, Stolen et al. 2013).

3.2. Anthropogenic trauma

a) Ship strike

Cases of collisions between vessels and marine mammals, especially manatees, pinnipeds, and large cetaceans, are highly reported and threaten some population of endangered species (Laist et al. 2001, Kraus et al. 2005). In cetaceans, these fatal encounters are more prone in specific locations, with high traffic and whale concentrations. In Europe, these areas are mainly the proximity or in the Pelagos Sanctuary (Panigada et al. 2006), the Strait of Gibraltar (De Stephanis & Urquiola 2006), and the Canary Islands (Carrillo & Ritter 2010). Nevertheless, animals suffer the consequences of the hit, as human injury and property damage had been described (Carrillo & Ritter 2010, Neilson et al. 2012). As these encounters are increasing, the International Whale Commission (IWC) developed a strategic plan to mitigate these impacts (Cates et al. 2017).

At least 11 cetacean species are reported hit by ships. Of them, fin whales, right whales (*Eubalaena spp.*), humpback whales, sperm whales, and gray whales (*Eschrichtius robustus*) are the species most commonly affected (Laist et al. 2001). Every age class is susceptible to ship strikes, and both sexes are equally affected. However, young animals seem to be more prone to these fatal encounters in some species (Knowlton & Kraus 2001, Laist et al. 2001, Vanderlaan & Taggart 2007,

Douglas et al. 2008, Carrillo & Ritter 2010, Neilson et al. 2012). Young whales are slower than adults and not fully adapted to dive (Papastavrou et al. 1989, Laist et al. 2001). In blue whales, ship strikes primarily involved adults (Laist et al. 2001). In nursing groups of sperm whales, mothers spend more time on the surface with their offspring than the rest of the adults (Whitehead 1996). Also, protective behaviors as mother sperm whale moving towards a boat have been described (Tregenza et al. 2002). These factors may expose them to a higher risk of ship strikes (Arregui et al. 2019).

It is crucial to determine whether the strike occurred ante- or post-mortem (Moore et al. 2013). In the case of alive strikes, the severity and type of trauma resulting from a ship strike vary depending on the injury's anatomic site, the angle of impact, the vessel speed, and size (Laist et al. 2001, Vanderlaan & Taggart 2007). Because of that, non-lethal superficial abrasions and contusions to fatal injuries can be described (Campbell-Malone et al. 2008). Lethal or severe injuries are caused by ships of 80 meters in length or longer, traveling at speeds of 14 knots or faster (Laist et al. 2001).

As a consequence of these encounters, different trauma can be observed: blunt force traumas, due to direct contact with a non-rotating feature of the vessel (p. ej., bow, hull, rudder, or skeg); sharp trauma, resulting from contact with rotating propeller; or a combination of both producing a chop wound (Moore et al. 2013).

In blunt force traumas, gross findings include swelling and elevation of the affected area, depressions, superficial abrasions, erosions, and lacerations. On reflection of the skin, subcutaneous tissue shows well-defined focal or multifocal hemorrhage and edema. Laceration, shredding or rupture of skeletal muscle with associated hemorrhages; bone fracture, luxation, and subluxation; visceral displacement, herniation, or rupture of internal organs; and massive hemoperitoneum or hemothorax is also frequent (Rommel et al. 2007, Campbell-Malone et al. 2008, Moore et al. 2013). If there is blood in internal cavities, fibrin strands on serosal surfaces or spontaneous clot formation on-air exposure indicate antemortem hemorrhage. Prolapse of internal viscera can be observed. If the impact is on the head, brain swelling, and occasional herniation of the brain stem and posterior cerebellum through the foramen magnum can be seen. In the vertebrae, lateral or dorsal processes may also be comminuted fractured (Moore et al. 2013). However, blunt force lesions are not pathognomonic for ship strikes as they can be found in different pathological entities in small and large cetaceans (Arbelo et al. 2013, Moore et al. 2013, Díaz-Delgado et al. 2018). Sharp-trauma injuries can be from mild superficial nicks to severe amputations and lethal wounds (Campbell-Malone et al. 2008). Grossly, the skin presents simple to multiple parallel and equidistant incising wounds, from linear to abruptly curvilinear or sigmoid (S- or

anti-S shaped). The wound's depth is related to the type, radius, and proximity of the propeller to the animal (Rommel et al. 2007). Subcutaneous and skeletal muscle can be exposed, red to pink (if acute) associated with hemorrhages, or gray to tan/white (if more chronic) muscle coloration is observed. Abdominal organs can be exposed, and pneumothorax may also be present. In the case of amputations, parts, or entire appendages, trunk or head may be lost. In mild lesions, wound repair around the margins associated with parasites (i.e., cyamid) may be observed (Moore et al. 2013). These wounds have been observed in an array of small and large stranded cetaceans, ranging from delphinids to mysticetes (Arbelo et al. 2013, Moore et al. 2013, Díaz-Delgado et al. 2018). Rests of propeller piece or hull paint transfer may appear as foreign bodies within the wound and should be well-documented (Moore et al. 2013).

Histologically, the affected skin and subcutaneous tissue present focal extensive hemorrhage and edema, with the occasional presence of fibrin deposition in the subcutis and subjacent musculature. In general, skeletal muscle (taken from the incision site and other locomotor muscle, like the *longissimus dorsi*) presents peracute, monophasic myocyte degeneration. The sarcoplasm often shows flocculent, granular or/and hyalinized eosinophilic. Images of segmentary degeneration, contraction band necrosis, and fragmentation of the myofibers are common findings. In this pathological entity, both fiber-types (I and II) are affected by acute degenerative changes, unlike in stress-related myopathy in which type I fibers have been reported as preferentially affected. Discoid degeneration is exclusive to the cases in which very deep incisions affects large-caliber blood vessels causing hypovolemic shock (Campbell-Malone et al. 2008). These features are detectable even in very decomposed carcasses (Sierra et al. 2014). Hemorrhages and fibrin deposition due to visceral ruptures and draining hemorrhage in regional lymph nodes may be found. In sharp traumas, the absence of blood in the wound may be explained by prolonged wound exposure to water, resulting in hemolysis and leaching of blood from wounds. Besides, in severe cases in which large-caliber blood vessels are affected, peracute death may explain the absence of histologic lesions and intravascular gas presence (Moore et al. 2013). Furthermore, predation on the exposed tissue can partially or entirely mask the lesions (Sierra et al. 2014).

The presence of fat embolism in lung microvasculature is a tool to determine antemortem collisions. In the Canary Islands, 83% of the studied stranded sperm whales with evidence of ship strikes were alive when a strike occurs. To detect it histologically, osmium tetroxide (OsO₄), chromic acid, and Oil Red O frozen techniques were valuable, even in samples in advanced autolysis and had been stored in

formaldehyde for years (Arregui et al. 2019, 2020). However, rapid cardiovascular collapse or not homogeneously sample of the lung tissue could explain the lack of detectable fat emboli in suspicious cases (Kinra & Kudesia 2004, Arregui et al. 2019). Thus, further studies of pulmonary blood circulation and fat emboli distribution should be done for accurate fat emboli detection (Arregui et al. 2019).

In the Canary Islands, most cetaceans affected by ship collisions are deep divers (Arbelo et al. 2013, Sierra et al. 2014, Díaz-Delgado et al. 2018). It might be explained by the prolonged period of recuperation at the surface after one or more apnea episodes (Arbelo et al. 2013). Most of them were young animals that appeared floating or stranded along Tenerife's east coast (Carrillo & Ritter 2010, Arregui et al. 2019). In this archipelago, an overlap between stranding location and fast-ferry transect has been reported, and the impact of ship strikes on the sperm whale population is concerning (Carrillo & Ritter 2010, Arregui et al. 2019).

b) Fishery interaction

i. Interaction with an active fishing gear

The major global anthropic threat to cetacean populations are fishery interactions (Reeves et al. 2013). NOAA Fisheries described accidental captures or “bycatch” as “discarded catch of marine species and unobserved mortality due to a direct encounter with fishing vessels and gear”. Bycatch is the highest widespread risk in cetaceans and responsible for thousands of deaths annually, although its prevalence might be underreported (Young & Iudicello 2007, Dolman & Moore 2017). The harbor porpoise, the bottlenose dolphin, the common dolphin, and the striped dolphin are the most commonly affected species worldwide (i.e., Bjørge et al. 2013, Reeves et al. 2013, Peltier et al. 2016). At least 75% of odontocete and 64% of mysticetes species have been recorded trapped in gillnet over the last 20 years (Reeves et al. 2013). They appear trapped in different fishing gears, such as gillnets, trawls, purse seines, fish traps, and longlines (Read et al. 2006). When trapped small cetaceans often die because they are not strong enough to break free to breathe. Meanwhile, large whales able to break free remain entangled for long periods, leading to debilitating injuries and even slow death (Moore et al. 2013).

Gross features in bycaught cetaceans are not pathognomonic, except for the presence of gear cuts and impressions on the skin (mainly over the head, flippers, tail, and body). Thus, to identify bycatch, it is essential to discard other possible causes of death (Kuiken 1996, Moore et al. 2013). Bycaught dolphins are often healthy individuals, in good body condition, found dead underwater with pathologic findings consistent with peracute underwater entrapment (PUE) (Moore et al.

2013). Some common findings in bycaught cetaceans are: froth in airways, lung edema, emphysema and atelectasis, non-digested food remains in the forestomach, reddish or bulging eyes, congestion, and disseminated gas bubbles (Moore et al. 2013, Bernaldo de Quirós et al. 2018). Related to recent feeding, the presence of chyle in the thoracic conduct, mesenteric lymphatic vessels, and other associated lymph nodes are noticed (Arbelo et al. 2013, Díaz-Delgado et al. 2018). In the case of animals brought to the deck that suffered from aggressive handling, severe traumatism, stabs, amputations, and gunshot might be found. The extraction of the *longissimus dorsi* muscle for bait use has been observed occasionally in stranded carcasses. Besides, abdominal cuts to sink the carcasses can also be noticed (Goldstein et al. 1999, Read & Murray 2000, Byard et al. 2006). Eventual larynx strangulation related with fishing gears were described in stranded bottlenose dolphins (Gomerčić et al. 2009, Levy et al. 2009). Gas scores and gas analysis of bycatch dolphins reveal greater numbers than in stranded animals being nitrogen the bubbles' main component (Bernaldo de Quirós et al. 2013, 2018).

Histologically, skeletal muscle shows signs of physical struggle and exertion, similar to the ones observed in severe agonic stressful situations due to adrenocortical response (Cowan & Curry 2002, 2008, Moore et al. 2013) such as live stranding (Herráez et al. 2007, 2013) or ship strikes (Arbelo et al. 2013, Sierra et al. 2014, 2017, Díaz-Delgado et al. 2018). Cardiac changes (Cowan & Curry 2002, 2008, Câmara et al. 2019) and hyaline globules in hepatocytes' cytoplasm (Jaber et al. 2004, Herráez et al. 2013, Díaz-Delgado et al. 2018) are also common. Inflammatory cells can be observed as part of the granulation process of the injuries (Díaz-Delgado et al. 2015). Also, the concomitant presence of other pathologic findings, such as parasitic pneumonia, may support the hypothesis that ill cetaceans may be also predisposed to interact with fisheries (Arbelo et al. 2013, Díaz-Delgado et al. 2018).

In previous retrospective studies in the Canary Islands, a low prevalence of fishery interactions was detected, mostly bycatch. The Atlantic spotted dolphin was the most affected species (Arbelo et al. 2013, Díaz-Delgado et al. 2018). Bycatch-associated lesions, entanglements and other traumatic lesions associated to aggressive handling were described. In the case of ingested longline hooks, Díaz-Delgado et al. (2018) firstly described the lesions associated to a hook piercing in the mandible of an Atlantic spotted dolphin. In previous studies in this geographical area, no gunshot nor larynx strangulation with fishing gears were described in stranded cetaceans.

ii. Chronic entanglement with fishing gears

Entanglements can be produced by fishing gears or marine debris. Regarding fishing gears, it is difficult to determine if an entangled

cetacean has been trapped by an active or an abandoned, lost or otherwise discarded fishing gear (Laist 1997, Macfadyen et al. 2009). Despite this, it is estimated that 97% of entangled cetaceans carry fishing gear (Baulch & Perry 2014). Entanglement in marine debris is less frequently reported. A total of 27 cetacean species in 78 incidences were reported entangled worldwide (Baulch & Perry 2014, Kühn et al. 2015).

The potential effects of chronic entanglements in different cetacean populations remain unknown and maybe underreported (Fossi et al. 2018). Primary, because the total of the population should be known; second, the same individual can become entangled multiple times (Knowlton et al. 2012), and then, entangled carcasses tend to sink (Laist 1997), among others. Due to the increasing number of sightings of entangled cetaceans, three times greater than in 1990 (Baulch & Perry 2014), international entanglement response and monitoring programs have been set up (Dolman & Brakes 2018, IWC 2018).

Previous studies in the Canary Islands reveals a low rate of entangled cetacean stranded on their coasts (Arbelo et al. 2013, Díaz-Delgado et al. 2018). Similar to other regions, mysticetes seem to be the most affected. At least half of whale deaths are attributed to chronic entanglements in the Atlantic Ocean (Northridge et al. 2010). Minke whales seem significantly affected (Lien 1994).

In whales, ropes and nets often affect the oral cavity, flippers, and tail. Entangled odontocetes appear with longlines, recreational fishing gear, or lures. Remains of gear over the body, gear impressions/cuts, multiple unhealed abrasions, lacerations and contusions, and damaged baleen or teeth suggest entanglement (Moore et al. 2013). No single mark is likely to be conclusive. Whether the area is mobile, or the defect is too large, exposed granulation tissue and possible contraction may be observed. Repair may occur if border margins are opposed. Other common findings are baleen malocclusion, amputations, and ischemia due to tissue constriction (Moore et al. 2013). Scars of healed wounds can be observed in detached animals (Heyning & Lewis 1990). It is essential to understand that even if a gear detaches, death from chronic entanglements may occur due to open wounds, leading to septicemia, or impaired movements due to atrophy that contributes to a negative energetic burden (Moore et al. 2013). Deficient body condition, cyanid spread, and other skin lesions are commonly observed (Pettis et al. 2004).

Histological changes include fibrosis, acute to chronic inflammatory cells infiltration, hemorrhage, fibrin, vascular thrombosis, myofiber degeneration, and bony inflammation. In animals in poor body condition, fat edema and muscular atrophy can be found. Besides, cardiac and skeletal muscle contraction bands, adrenal cortical

hypertrophy, medullary hyperplasia, and adrenal cortical lipoidal degeneration due to stress may be observed (Moore et al. 2013).

c) *Marine debris ingestion (macro and micro debris ingestion)*

The primary source of marine debris is represented by intentional illegal discard or accidental losses of anthropic items from land. There also exists a directly thrown of debris to the sea, for example, fishery household items such as supermarket plastic bags, water bottles, and food packaging are often dumped (Richardson et al. 2017). Dumping from touristic beaches and leisure cruise ships should not be discarded as potential ocean plastics pollution (Herz & Davis 2002). As were introduced in the previous section, abandoned, lost, or otherwise discarded fishing gears, known to produce "ghost fishing", are also part of marine debris. As an example, in the Atlantic Ocean, it is estimated that a large percentage of marine debris consists of derelict fishing gears (>40%), most of them made of plastic (Monteiro et al. 2018). Plastic is the most common widespread item found in the open ocean. Plastics have been found throughout the water column, floating on the sea surface, accumulating in deep-sea trenches and sediment, and sequestered in polar sea ice (Obbard et al. 2014, Woodall et al. 2014, Cózar et al. 2014, Fossi et al. 2018). Marine plastic pollution is abundant in the Atlantic Ocean, and it is present in the waters surrounding the Canary Islands (Monteiro et al. 2018). In these waters, the 'Canary Current' brings marine debris from the open Atlantic Ocean (Baztan et al. 2014). In shallow waters, plastic debris concentration is approximately 200-500 g.km⁻² (Cózar et al. 2014).

Since 1960, more than 800 species have been reported interacting with marine debris, and almost all of these encounters involved plastics (Secretariat of the Convention on Biological Diversity 2016). Ingestion of foreign bodies has been reported in cetacean species worldwide (Derraik 2002, Gregory 2009, Baulch & Perry 2014, Fossi et al. 2018, Lusher et al. 2018). Deep divers, especially sperm whales, seem to be significantly affected (Jacobsen et al. 2010, de Stephanis et al. 2013, Baulch & Perry 2014, Unger et al. 2016). In recent years, an increasing trend of these fatal interactions has been recorded in scientific publications and social media. However, the real impact on cetaceans populations remains unknown (Williams et al. 2011, Simmonds 2012). The scientific committee of the International Whaling Commission (IWC) encourages long-term studies about foreign body ingestions and its clinicopathological consequences, as they are considered a threat to cetacean populations (IWC 2019).

Diverse hypotheses have been formulated to explain the ingestion of marine debris: confusion with a prey (Carpenter & Smith 1972), not echolocating at the ultimate approach to the prey, proximity to the debris with a prey target (Ross 1984, Mead 1989), inexperience

in young animals (Di Benedetto & Ramos 2014), suction mechanism of prey capture in beaked whales (MacLeod et al. 2007), playful and curiosity (Laist 1987), disease factors, and ingestion during stranding events (Walker & Coe 1989). Recently, in a retrospective study of stranded cetaceans in Catalonia's coast, CNS lesions and maternal separation have been identified as potential risk factors for ingestion of foreign materials in odontocetes (Lacombe et al. 2020).

Marine debris have been found in over 60% of all cetacean species (Baulch & Perry 2014). As in the ocean, plastics are the main component of foreign bodies found in stranded cetaceans (Baulch & Perry 2014), from domestic objects to agricultural items and fishing gears (Derraik 2002, Jacobsen et al. 2010, de Stephanis et al. 2013, Unger et al. 2016, 2017). Its presence can damage the digestive tract and related systems.

The pathologies associated with foreign bodies' presence are known as "foreign body associated pathology" and can lead to the animal's death. Previous studies in the Canary Islands reveals a low rate of foreign body ingestion in stranded cetaceans, mainly odontocetes (Arbelo et al. 2013, Diaz-Delgado et al. 2018).

Starvation and malnutrition appear due to impairment of foraging (Secchi and Zarzur, 1999). Suffocation has also been reported (Laist 1997). Reduced growth rates, longevity, and reproductive capacity of affected animals are also reported in different species (i.e., Laist 1987, McCauley & Bjorndal 1999, Gregory 2009). Common gross features during the necropsy are loss of body condition, bleeding ulcers, obstructions, impaction and perforation of the digestive tract with associated peritonitis and septicemia (i.e., Walker & Coe 1989, Laist 1997, Abollo et al. 1998, Stamper et al. 2006, Unger et al. 2017). Histology must confirm inflammatory and vascular changes in affected systems. In affected cetaceans, morbilliviral encephalitis, neurobrucellosis or encephalomalacia have been described (Lacombe et al. 2020). In some cases, no lesions in the digestive tract have been detected related to foreign bodies, maybe because of recent ingestion during the stranding event (Denuncio et al. 2011, Mazzariol et al. 2011, Unger et al. 2016). However, long-term deleterious effects of foreign body ingestion have been less studied.

Smaller plastic pieces, less than 5 millimeters long, are called microplastics (Kershaw 2015). As macro debris, microplastics are ubiquitous and appear spread into different and remote environments interacting with the aquatic fauna. The study of microplastic in cetaceans is challenging, as it has to combine necropsy protocols and simultaneous analysis of large volumes of stomachs and gut content, particularly in large cetaceans (Fossi et al. 2018). Because of that, only a few studies have reported microplastic in cetaceans' digestive tracts,

comparing to macro litter ingestion. Both odontocetes and mysticetes species have been reported with ingested microplastics (i.e., Lusher et al. 2015, 2018, Besseling et al. 2015, Hernández-González et al. 2018, Van Franeker et al. 2018, Xiong et al. 2018, Nelms et al. 2019, Moore et al. 2020). Recently, a protocol for study microplastic intake in odontocete species has been performed in stranded cetaceans in the Canary Islands (Montoto-Martínez et al. 2020), based on previous protocols of microplastics from open water (Lusher et al. 2014). Multiple routes of microplastic uptake in cetaceans include the ingestion from the water column while feeding, inhalation at the air-water interface, or from prey with microplastics in their bodies, within others (IWC 2013, Fossi & Panti 2018, Panti et al. 2019). Fossi et al. (2014), estimated that an adult Mediterranean fin whale could consume more than 3,000 microplastic particles per day in the Ligurian sea. Adverse effects of microplastic ingestions include plastic-associated toxins and additives such as phthalates (Fossi et al. 2018). However, no long-term studies regarding microplastic ingestion and health consequences in cetaceans have been published yet. The IWC urgent the monitoring of microplastics and plastic-associated toxins in certain cetaceans species as the Mediterranean fin whale (IWC 2019).

2) Direct catches (whaling, bush meat, bait use, shooting)

In 1982, the IWC established a moratorium from the 1985/1986 whaling season onwards. This pause remains in place today. However, certain countries objected to this moratorium: Norway, Iceland, and the Russian Federation. Nowadays, only Norway and Iceland take whales commercially. Catch limits are established by them, but these countries must provide information on their catches to the IWC. In addition, The International Convention for the Regulation of Whaling (1946) from the IWC, in the Article VIII, permits to kill whales for scientific research purposes. These catches are responsibility (setting and regulating) of the individual governments, not the IWC. In Spanish waters, the killing cetaceans for commercial purposes (bushmeat trade) or scientific purposes are not allowed [Artículo 52.3 Ley 42/2007 del Patrimonio Natural y de la Biodiversidad].

Lethal traumas associated with whaling or direct catches in cetaceans are those caused by harpoon, shot or grenade. In some cases, whales manage to free out harmed (Knowles & Butterworth 2006). There was no report of a harmed whale with indicative lesions of whaling in the Canary Islands during this study period.

However, the appearance of stranded cetaceans suspicious of fishery interaction with anthropic cuts and tissue depletion (i.e., epaxial muscle dissection) is not unusual in the Spanish cetacean stranded networks. Cetaceans meat might be used as bait in illegal fishery.

Finally, in other stranding locations, cetaceans show traumas associated with aggressive behaviours towards them, like shoots and amputations (Moore et al. 2013). However, no evidence of gunshot nor amputation was present in stranded cetaceans in this retrospective study.

Material and Methods



Material and Methods

To achieve the main and specific objectives of this thesis project, the anatomopathological reports of cetaceans stranded in the Canary Islands, elaborated by the pathologist of the ACCR, were reviewed. During the thesis project, the PhD student participated in cetacean postmortem studies carried out by the veterinary pathologists specialized in cetacean of the ACCR with the permit of the Ministry of Agriculture and Fisheries, Food and Environment (2015-2021).

The studied cetaceans were necropsied following the protocol of Kuiken & García-Hartmann (1991). All organs and tissues were systematically observed and dissected. External and internal injuries were described, photographed, and sampled. The samples were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin for their histological study. No live animal experiments were performed.

As part of this thesis project, three retrospective studies have been published relating three pathological entities that implies traumatic lesions: foreign bodies associated pathologies (publication 1), traumatic intra-interspecific interactions (publication 2), and interaction with fisheries (post 3). The material and methods has been unified in this section, highlighting the most notable differences between studies.

First, the study period. These retrospective studies covered different periods:

- First publication: 465 cases of cetaceans stranded between January 2000 and December 2015; 36 cetaceans presented foreign body ingestion.
- Second publication: 540 cases of cetaceans stranded between January 2000 and December 2017; 27 cetaceans died due to traumatic intra-interspecific interactions.
- Third publication: 586 cases of cetaceans stranded between January 2000 and December 2018; 32 died due to the pathological consequences of fishery interaction.

Second, the epidemiological data. In these retrospective studies, epidemiological data were collected for each stranded cetacean (date, location, and type of stranding), as well as biological variables (species, physical development, sex, and sexual maturity), body condition, degree

of decomposition of the animal and pathological entity. For certain variables, a categorization was carried out:

- Physical development: It was determined according to the total length of the animals (Würsig et al. 2009) and the histological study of the gonads (Geraci & Lounsbury 2005). In publications 2 and 3, the osteological characteristics studied by Tejedor (2016) were also used. This category was subdivided into neonate, young, juvenile, subadult, and adult.

- Body condition: It was estimated according to the external physical conformation of the cetaceans following the criteria of Joblon et al. (2014). This category was subdivided into very poor, poor, moderate, and good body condition.

- State of conservation: In publication 1 it was determined following the protocol of Kuiken & García-Hartmann (1991), with the exception of code 1, that was used for live stranded cetaceans that died during care or were euthanized, and presented a very fresh status at the necropsy. This change is already reflected in the protocol of IJsseldijk et al. (2019), referenced in publications 2 and 3.

In these studies, the term “deep diver” refers to those species that reach depths of more than 500 m in search of food (*Kogia* spp, *Physeter macrocephalus*, *Ziphius cavirostris*, *Mesoplodon* spp, *Globicephala macrorhynchus* and *Grampus griseus*) (Gannier 1998, Astruc & Beaubrun 2005, Aguilar de Soto 2006, Tyack et al. 2006, Watwood et al. 2006, West et al. 2009).

All the necropsy reports of the stranded cetaceans were systematically reviewed. For this, photographic record of the cases and histological samples were also reviewed in the cases that were necessary. Necropsy cases in which there was insufficient forensic evidence to determine a probable cause of death were classified as undetermined pathological entity.

The causes of death were grouped into the pathological entities described by Arbelo (2013) as follows:

- Natural pathology entities or not directly anthropogenic: intra-interspecific traumatic interaction, natural pathology associated with a significant loss of body condition, natural pathology associated with a moderate/good body condition, neonatal-perinatal pathology, and active stranding.
- Anthropogenic entities: fishery interaction, pathology associated with the ingestion of a foreign body, ship strike, and military manoeuvres associated with the use of sonar.

In publications 2 and 3, the results of the histological study of certain tissues (i.e., skeletal muscle, heart muscle, lung, liver, kidney, adrenal gland, brain) were reviewed and compared to find stress-related lesions.

In publication 3, the gas score and the determination of gas composition were carried out in one animal (case 26 of the study) following standardized protocols (Bernaldo de Quirós et al. 2011, 2013).

Statistical analysis was used to identify statistical significance between the variables and the different pathological entities of each study. Categorical variables (i.e., species, diving behaviour, age, sex, sexual maturity, body condition, location of stranding) were expressed as frequencies and percentages, and were appropriately compared using the Chi square test (χ^2) or the exact Fisher test. Statistical significance was set at $p < 0.05$. In publication 1, in order to identify factors that maintained an independent association with the entity, a multivariate logistic regression analysis was performed. The variables that showed a significant association with the entity in the univariate analysis were entered in the multivariate analysis. Selection of variables based on complete enumeration algorithm and Bayes Information Criterion (BIC) was then performed. The models were summarized as coefficients (SE), p-values (likelihood ratio test) and odds ratios which were estimated by confidence intervals at 95%. The data of all the analyses were treated with the R package, in version 3.3.1. for publication 1 and 2, and in version 3.6.1 for article 3.

For statistical reasons, the different categories of these variables were regrouped as follows:

- Physical development: neonate-young, juvenile-subadult, and adult.
- Body condition: very poor-poor and moderate-good.
- Stranding location: In publication 1, the islands were regrouped by geographical proximity into eastern islands (Fuerteventura, Lanzarote, and La Graciosa), western islands (El Hierro, La Gomera, and La Palma) and Tenerife, and Gran Canaria. In publications 2 and 3, the islands were regrouped based on the presence of resident cetacean populations in eastern islands (Fuerteventura, Lanzarote, and La Graciosa), western islands (El Hierro and La Palma), La Gomera together with Tenerife, and Gran Canaria.

Result and Discussion: Scientific Publications



Results and Discussion:

Scientific Publications

- 1. Retrospective study of foreign body-associated pathology in stranded cetaceans, Canary Islands (2000- 2015)*



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Retrospective study of foreign body-associated pathology in stranded cetaceans, Canary Islands (2000–2015)[☆]



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ABSTRACT

Marine pollution, overrepresented by plastic, is a growing concern worldwide. However, there is little knowledge on occurrence and detrimental impacts of marine debris in cetaceans. To partially fill in this gap of knowledge, we aimed to investigate the occurrence and pathologies associated with foreign bodies (FBs) in a large cohort of cetaceans (n = 465) stranded in the Canary Islands. The Canary Islands shelter the greatest cetacean biodiversity in Europe, with up to 30 different species, of which nine are regularly present year around. We found at least one ingested FB in 36 out of 465 (7.74%) studied cetaceans, involving 15 different species, including eight out of the nine (80%) cetacean species present year-round in the Canary Islands. Risso's dolphin was the species most affected, followed by sperm whale, beaked whale and mysticetes. Plastic FB were the most common item found (80.56%). FB was directly associated with death in 13/36 (36.11%) animals. Poor body condition and deep diving behavior were found to be risk factors for FB ingestion, whereas the adult age was a protective factor. To the authors knowledge this is the first study that use statistical analysis to investigate risk and protective factors for FB ingestion. This study also provides insights of the potential impact caused by ingested FBs on the animal's health and mortality. This knowledge is critical to better understand and assess the impact of FB in cetaceans setting the scientific basis for prospective impact monitoring and future conservation policies.

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1. Introduction

The current period has been coined the 'Plastic Age' (Yarsley and Couzens, 1945) from a merging anthropological and ecological standpoint, as plastic has become the most prevalent and widespread element of marine litter (Cozar et al., 2014). Three hundred million tons of plastic are produced each year (Plastics Europe, 2015), from which up to 12.7 million tons are dumped into the ocean annually (Jambeck et al., 2015). It has been estimated that

250,000 tons of plastic are floating in the oceans (Eriksen et al., 2014), representing more than 50% of marine litter (Laist, 1987; Derraik, 2002; Moore, 2008; Simmonds, 2012; Di Benedetto and Ramos, 2014). The accumulation of marine debris is a growing global concern and an important threat to marine biodiversity (European Parliament and Council, 2008). At least 693 species have been described interacting with marine debris and 92% of encounters involved plastics (Gall and Thompson, 2015). Thousands of marine debris interactions, primarily involving entanglement and ingestion, have been reported in many cetacean species worldwide to date (Derraik, 2002; Gregory, 2009; Baulch and Perry, 2014; Lusher et al., 2018). Both above-mentioned interactions have proven devastating for individual cases and alarming for certain cetacean populations (Baulch and Perry, 2014; Fossi et al., 2016; Lusher et al., 2018).

[☆] This paper has been recommended for acceptance by Maria Cristina Fossi.

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Since the first report in 1970 (Laist, 1987), at least 462 cetaceans from 48 species ingested marine debris, being plastic the most commonly observed item (46%) (Baulch and Perry, 2014). The number of cases of foreign body (FB) ingestion in cetaceans increases each year all over the world (Baulch and Perry, 2014; Lusher et al., 2015, 2018; Unger et al., 2016, 2017). Unknown cetacean population mortality rates and partial necropsy examinations of stranded animals may result in an underestimation of interactions between cetaceans and marine litter (Williams et al., 2011). The scientific committee of the International Whaling Commission (IWC) encourages intensifying the studies on interaction between marine debris and cetaceans (IWC, 2016). Recognized immediate and chronic foreign body (FB) associated clinicopathological consequences include: starvation, malnutrition, loss of body condition (Laist, 1997), limited predator-avoidance capabilities (Secchi and Zarzur, 1999), reduced growth rates, reduced longevity, and reduced reproductive capacity (Laist, 1987; McCauley and Bjorndal, 1999; Gregory, 2009), as well as general debilitation mainly secondary to bleeding ulcers, obstructions, impaction and/or perforation of the digestive tract (Walker and Coe, 1990). Nevertheless, mortality rates and sublethal consequences in cetaceans remain poorly understood (Simmonds, 2012), especially in mysticetes (Fossi et al., 2016). Some reports have indicated no apparent pathology or health impact in individuals that ingested FBs (Denuncio et al., 2011; Mazzariol et al., 2011; Unger et al., 2016); however, their long-term deleterious effects have been less studied (Jacobsen et al., 2010; Simmonds, 2012).

Several hypotheses have been formulated to explain FB ingestion: 'mistaken identity' with a prey (Carpenter and Smith, 1972), close proximity to the debris with a prey target or not echolocating at the ultimate approach to the prey (Ross, 1984; Mead, 1989), juvenile inexperience (Di Benedetto and Ramos, 2014), prey capture mechanism in beaked whales (MacLeod, 2007), playful and curious behavior (Laist, 1987), disease factors, and the stranding events (Walker and Coe, 1990).

The Canary Islands are located within the Macaronesia and shelter the greatest cetacean biodiversity in European waters, with up to 30 cetacean species recorded (Banco de Datos de Biodiversidad de Canarias). Four of these species are classified as endangered by the International Union for Conservation of Nature (IUCN): North Atlantic right whale (*Eubalaena glacialis*), blue whale (*Balaenoptera musculus*), sei whale (*Balaenoptera borealis*), and fin whale (*Balaenoptera physalus*). Nine cetacean species are regularly present year-round: common bottlenose dolphin (*Tursiops truncatus*), short-finned pilot whale (*Globicephala macrorhynchus*), striped dolphin (*Stenella coeruleoalba*), Risso's dolphin (*Grampus griseus*), sperm whale (*Physeter macrocephalus*), pygmy sperm whale (*Kogia breviceps*), Cuvier's beaked whale (*Ziphius cavirostris*), Blainville's beaked whale (*Mesoplodon densirostris*) and Gervais' beaked whale (*Mesoplodon europaeus*) (Martin et al., 2009). Recent reports revealed that the 'Canary Current' brings marine debris from the open Atlantic Ocean to the Canary Islands (Baztan et al., 2014). The concentration of plastic debris in the shallow waters of this archipelago is approximately 200–500 g.km⁻² (Cozar et al., 2014).

This study aims to investigate the pathologic findings associated with foreign bodies (FB associated pathology) and to study retrospectively the interaction between cetaceans and marine debris in the Canary Islands based on *postmortem* examinations.

2. Materials and methods

Four hundred and seventy-five cetaceans, stranded dead or alive along the coasts of the Canary Islands between January 2000 and December 2015, were necropsied following standardized protocols (Kuiken and García Hartmann, 1991). Stranding epidemiology

(location and date), life history data (species, age category, gender, gonad maturation, were systematically recorded following standardized protocols (Geraci and Lounsbury, 2005). During the necropsy, the body condition and the decomposition code of the carcass were evaluated. The digestive tract (i.e.: mouth, pharynx, esophagus, gastric chambers, intestines, and anus) was carefully dissected and examined. Gastric contents were routinely photographed, visually analyzed, described, and collected. Representative tissue samples were collected, fixed in 10% neutral buffered formalin, embedded in paraffin, processed and stained with hematoxylin and eosin as routine for histopathologic analysis.

Age categories were established based on total body length (Perrin et al., 2009) and histologic gonadal examinations (Geraci and Lounsbury, 2005) in: neonate or newborn (i.e.: animals with vibrissal hairs or vibrissal crypts, unhealed navel, fetal folds over the body, and soft and folded dorsal fin and tail flukes), calf (i.e.: animal with presence of milk in its stomach, or about the size of a nursing calf), juvenile, (i.e.: sexually immature animal with a body length smaller than the adult but larger than a calf), subadult, (i.e.: body length of an adult but immature gonads), and adult (i.e.: mature gonads).

The conservation condition of the animal was determined following Kuiken and García Hartmann, 1991 classification, with a small modification for code 1: we defined code 1 as an animal that has recently died or euthanized. The other codes remained the same: fresh (code 2), slight skin dryness, corneal opacity, onset of rigor mortis and/or presence of cadaveric lividity that disappears by digital pressure; moderate (code 3), also with delimited small red imbibition spots; advanced (code 4), skin slippage and blistering, sunken eyeball, tympanization due to gas formation, thickening of the tongue, protrusion of genitals and large imbibition spots; and very advanced autolysis (code 5), skin losing, ocular protrusion, tympanization with protrusion and/or evisceration of abdominal organs, liquefaction, mummification or adipocera.

Body condition was estimated based on the external physical conformation: the degree of concavity or convexity ventrolateral to the dorsal fin, the degree of depression posterior to the blowhole, the visibility of the ribs and transverse apophysis, as well as the presence or absence of nuchal and epicardial fat (Joblon et al., 2014). Four categories were established: very poor, animals with extremely concave dorsal profile, visible costal reliefs, body fat low or absent, and fatty serous atrophy; poor, animals with concave dorsal profile, low body fat and the ribs can be noted by palpation; fair, animal with dorsal profile straight or slightly convex and moderate body fat; and good, animals with a dorsal convex profile and abundant body fat.

Pathological categories (Arbelo et al., 2013) were established for each case according with anatomopathological findings in: anthropogenic pathologies [i.e.: FB associated pathology (when the lesions due to FB presence are directly related to the death of the animal), ship collisions, interaction with fishing activities, military maneuvers using sonar, and atypical mass stranding], natural or "not directly anthropogenic pathologies" [i.e.: pathology associated with significant loss of body condition, with good body condition, neonatal-perinatal pathology, intra/interspecific traumatic interactions, and typical mass stranding] and not determined.

For statistical purposes, species with very low stranding events were removed (n = 10) (Table 1). In addition, age categories were regrouped in the following categories: neonate/calves, juvenile/subadults and adults. Body condition categories were regrouped in two categories: poor/very poor and good/fair. Finally, the strandings were also studied by location. For this purpose, we grouped Fuerteventura and Lanzarote coasts (eastern islands), as well as El Hierro, La Palma and La Gomera coasts (western islands), and left alone Tenerife and Gran Canaria coast.

We defined deep diving species as those known to dive deeper than 500m for foraging (*Kogia* spp, *Physeter macrocephalus*, *Ziphius cavirostris*, *Mesoplodon* spp, *Globicephala macrorhynchus* and *Grampus griseus*) (Gannier, 1998; Astruc and Beaubrun, 2005; Aguilar de Soto, 2006; Tyack, 2006; Watwood et al., 2006; West et al., 2009).

2.1. Statistical analysis

In order to identify the factors associated with the presence of FB and FB associated pathology, categorical variables (i.e.: age, sex, sex mature, species, coast, FB presence, FB associated pathology, body condition) were expressed as frequencies and percentages, and compared as appropriate, using the Chi-square (χ^2) test or the exact Fisher test (Table 2).

In order to identify factors that maintain independent association with the outcome, a multivariate logistic regression analysis was performed. The variables that showed significant association with the corresponding outcome in univariate analysis were entered into the multivariate analysis. Selection of variables based on complete enumeration algorithm and Bayes information criterion (BIC) was then performed. The models were summarized as coefficients (SE), p-values (likelihood ratio test) and odds ratios which were estimated by confidence intervals at 95%. Statistical significance was set at $p < 0.05$. Data were analyzed using the R package, version 3.3.1 (R Development Core Team, 2016).

3. Results

This sixteen-year retrospective study revealed that FBs were found in 36 cetaceans (7.74% of stranded and necropsied cetaceans in the archipelago); being plastic the most prevalent item found (80.56%). Fifteen cetacean species were affected by marine debris ingestion. We described ulcerative gastritis with digested blood (14/36; 38.89%), impacted stomach (9/36; 25%) and gastro-intestinal perforations (3/36; 8.33%), as the main lesions contributing to the death of 13 animals. High statistical significance differences for FB presence were detected between species, age, body conditions and diving behavior. Poor body condition and deep diving were found to be risk factors for FB ingestion; meanwhile the adult age was a protective variable.

3.1. Foreign bodies found in stranded cetaceans

In the present study, 36/465 (7.74%) cetaceans presented FB ingestion. Plastic debris, mainly plastic bags, but also plastic caps, nylon wires, and cylindrical plastic items were found within the gastric contents in 29/36 (80.56%) animals. This represented 29/465 (6.24%) of total necropsied animals considered in the study. Ropes and threads (7/36; 19.44%), metal filaments (3/36; 8.33%), electric wire (1/36; 2.78%), fragments of cloth (1/36; 2.78%) and glass fragments (1/36; 2.78%) were also observed. Nine animals had more than one item in the digestive tract. (Table 1. Fig. 1).

3.2. Foreign body-associated lesions

FBs were found in the oral cavity (tongue, maxilla, jaw), esophagus, first gastric compartment and intestine. Two entangled whales were included in the FB cases, since the ropes and net were also affecting the digestive tract: a fin whale with a thick and long rope around the tongue that reached the stomach and a common minke whale with a 5.5 m long net entangled in the first third of the jaw and right pectoral fin as well as the dorsal fin.

Ulcerative gastritis with presence of luminal blood (14/36; 38.89%) and impacted stomach (9/36; 25.00%) were observed in

animals with FB ingestion. Also, gastro-intestinal perforations (3/36; 8.33%), ulcerative glossitis (2/36; 5.56%), stomatitis (2/36; 5.56%), healed ulcers (2/36; 5.56%) and petechiae (1/36; 2.78%) were observed. Pathological findings related to FB were absent in ten necropsied animals with presence of FBs (Table 1. Fig. 1).

3.3. Pathological categories

Pathological categories identified in the set of animals with FBs included: FB associated pathology (13/36; 35.14%); pathology associated with a significant loss of body condition (12/36; 33.33%); ship collision (5/36; 13.89%); intra- or interspecific traumatic interaction (2/38; 5.26%); pathology associated with a good body condition (3/36; 8.33%) and fishing interaction (1/36; 2.78%). The cause of death could not be determined in one animal that had FBs due to its advanced decomposition code.

The cause of death of 13 out of 36 cetaceans with FBs (35.14%) was related to gastrointestinal impaction (11/36; 30.56%) or gastro-intestinal perforations (2/36; 5.56%) (Table 1; Fig. 1). These cases represented 2.8% of total necropsied animals (13/465).

3.4. Statistical study of factors

3.4.1. Temporality of stranding events

The yearly average occurrence of FB ingestion in this study was 2.25 animals, 36 cases over 16 years. A higher number of animals ($n = 6$) was detected in 2014. Despite this focally increased number of FB ingestion in 2014, the statistical analysis discarded any trend, either in FB ingestion (Fig. 2) or FB associated pathology.

3.4.2. Location of stranded cetaceans

Statistically, stranding location is not a relevant epidemiologic factor regarding FB presence (p -value = 0.253) (Table 2). In decreasing order, animals with FBs stranded mostly in Tenerife (14/36; 38.89%), Gran Canaria (11/36; 30.56%), Fuerteventura and Lanzarote (11/36; 30.56%). Stranding location of FB associated pathology cases presented the same trend: Tenerife (5/13; 38.46%), Gran Canaria (4/13; 30.77%), Fuerteventura and Lanzarote (4/13; 30.77%). These results showed no significant difference for FB associated pathology and stranding location (p -value = 0.718). Western islands did not present FB or FB associated pathology cases. (Table 2).

3.4.3. Species

A total of 15 species presented FBs (Tables 1 and 2). Eight out of nine cetacean species regularly present year around in the Canary Islands presented FBs: Risso's dolphin (4/12; 33.33%), Blainville's beaked whale (2/6; 33.33%), sperm whale (6/28; 21.43%), Cuvier's beaked whale (5/33; 15.15%), Gervais' beaked whale (1/11; 9.09%), pygmy sperm whale (1/28; 3.57%), striped dolphin (3/92; 3.26%) and common bottlenose dolphin (1/40; 2.5%). In this study 34/448 (7.59%) odontocetes and 2/17 (11.76%) mysticetes were affected by FB ingestion.

Statistically significant differences were found between species with FBs presence (p -value < 0.001) (Table 2). Species with the highest prevalence of FB ingestion were Risso's dolphin (4/12; 33.33%), sperm whale (6/28; 21.43%) and *Mesoplodon* sp. (4/19; 21.05%). No statistically significance differences were found between species presenting with FB associated pathology (p -value = 0.105) (Table 2).

3.4.4. Diving behavior

Statistically significant differences were found between deep diving and shallow diving species and FB presence (p -value = 0.004) (Table 2), deep divers (21/36; 58.3%) presented the

Table 1

Cetaceans stranded with foreign body (FB) on the Canary Islands (January 2000 to December 2015). For each animal (36) we describe the species; stranding date (day/month/year) and location (F: Fuerteventura; GC: Gran Canaria; L: Lanzarote; T: Tenerife); gender (F: female; M: male); age; body condition (1: very poor; 2: poor; 3: fair; 4: good); decomposition code (1: very fresh, 2: fresh; 3: moderate autolysis; 4: advanced autolysis); FB observed; stomach contents; pathological findings and pathological categories (FBAP: foreign body associated pathologies; SC: Ship collision; FI: Fishing interaction; I: Intra/interspecific interaction; NPGBC: Natural pathology associated with good body condition; NPALBC: Natural pathology associated with significant loss of body condition; N.D.: Not determined). Species with very low stranding events within the study period were removed from the study for statistical purposes: rough-toothed dolphins (*Steno bredanensis*; n = 3) Fraser's dolphin (*Lagenodelphis hosei*; n = 3), false killer whale (*Pseudorca crassidens*; n = 2); Orca (*Orcinus orca*; n = 1) and harbour porpoise (*Phocoena phocoena*; n = 1). Of these animals, only one Fraser's dolphin presented with FB.

Case	Species	Stranding date	Stranding location	Gender	Age	Body condition	Decomposition code	FB	Stomach contents	Pathological findings	Pathological category
1	<i>Balaenoptera physalus</i>	15/04/2000	F	F	Adult	1	2	Rope	N.D.	Glossitis and impacted stomach	FBAP
2	<i>Physeter macrocephalus</i>	12/06/2000	T	M	Calf	4	4	Plastic bags	Squid beaks	No	SC
3	<i>Ziphius cavirostris</i>	16/07/2000	T	F	Juvenile	2	3	Plastic bags	Squid beaks	Impacted stomach. Digested blood in stomach	FBAP
4	<i>Stenella frontalis</i>	10/03/2001	T	M	Juvenile	2	3	Plastic bags	N.D.	Impacted stomach. Digested blood in stomach	FBAP
5	<i>Kogia breviceps</i>	15/01/2002	GC	M	Juvenile	4	4	Plastic debris	Squid beaks and digested fishes	No	FI
6	<i>S. frontalis</i>	25/01/2002	GC	M	Adult	N.D.	4	Plastic bags	N.D.	Impacted stomach. Digested blood in stomach	FBAP
7	<i>P. macrocephalus</i>	06/02/2003	T	M	Adult	1	3	Plastic wire	Squid beaks and lens	Ulcerative stomatitis with associated necrosis and fibrosis	FBAP
8	<i>Mesoplodon densirostris</i>	18/04/2004	T	M	Adult	2	1	Plastic debris and stone	Fishes	No	NPALBC
9	<i>S. coeruleoalba</i>	26/04/2004	T	M	Subadult	2	2	Plastic bag	N.D.	Impacted stomach	FBAP
10	<i>Steno bredanensis</i>	06/08/2004	GC	M	Juvenile	1	2	Plastic debris	Squid beaks and not digested food	Congestion and ulcerative gastritis	NPALBC
11	<i>P. macrocephalus</i>	11/05/2005	F	F	Subadult	4	4	Plastic debris	Squid beaks and lens	Perforation and ulcerative gastritis.	SC
12	<i>S. frontalis</i>	10/12/2005	GC	M	Adult	2	2	Hook and glass fragment	Absence	No	NPALBC
13	<i>M. europaeus</i>	06/04/2006	GC	F	Juvenile	3	2	Thread and metal filament	Squid beaks	Perforation and ulcerative gastritis.	FBAP
14	<i>Z. cavirostris</i>	06/07/2006	T	ND	Juvenile	3	3	Plastic debris and plastic wire	Squid beaks	Impacted stomach. Digested blood in stomach	FBAP
15	<i>S. frontalis</i>	01/01/2007	L	F	Subadult	3	1	Plastic bags	Absence	Impacted stomach. Digested blood in stomach. Ulcerative gastritis.	FBAP
16	<i>M. bidens</i>	16/04/2007	L	M	Adult	1	2	Plastic plug	Absence	Healed ulcers	SC
17	<i>Delphinus delphis</i>	28/04/2007	T	M	Adult	2	3	Plastic debris and ropes	Fishes	No	NPALBC
18	<i>Grampus griseus</i>	20/04/2008	T	M	Juvenile	2	2	Ropes, plastic debris and metal filaments	N.D.	Ulcerative gastritis	NPALBC
19	<i>St. frontalis</i>	26/04/2008	F	F	Calf	2	3	Plastic debris	Squid beaks and lens	No	NPALBC
20	<i>G. griseus</i>	07/11/2008	F	F	Calf	1	2	Thread	Squid beaks	Healed ulcers	I
21	<i>S. bredanensis</i>	18/11/2008	GC	M	Adult	1	2	Plastic bag	Squid beaks	Digested blood and ulcerative gastritis	NPALBC
22	<i>S. coeruleoalba</i>	22/01/2009	F	F	Adult	2	2	Metal filament	N.D.	Congestion and ulcerative gastritis	NPALBC
23	<i>B. acutorostrata</i>	09/04/2009	GC	F	Calf	1	2	Netting remains	Digested food	Ulcerative glossitis and stomatitis	FBAP
24	<i>P. macrocephalus</i>	27/06/2009	T	F	Adult	3	3	Rope	Absence	Digested blood, ulcerative gastritis and congestion.	SC
25	<i>P. macrocephalus</i>	16/03/2010	T	F	Calf	3	3	Plastic bags and stones	Squid beaks and digested food	Petechiae	I
26	<i>G. griseus</i>	22/04/2010	T	M	Juvenile	2	1	Plastic bag and sand	Squid beaks (3)	Ulcerative gastritis	NPALBC
27	<i>Z. cavirostris</i>	13/06/2011	T	M	Subadult	3	4	Plastic debris	N.D.	No	SC
28	<i>P. macrocephalus</i>	07.09.2011	T	F	Adult	3	4	Electric wire, nylon wire	Squid beaks	No	N.D.
29	<i>G. griseus</i>	03/11/2012	F	M	Subadult	2	3	Plastic bags and rope	N.D.	Impacted stomach. Digested blood in stomach. Ulcerative gastritis. Forestomach mucosa hyperplasia.	FBAP

Table 1 (continued)

Case	Species	Stranding date	Stranding location	Gender	Age	Body condition	Decomposition code	FB	Stomach contents	Pathological findings	Pathological category
30	<i>Z. cavirostris</i>	09/02/2013	L	M	Juvenile	3	3	Plastic debris	Squid beaks and lens	Intestinal perforation and ulcerative gastritis.	FBAP
31	<i>M. densirostris</i>	12/02/2014	L	F	Adult	4	3	Rope and plastic debris	Squid, squid beaks and fishes	Ulcerative gastritis and petechiae	NPAGBC
32	<i>K. breviceps</i>	20/03/2014	GC	F	Calf	3	2	Plastic debris	N.D.	Impacted stomach. Digested blood in stomach. Congestion and ulcerative gastritis	FBAP
33	<i>S. coeruleoalba</i>	23/03/2014	GC	F	Subadult	3	1	Plastic debris	N.D.	Ulcerative gastritis	NPAGBC
34	<i>S. bredanensis</i>	01/05/2014	GC	M	Calf	2	1	Plastic debris, sand	Sea sponges	No	NPALBC
35	<i>Z. cavirostris</i>	06/06/2014	L	F	Adult	3	3	Plastic debris	Squid beaks and crustaceans	Congestion and digested blood	NPAGBC
36	<i>Tursiops truncatus</i>	23/07/2014	GC	F	Adult	2	2	Clothes (bra)	Fishes	No	NPALBC

Table 2

Characteristics of the cetaceans according to presence/absence of FB and FB associated pathology. Categorical variables are expressed as frequencies and percentages - in brackets- and were compared, as appropriate, using the Chi-square (χ^2) test or the exact Fisher test.

	Overall	FB presence		FB Associated Pathology			
	N = 465	No N = 429	Yes N = 36	No N = 452	Yes N = 13	p-value	Test
Age						0.053	Chi ²
Neonate/Calve	126 (27.1)	114 (26.6)	12 (33.3)	121 (26.8)	5 (38.5)		
Juvenile/Subadult	135 (29.0)	120 (28.0)	15 (41.7)	128 (28.3)	7 (53.8)		
Adult	204 (43.9)	195 (45.5)	9 (25.0)	203 (44.9)	1 (7.7)		
Sex						0.913	Chi ²
Female	216 (47.7)	199 (47.6)	17 (48.6)	210 (47.6)	6 (50.0)		
Male	237 (52.3)	219 (52.4)	18 (51.4)	231 (52.4)	6 (50.0)		
Sex mature						0.067	Chi ²
Immature	228 (49.2)	205 (48.0)	23 (63.9)	218 (48.4)	10 (76.9)		
Mature	235 (50.8)	222 (52.0)	13 (36.1)	232 (51.6)	3 (23.1)		
Species						<.001	Fisher
Misticets	17 (3.7)	15 (3.5)	2 (5.6)	15 (3.3)	2 (15.4)		
<i>Kogia</i> spp.	34 (7.3)	32 (7.5)	2 (5.6)	33 (7.3)	1 (7.7)		
<i>Mesoplodon</i> spp.	19 (4.1)	15 (3.5)	4 (11.1)	18 (4.0)	1 (7.7)		
<i>Delphinus delphis</i>	45 (9.7)	44 (10.3)	1 (2.8)	45 (10.0)	0		
<i>Globicephala macrorhynchus</i>	41 (8.8)	41 (9.6)	0	41 (9.1)	0		
<i>Grampus griseus</i>	12 (2.6)	8 (1.9)	4 (11.1)	11 (2.4)	1 (7.7)		
<i>Physeter macrocephalus</i>	28 (6.0)	22 (5.1)	6 (16.7)	27 (6.0)	1 (7.7)		
<i>Stenella coeruleoalba</i>	92 (19.8)	89 (20.7)	3 (8.3)	91 (20.1)	1 (7.7)		
<i>S. frontalis</i>	83 (17.8)	78 (18.2)	5 (13.9)	80 (17.7)	3 (23.1)		
<i>Steno bredanensis</i>	21 (4.5)	18 (4.2)	3 (8.3)	21 (4.6)	0		
<i>Tursiops truncatus</i>	40 (8.6)	39 (9.1)	1 (2.8)	40 (8.8)	0		
<i>Ziphius cavirostris</i>	33 (7.1)	28 (6.5)	5 (13.9)	30 (6.6)	3 (23.1)		
Coast						0.253	Fisher
Hierro/Palma/Gomera	22 (4.7)	22 (5.1)	0	22 (4.9)	0		
Tenerife	130 (28.0)	116 (27.0)	14 (38.9)	125 (27.7)	5 (38.5)		
Gran Canaria	137 (29.5)	126 (29.4)	11 (30.6)	133 (29.4)	4 (30.8)		
Fuerteventura/Lanzarote	176 (37.8)	165 (38.5)	11 (30.6)	172 (38.1)	4 (30.8)		
Body condition						0.002	Chi ²
Poor/Very poor	140 (33.6)	120 (31.4)	20 (57.1)	133 (32.8)	7 (58.3)		
Good/Fair	277 (66.4)	262 (68.6)	15 (42.9)	272 (67.2)	5 (41.7)		
Diving behavior						0.004	Chi ²
Shallow diver	298 (64.1)	283 (66.0)	15 (41.7)	292 (64.6)	6 (46.2)		
Deep diver	167 (35.9)	146 (34.0)	21 (58.3)	160 (35.4)	7 (53.8)		

highest prevalence while shallow divers the lowest (15/36; 41.7%). Regarding with the total deep-diving necropsied cetaceans, 21/167 (12.57%) of them presented FB.

Deep diving behavior was found to be a risk factor for the presence of FB (OR = 3.330; 95%CI = 1.470; 7.546) (Table 3). In contrast, the relationship between diving behavior and animals with FB associated pathology was not as clear (p-value = 0.172): deep diver (7/13; 53.8%) and shallow diver (6/13; 46.2%) (Table 2).

3.4.5. Sex and gonads maturation

No significant difference (p-value = 0.913) regarding sex and FB presence [females (17/36; 48.6%); males (18/36; 51.4%)] nor sex and FB associated pathology (p-value = 0.871) [(females (6/13; 46.15%); males (6/13; 46.15%)] were found (Table 2). The sex could not be determined in one animal with FB ingestion due to evisceration with loss of reproductive tissues produced by shark bites.

Quasi statistically significant differences (p-value = 0.067) for

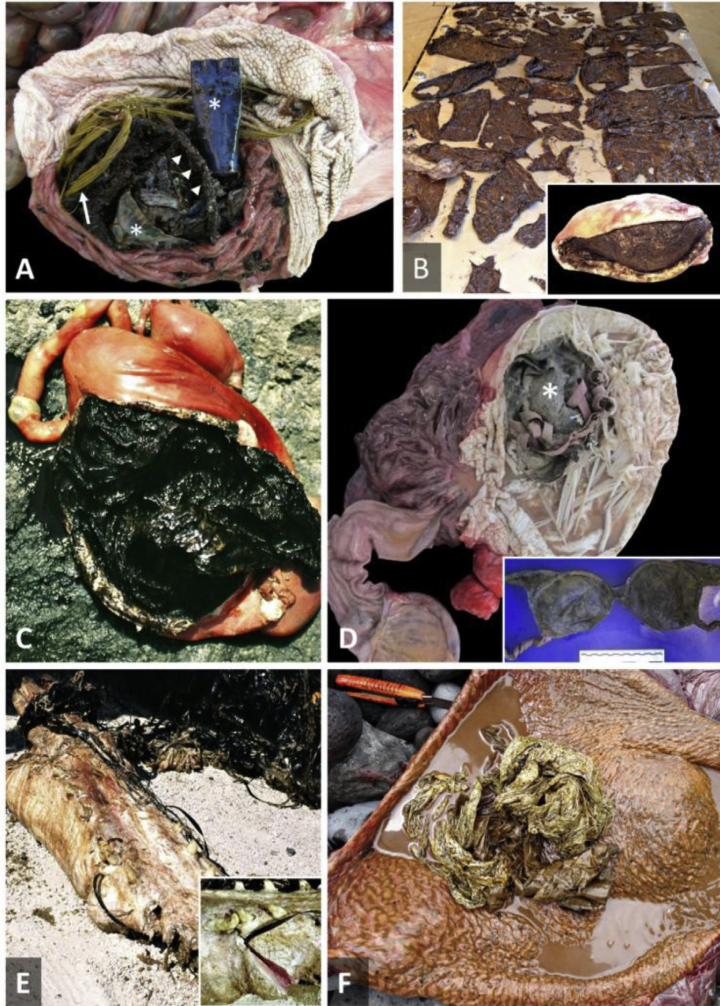


Fig. 1. A, Obstruction of the forestomach caused by plastic debris (asterisk), metal filament (arrow) and ropes (arrowheads), *Grampus griseus*, case 20; B, Approximately 20 digested plastic bags found in the forestomach, stomach impaction (inset), *Stenella frontalis*, case 15; C, Impaction of the forestomach caused by plastic debris, *S. frontalis*, case 4; D, Foreign body (a bra) with digested fishes in the forestomach, exposed mainstomach and pyloric stomach, full bra found in the forestomach (inset), *Tursiops truncatus*, case 36; E, Plastic packing tape in oral cavity wrapped with pieces of ropes and nets and large quantity of marine organisms (algae, crustaceans, molluscs), penetrating lesion into oral mucosa caused by the foreign body (inset), *Physeter macrocephalus*, case 7; F, Plastic debris in the main stomach, congestion and digested blood, *Ziphius cavirostris*, case 14.

presence of FBs were found between sexually immature (23/36; 63.89%) and mature (13/36; 36.11%) animals (Table 2). This difference was statistically significant for FB associated pathology (p-value = 0.043), where the majority of the animals dead due to FB ingestion were immature (10/13; 76.92%). Mature animals affected by FB ingestion represented only 3/13 (23.08%) of the FB associated pathology cases (Table 2).

3.4.6. Age

Quasi statistically significant differences (p-value = 0.053) for presence of FBs were found between age categories. Highest prevalence was detected in juveniles/subadults (15/36; 41.7%) followed by calves (12/36; 33.33%), being adults the lowest (9/36; 25.0%) (Table 2). There was an absence of FB in neonates.

The difference between age categories for FB associated pathology was statistically significant (p-value = 0.024) (Table 2). The highest prevalence in FB associated pathology was in juveniles/subadults (7/13; 53.85%). Only one adult died by FB associated pathology (1/13; 7.69%). The adult age showed independent association with the presence of FB and was found to be a protective factor (OR = 0.233; 95%CI = 0.084; 0.641) (Table 3).

3.4.7. Body condition

The results show a high statistical significance difference between the presence of FB and body condition (p-value = 0.002) (Table 2), being poor condition (13/36; 36.11%) the highest prevalence and good the lowest (4/36; 11.11%). Poor body condition was found to be a risk factor for the presence of FB (OR = 4.080; 95%

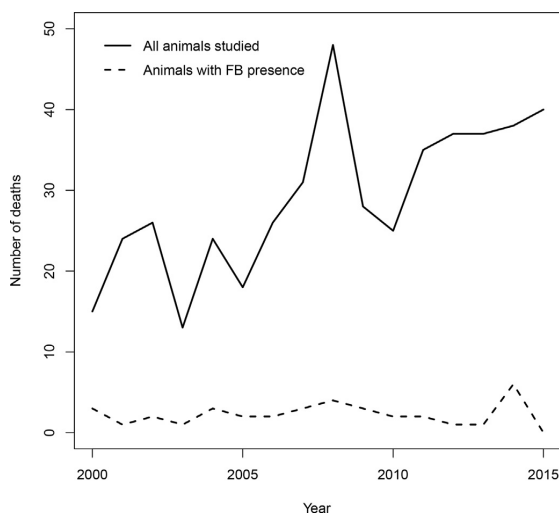


Fig. 2. Total studied cetaceans and foreign body cases per year of studied period (2000–2015).

Table 3

Multivariate logistic regression for the presence of foreign body. Odds ratio (OR), confidence interval (CI).

	Coefficient (SE)	P	OR (95% CI)
(Intercept)	−3.211 (0.416)	<0.001	0.040 (0.018; 0.091)
Adult age	−1.459 (0.517)	0.005	0.233 (0.084; 0.641)
Poor body condition	1.406 (0.419)	<0.001	4.080 (1.794; 9.278)
Deep diving behavior	1.203 (0.417)	0.004	3.330 (1.470; 7.546)

CI = 1.794; 9.278) (Table 3). In contrast, the relationship between body condition and animals with FB associated pathology was not as clear (p -value = 0.065) (Table 2): poor/very poor (7/13; 53.85%) and good/fair (5/13; 38.46%). In one case body condition could not be determined due to its advanced decomposition state.

4. Discussion

Plastic has been described as the most ubiquitous and prevalent debris in the ocean (Cozar et al., 2014). In this study, the FB most frequently found in stranded cetaceans was plastic (80.56%) as in the literature (e.g. Baulch and Perry, 2014). Other marine debris found in stranded cetaceans are nets, threats or ropes used by fishing activities (e.g. Jacobsen et al., 2010). Domestic and agricultural items have also been reported (e.g. De Stephanis et al., 2013; Unger et al., 2017).

FB ingestion caused fatal lesions in 13/36 (36.11%) animals. Similar lesions associated with ingested marine debris leading to eventual death have been previously described in the literature (e.g. Abollo et al., 1998; Stamper et al., 2006; Arbelo et al., 2013). In addition, ten animals with presence of FB had neither grossly nor microscopically evident lesions associated with FB ingestion. In a similar way Unger et al., 2016 described the presence of FB with absence of event lesions in a mass stranding of sperm whales along the North Sea coast in 2016. Mortality rates and sublethal harmful effects due to FB ingestion remain poorly understood (Williams et al., 2011; Simmonds, 2012), so further research is needed. It is important to consider that 50% of species reported in this

archipelago were affected by FB ingestion. A fin whale and six sperm whales, cataloged as endangered and vulnerable species at IUCN red list, were affected by marine debris pollution. Scarce reports have described FB in cetaceans of the Canary Islands before (Fernández et al., 2009; Arbelo et al., 2013), increasing from 8 to 15 affected species in this study. The impact of marine debris in cetaceans is of major conservation concern and should be further analyzed and compared with other geographical areas (Besseling et al., 2015; Unger et al., 2016). In this study, both odontocetes and mysticetes were affected by FB ingestion. High discrimination capacity has been assumed in odontocetes, which exhibit an active feeding linked to a highly developed echolocation system used for predation and orientation (Walker and Coe, 1990). By contrast, mysticetes present passive feeding behavior, filter the water to catch preys. Thus, baleen whales have been used as a ‘monitoring species’ because of their exposure to marine debris and microplastics directly from the water column as well as via prey species (Fossi et al., 2012, 2014, 2016). According to the similar percentages of affected animals found between both groups, we propose that odontocetes species be considered as sentinels of ocean macrolitter pollution.

Our results suggest that deep diving species ingest more FB than shallow diving species, as previously reported (Lusher et al., 2018). Species with some degree of association with the sea bottom are suggested to be bioindicators of marine debris pollution in previous studies (Di Benedetto and Ramos, 2014). However, this is the first study that has statistically analyzed the relationship between diving behavior and FB ingestion. The depth where the FB ingestion occurs is still unknown. No statistical significance was found between diving behavior and FB associated pathology. This could be explained by the small number of FB associated pathology cases.

This is the first study that has statistically analyzed the relationship between age categories and FB ingestion. Our results suggested that the adult age is a protection factor. Juvenile and subadult animals were the most affected ages by FB ingestion, followed by calves, while there was an absence of FB in neonates. Some authors have pointed out a ‘mistaken identity’ with a prey (Carpenter and Smith, 1972) or the foraging inexperience of immature animals (Di Benedetto and Ramos, 2014) as a possible cause of FB ingestion. In cetaceans, it is well known that juveniles are more curious about marine debris and interact more often with it (Laist, 1987). In other species of marine mammals, the presence of FB were most commonly found in juveniles (Unger et al., 2017). Neonates with FB have not been reported. Their feeding and social maternal dependence may explain this lack of interaction.

This study is also the first one in studying statistically the relationship between body condition and FB presence in cetaceans. Previously, starvation, malnutrition (Laist, 1997) or limited predator-avoidance capabilities (Secchi and Zarzur, 1999) were described as consequence of FB ingestion. Other authors proposed loss body condition as a risk factor for FB ingestion (Baird and Hooker, 2000; de Meirelles and do Rego Barros, 2007; Unger et al., 2017) associated or not with pre-existing disease factors, such as parasitism (Walker and Coe, 1990). Our study showed high statistical significance between body condition and presence of FB. Poor body condition was found to be a risk factor for FB ingestion but not for FB associated pathology. Acute lethal lesions, such as perforation or impaction, caused the death of the animals without loss of body condition in 5 cases (5/13; 38.46%). Loss of body condition can also occur as a consequence of FB ingestion in chronic cases. Though, in other cases the presence of FB was not associated with lesions or a deficient body condition, as previously reported (Unger et al., 2016). The long-term deleterious effects of FB ingestion have been less studied (Jacobsen et al., 2010; Simmonds, 2012) and need further research.

Additional investigations should take special emphasis on microplastics, plastic fragments smaller than 5 mm. Their toxicological potential is due to persisted organic pollutants (POP's) added during plastics' manufacture and other contaminants (e.g. polyethylene, polypropylene and phthalates) adsorbed from the surrounding seawater. Those pollutants are considered human and wildlife health risks that can potentially affect the physiology of organisms (Teuten et al., 2007; Latini, 2005) and be bioaccumulated on the food-chain (Carpenter and Smith, 1972; Teuten et al., 2009; Cole et al., 2011). Microplastics have been found in at least 180 marine species, including a True's beaked whale (*Mesoplodon mirus*) and a humpback whale (*Megaptera novaeanglia*) (Besseling et al., 2015; Lusher et al., 2015). Also, the presence of leached plastic additives has indicated chronic exposure in Mediterranean fin whales (Fossi et al., 2012).

5. Conclusions

The results from this study provided insights of the potential impact cause by ingested FBs on the animal's health and mortality. Marine debris were found affecting many different species of both shallow and deep diving behavior, the latter group being the most affected. Most FBs were of plastic nature. Ingested FB were associated with cause of death in more than one third of the cases, due to lethal lesions such as impactions and gastrointestinal perforations. To the authors knowledge this is the first study that used statistical analysis to investigate risk and protective factors for FB ingestion: poor body condition and deep diving behavior were found to be risk factors for FB ingestion, meanwhile the adult age was a protective factor. The results from this study contributes to increase the knowledge of FB impact in cetacean's health. This is critical to set the scientific basis for prospective impact monitoring and future conservation policies.

Declaration of interests

None.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2018.09.012>.

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2. *Retrospective study of traumatic intra-
interspecific interactions in stranded cetaceans,
Canary Islands*



Retrospective Study of Traumatic Intra-Interspecific Interactions in Stranded Cetaceans, Canary Islands

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Aggressive encounters involving cetacean species are widely described in the literature. However, detailed pathological studies regarding lesions produced by these encounters are scarce. From January 2000 to December 2017, 540 cetaceans stranded and were necropsied in the Canary Islands, Spain. Of them, 24 cases of eight species presented social traumatic lesions produced by cetaceans of the same or different species. All the cases presented severe multifocal vascular changes, 50% (12/24) presented fractures affecting mainly the thoracic region, 41.7% (10/24) acute tooth-rake marks, 37.5% (9/24) undigested food in the stomach, 33.3% (8/24) tracheal edema, and 12.5% (3/24) pulmonary perforation. In 10 cases with tooth-rake marks, the distance between the teeth, allowed us to further identify the aggressor species: four cases were compatible with killer whales (*Orcinus orca*) affecting three species [pigmy sperm whale (*Kogia breviceps*), Cuvier's beaked whale (*Ziphius cavirostris*), and short-finned pilot whale (*Globicephala macrorhynchus*)] and four cases compatible with common bottlenose dolphins (*Tursiops truncatus*) affecting two species [short-beaked common dolphin (*Delphinus delphis*) and Atlantic spotted dolphin (*Stenella frontalis*)]. We also described two cases of intraspecific interaction in striped dolphin (*Stenella coeruleoalba*). Microscopically, 70.8% (17/24) of the cases presented acute degenerative myonecrosis, 66.7% (14/21) presented vacuoles in the myocardiocytes, 36.8% (7/19) pigmentary tubulonephrosis, 31.6% (6/19) cytoplasmic eosinophilic globules within hepatocytes, 21.4% (3/14) hemorrhages in the adrenal gland, and 17.3% (4/23) bronchiolar sphincter contraction. The statistical analysis revealed that deep divers, in good body condition and nearby La Gomera and Tenerife were more prone to these fatal interactions. Additionally, in this period, three animals died due to an accident during predation: a false killer whale (*Pseudorca crassidens*) died because of a fatal attempt of predation on a stingray, and two Risso's dolphins (*Grampus griseus*) died as a consequence of struggling while predating on large squids.

Keywords: tooth-rake marks, social interaction, prey interaction, dolphin, aggression, trauma

INTRODUCTION

The term “intra-interspecific interactions” refers to interaction with individuals of the same species (intraspecific) or with other species (interspecific). When the interactions become aggressive it may lead to serious injuries and/or death of the animal (1–4).

Social intra-interspecific interactions can produce mild multifocal lesions over the skin known as “tooth-rake marks” (external linear and parallel erosions on the skin inflicted by teeth), frequently observed healed in stranded animals. However, when interactions became aggressive, tooth-rake marks could be severe and ulcerate the skin affecting the subcutaneous and muscle tissue. Other lesions that had been reported in fatal encounters include: blunt traumas with subcutaneous focal or multifocal extensive hemorrhages, hematomas, tearing of the blubber, vertebral and/or ribs fractures, myonecrosis, tearing of the parietal pleura with associated-pulmonary hemorrhages, hemothorax, retroperitoneal hemorrhages, perforation of the abdominal wall, and liver rupture (3–6). Histologically, acute monophasic degeneration and hemorrhages in the muscle are common findings (4, 7). Pulmonary fat emboli (8) and myo-/hemoglobinuric nephrosis (4) can be observed by specific staining.

Aggressive encounters involving individuals of the same species are largely described in the literature [e.g., (9, 10)], including the formation of male alliances (11–13). Male alliances are responsible for violent kidnappings (“herding events”) of non-pregnant females to increase their mating opportunities as well as of infanticides in different cetacean species such as the Amazon river dolphin (*Inia geoffrensis*) (14), Indo-Pacific humpback dolphin (*Sousa chinensis*) (15), killer whale (16), tucuxi dolphin (*Sotalia guianensis*) (17), and bottlenose dolphin (*Tursiops* spp.) (1, 2, 18, 19). In Mysticetes, males humpback whales (*Megaptera novaeangliae*) have been reported escorting receptive females and threatening other males by thrashing of their flukes or signing as communication signals in the context of male competition (20). Although male coalitions have also been observed in whales, aggressive reactions are not usual, and fights rarely result in serious injury or death (10).

Violent interspecific interactions with other species may occur for reasons other than sexual competition, such as prey competition (21), fight practice (6), or predation on cetaceans and non-cetacean species. Killer whales (*Orcinus orca*) have been observed attacking or harassing about 20 different species of cetaceans, including both, odontocetes and Mysticetes (22–28). In addition, false killer whales (*Pseudorca crassidens*) predate on species of the genus *Stenella* spp. and short-beaked common dolphins (29).

The Canarian waters are known for their particular oceanographic features and their enormous diversity of cetacean species, with 30 species described so far (Banco de Datos de Biodiversidad de Canarias), some of them regularly seen year-round (30). Although there is evidence of habitat partitioning in the waters used by several cetacean species in La Gomera (31), most species coexist in other areas of the Canary Islands. This confluence is motivated by factors such as temperature, deep waters near the coast, an abundance

of food resources, and calm waters in southwestern regions. Thus, numerous interactions between different cetacean species inhabiting these waters are expected.

This study aims to investigate the prevalence and the pathologic findings associated with social traumatic interactions between cetacean species and foraging fatalities in the Canary Islands, based on postmortem examinations.

MATERIALS AND METHODS

Post-mortem examinations following standardized protocols (32) were carried out on 540 stranded cetaceans in Canary Islands, Spain, from 2000 to 2017. Required permission for the management of stranded cetaceans was issued by the environmental department of the Canary Islands’ Government and the Spanish Ministry of Environment. Experiments on live animals were not performed.

Epidemiology of the stranding (i.e., location and date), life history data (i.e., species, age class, sex, gonad maturation), and body condition were systematically recorded following standardized protocols (33). Age class (i.e., neonate, calf, juvenile, subadult, and adult) was established based on total body length (20), histologic gonadal examinations (33), and in some cases, osteological studies (34). Body condition was estimated based on the external physical conformation (the degree of epiaxial concavity or convexity, nuchal depression, the visibility of the ribs and vertebral transverse processes, as well as the presence or absence of nuchal and epicardial fat) in very poor, poor, fair and good body condition (35). For decomposition status, five codes were applied following IJsseldijk (36) classification: very fresh (code 1), fresh (code 2), moderate autolysis (code 3), advanced autolysis (code 4), or very advanced autolysis (code 5).

External and internal lesions were fully described, photographed, and sampled. Tissue samples were immersed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, processed, sectioned at 5 μ m and stained with hematoxylin and eosin for histopathologic analysis.

For the diagnosis of traumatic intra-interspecific interactions we took a conservative approach based on previous references (5, 6, 37) excluding the cases in which other possible traumatic causes of death such as fisheries interaction, vessel collision, or a live stranding (38–42) could not be ruled out. Stress-related lesions were histologically studied in selected samples upon availability [skeletal muscle ($n = 24$), lung ($n = 23$), cardiac muscle ($n = 21$), liver ($n = 19$), kidney ($n = 19$), and adrenal gland ($n = 14$)].

To identify factors associated with death due to intra-interspecific interaction between cetaceans ($n = 24$), categorical variables (i.e., species, diving behavior, age, sex, maturity, location, and body condition) were expressed as frequencies and percentages and were compared, as appropriate, using the Chi-square (χ^2) test or the exact Fisher test. For statistical purposes, age classes were regrouped in neonate/calves, juvenile/subadults, and adults; and body condition categories were regrouped in poor/very poor and good/fair. Stranding locations were also regrouped based on geographical proximities and the presence

of high-site fidelity populations: Eastern islands (Fuerteventura and Lanzarote), Western Islands (El Hierro and La Palma), La Gomera and Tenerife, and Gran Canaria. Statistical significance was set at $p < 0.05$. Data were analyzed using the R package, version 3.3.1 (43).

RESULTS

Between January 2000 and December 2017, a total of 540 cetaceans stranded along the coasts of the Canarian archipelago

were necropsied. A pathological entity (category of cause of death) was identified in 432 cases. Of them, 27 individuals (6.3%) presented severe lesions consistent with aggressive intra-interspecific interactions. In 88.9% (24/27) of the cases, social traumatic interactions between cetaceans of the same or different species produced blunt-force traumas that led to death. In 11.1% (3/27) of the cases, the animals died due to fatal accidents while foraging on potential prey (squid or stingray) (Table 1). Two out of 27 affected cetaceans were found stranded alive (case no 14 and 18).

TABLE 1 | Twenty-seven cetaceans dead due to traumatic intra-interspecific interaction the Canary Islands (from January 2000 to December 2017), between cetaceans ($n = 24$) or because a failure in the predation ($n = 3$).

Case	Species	Diving behavior	Stranding date	Island	Stranding event	Sex	Age	Body condition	Decomposition state	Sexual maturity	Traumatic behavior
1	<i>Globicephala macrohynchus</i>	D	14.07.2003	FV	D	F	Adult	2	2	M	S
2	<i>Mesoplodon europaeus</i>	D	08.09.2003	TNF	D	M	Calf	2	3	I	S
3	<i>Stenella frontalis</i>	S	09.06.2004	TNF	D	M	Neonate	2	3	I	S
4	<i>Stenella coeruleoalba</i>	S	05.02.2005	LNZ	D	F	Adult	2	2	M	S
5	<i>Stenella coeruleoalba</i>	S	14.06.2005	TNF	D	F	Juvenile	2	3	I	S
6	<i>Kogia breviceps</i>	D	31.03.2006	LG	D	F	Adult	2	3	M	S
7	<i>Mesoplodon europaeus</i>	D	28.07.2006	TNF	D	M	Calf	2	3	I	S
8	<i>Globicephala macrohynchus</i>	D	30.11.2006	GC	D	M	Adult	ND	4	M	S
9	<i>Kogia breviceps</i>	D	06.04.2007	TNF	D	F	Adult	2	3	M	S
10	<i>Kogia breviceps</i>	D	29.08.2007	LNZ	D	F	Juvenile	1	3	M	S
11	<i>Globicephala macrohynchus</i>	D	07.09.2007	TNF	D	M	Neonate	ND	5	I	S
12	<i>Delphinus delphis</i>	S	14.01.2008	TNF	D	M	Calf	2	3	I	S
13	<i>Delphinus delphis</i>	S	08.03.2008	TNF	D	M	Calf	2	3	I	S
14	<i>Pseudorca crassidens</i>	S	11.03.2008	LNZ	A	M	Calf	1	2	I	P
15	<i>Delphinus delphis</i>	S	09.07.2008	FV	D	M	Calf	1	4	I	S
16	<i>Stenella coeruleoalba</i>	S	09.02.2009	GC	D	F	Calf	2	2	I	S
17	<i>Grampus griseus</i>	D	06.03.2009	FV	D	M	Subadult	2	2	I	P
18	<i>Globicephala macrohynchus</i>	D	06.07.2009	TNF	A	M	Subadult	2	2	M	S
19	<i>Stenella frontalis</i>	S	13.04.2010	TNF	D	F	Adult	2	2	M	S
20	<i>Grampus griseus</i>	D	17.09.2010	TNF	D	F	Adult	1	2	M	P
21	<i>Tursiops truncatus</i>	S	05.08.2011	TNF	D	F	Calf	ND	4	I	S
22	<i>Globicephala macrohynchus</i>	D	24.08.2011	FV	D	F	Calf	1	3	I	S
23	<i>Stenella frontalis</i>	S	19.03.2013	TNF	D	F	Adult	2	3	M	S
24	<i>Globicephala macrohynchus</i>	D	16.06.2013	TNF	D	M	Calf	2	4	I	S
25	<i>Globicephala macrohynchus</i>	D	25.02.2015	LNZ	D	F	Juvenile	2	4	M	S
26	<i>Globicephala macrohynchus</i>	D	20.05.2015	TNF	D	M	Adult	2	4	M	S
27	<i>Ziphius cavirostris</i>	D	22.05.2017	GC	D	M	Adult	ND	4	ND	S

The table shows the species, diving behavior (D, deep diver; S, shallow diver), stranding date (day.month.year), location (FV, Fuerteventura; GC, Gran Canaria; LG, La Gomera; LNZ, Lanzarote; TNF, Tenerife), the type of stranding event (D, death; A, alive), sex (F, female; M, male), age (neonate, calf, juvenile, subadult, adult) of each case ($n = 27$). Forensic studies allow us to know the following data: body condition (1: poor/very poor; 2: good/fair), decomposition state (2: fresh; 3: moderate autolysis; 4: advanced autolysis), sexual maturity (I, immature; M, mature), and the traumatic behavior that cause the death of the animal (S: social traumatic interaction between cetaceans of the same species or other; P: death due to an accident during predation).

SOCIAL TRAUMATIC INTERACTIONS BETWEEN CETACEANS

Gross Findings

All the animals diagnosed with intra-interspecific trauma (24/24) presented multifocal severe vascular changes such as hemorrhages in the blubber; 62.5% (15/24) presented hemorrhages and/or congestion in the central nervous system (Figure 1F); 54.2% (13/24) presented subcutaneous hematomas (Figures 1C,D); 50% (12/24) presented hemothorax; 29.2% (7/24) presented hemoabdomen, and 4.2% (1/24) presented hemopericardium (Table 2).

Healed tooth-rake marks (linear non-severe parallel superficial skin lesions) compatible with social intraspecific behavior were observed in 95.8% of the cases (23/24). Severe acute multifocal tooth-rake marks were found in 41.7% of the cases (10/24) (Figures 1A,B). Tooth-rake marks were compatible with killer whale interaction in two pregnant female pigmy sperm whales (cases 9 and 10), a calf short-finned pilot whale (case 22), and an adult Cuvier's beaked whale (case 27). In these cases, 28–43 mm separation between tooth-rake marks was observed but also punctures (Figure 1A). The other four animals, three calves of short-beaked common dolphin (cases 12, 13, and 15) and one adult of Atlantic spotted dolphin (case 19), presented 7–12 mm separation tooth-rake marks compatible with adult bottlenose dolphin interaction (Table 3). Also, intraspecific tooth-rake marks were present in two female striped dolphins (cases 4 and 5), mainly found in genital area (Figure 1B) and head (Table 3).

Semi-circular parallel multifocal tooth marks without inflammatory or vascular changes in the tissue, mainly in the dorsal or ventral part of the peduncle close to the perineal area, consistent with post-mortem shark bites, were found in 29.2% of the animals (7/24) (cases 8, 9, 10, 22, 24, 25, and 26) (Table 2).

Half of the cases diagnosed with intra-interspecific trauma (12/24) presented bone fractures, and in all of these cases, the fractures involved multiple bones and were bilateral in 5 of them. The thorax was the most affected body region with fractures involving the ribs (cases 2, 4, 5, 11, 12, 13, 15, 19, 23, 25, and 26), thoracic vertebrae (cases 2, 19, and 23), and the scapula (case 2). Other bones were also fractured such as the mandible (cases 2 and 24), the maxilla (case 2), the tympanic and the bones of the temporal region (case 18), and the lumbar vertebrae (case 19). In the case of the ribs, multiple contiguous unilateral rib fractures were most often detected. Only one individual had a single rib fracture (case 19) (Table 2).

Other macroscopic findings observed were: undigested food in the stomach in 37.5% (9/24) of the cases (cases 2, 4, 6, 7, 8, 9, 23, 25, and 26); tracheal edema in 33.3% (8/24) of the cases (cases 1, 4, 6, 9, 10, 16, 18, and 22); and pulmonary perforations in 12.5% (3/24) of the cases (cases 3, 11, and 23) (Figure 1E, Table 2).

Finally, regarding sexually mature animals, three polytraumatized adult female pigmy sperm whales were pregnant (cases 6, 9, and 10). The stranding records of this species in the Canary Islands showed that 85.7% (6/7) of the mature females were also pregnant.

Histological Findings

Histological findings in skeletal muscle included mild to severe acute myonecrosis (segmental degeneration with hyalinized eosinophilic sarcoplasm and hypercontraction) in 70.8% (17/24) of the cases (Figures 2A,B). These lesions were severe in 29.2% (7/24) of the cases, moderate in 25% (6/24) and mild in 16.6% (4/24). Regarding cardiac muscle, degenerative changes such as juxtannuclear vacuolization and increased acidophilic cytoplasm of the myocardiocytes were present in 66.7% (14/21) of the cases (Figure 2C), being in 4.8% (1/21) of the cases severe, in 28.6% (6/21) moderate, and in 33.3% (7/21) mild.

With respect to the kidney, pigmentary tubulonephrosis with orange-red homogeneous intratubular casts was found in 36.8% (7/19) of the cases (Figure 2D). This finding was severe in 10.5% (2/19) of the cases, moderate in 5.3% (1/19), and mild in 21% (4/19).

In the case of the liver, intracytoplasmic hepatocellular hyaline globules were found in 31.6% (6/19) of the cases, being severe in 10.5% (2/19) of the cases, moderate in 5.3% (1/19), and mild in 16.8% (3/19) of the cases.

Other mild to moderate histological findings included corticomedullary adrenal hemorrhages in 21.4% (3/14) of the animals and bronchiolar sphincter contraction in 17.3% (4/23) of the cases.

Statistical Analysis

Species

Eight different species presented lesions consistent with social traumatic intra-interspecific interaction (Table 4). The most affected species was the short-finned pilot whale with 33.3% of the cases (8/24); followed by the pigmy sperm whale, the short-beaked common dolphin, the striped dolphin and the Atlantic spotted dolphin with 12.5% of the cases each of them (3/24); Gervais' beaked whale with 8.3% of the cases (2/24); and Cuvier's beaked whale and common bottlenose dolphin with 4.2% of the cases each (1/24) (Table 4). The prevalence of traumatic intra-interspecific interaction was not statistically significant different between species ($p = 0.111$).

Regarding the most affected species, the short-finned pilot whale, 45 individuals stranded in 18 years, and 17.8% died due to intra-interspecific interaction (8/45). It was remarkable the high prevalence of social traumatic interaction in two infrequent stranded species: Gervais' beaked whale and pigmy sperm whale, with 18.2% (2/11) and 10.3% (3/29) of the necropsied individuals affected, respectively.

Diving Behavior

Although fewer deep-diving cetaceans were necropsied [34.1%; 184/540] compared to shallow diving cetaceans [65.9%; 356/540], more deep divers presented with traumatic intra-interspecific interaction [58.3%; 14/24] (Table 1). Comparing the affected animals with the number of necropsies of each group, 7.6% of deep divers (14/184) presented this pathological entity, while only 2.8% of shallow divers (10/356) were affected. This difference was found statistically significant ($p = 0.003$) (Table 4).

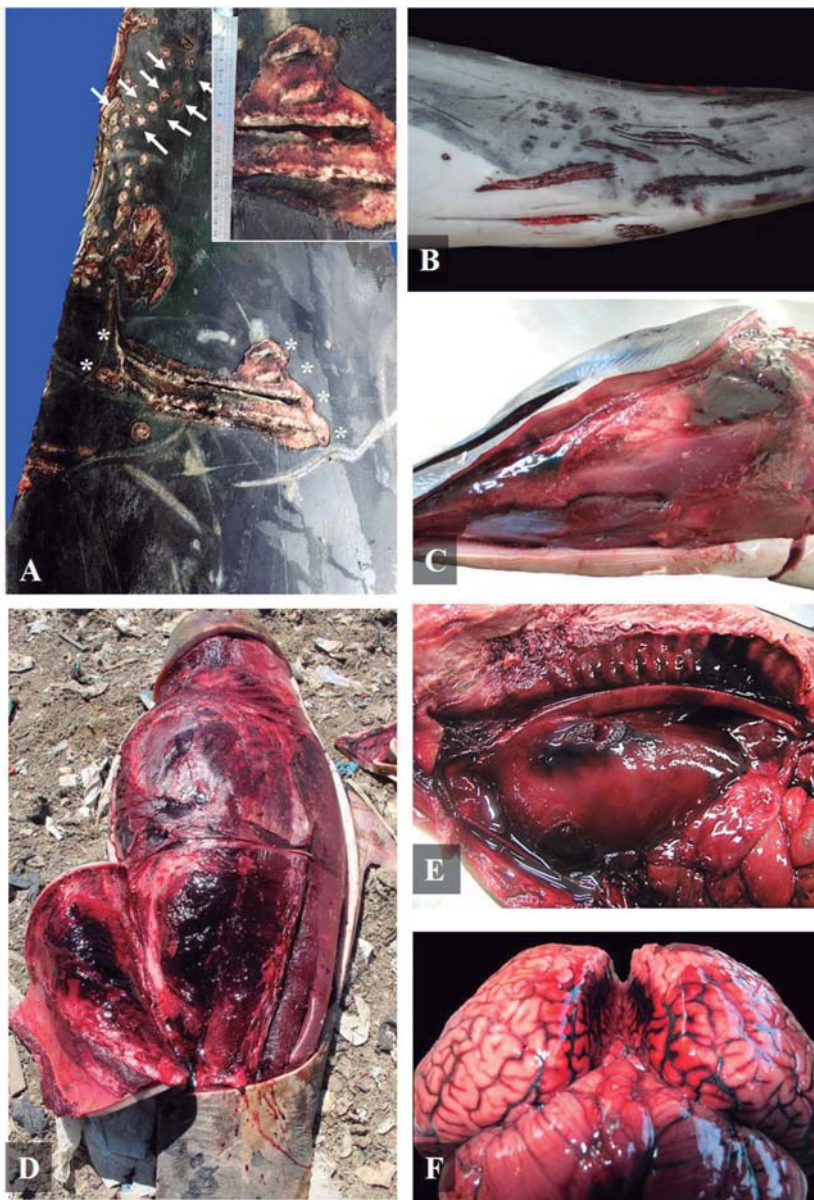


FIGURE 1 | Gross findings of social traumatic intra-interspecific interaction between cetaceans. **(A)** Right lateral view of the peduncle of an adult Cuvier's beaked whale with severe multifocal tooth-rake marks compatible with killer whale behind the dorsal fin (asterisk and inset) and along the dorsal part of the peduncle (arrows) (case 27); **(B)** Left ventrolateral view of the peduncle of a mature female striped dolphin with severe multifocal intraspecific tooth-rake marks (case 4); **(C)** Left ventrolateral view of head of a striped dolphin calf with a severe multifocal hematoma in the submandibular region (case 16); **(D)** Left ventrolateral view of a calf short-finned pilot whale with a subcutaneous and muscular hematoma in the abdominal region (case 24); **(E)** Left ventrolateral view of the thoracic cavity of a neonate of Atlantic spotted dolphin with a severe hemothorax associated to a perforation of the pleural and pulmonary parenchyma of the left lung, related to focally extensive hemorrhage on the adventitia of the aorta and in the rete mirabile (case 3); and **(F)** Caudal view of the brain of an Atlantic spotted dolphin adult with a severe diffuse vascular congestion of the meninges and bilateral hemorrhages between brain hemispheres (case 23).

TABLE 2 | Macroscopic findings in cases of social traumatic intra-interspecific interaction between cetaceans (n = 24).

Case	Species	Intra-specific tooth-rake marks	Healed rake marks	Skin erosion/laceration	Skin vascular changes	Postmortem shark bites	Hematomas		Fractures	Scapula		Hemothorax	Hemoabdomen	Hemopericardium	Lung perforation	Non-digested food	Tracheal edema	CNS vascular changes
							Cranium	Mandibles		Spine	Ribs							
1	<i>Gibbophala macrohynchus</i>	N	Y	N	Y	N	Y	N	N	N	N	N	N	N	N	N	Y	N
2	<i>Mesoplodon europaeus</i>	N	Y	N	Y	N	N	M (I)	M (R&L)	S (L)	Y	Y	Y	N	N	Y	N	Y
3	<i>Stenella frontalis</i>	N	N	N	Y	N	N	N	N	N	Y	N	N	N	Y	N	N	Y
4	<i>Stenella coeruleoalba</i>	Y	Y	N	Y	N	Y	N	M (R)	N	N	N	N	N	N	Y	Y	Y
5	<i>Stenella coeruleoalba</i>	N	Y	N	Y	N	Y	N	M (L: 5, 6, 7)	N	N	N	N	N	N	Y	Y	Y
6	<i>Kogia breviceps</i>	N	Y	Y	Y	N	Y	N	N	N	N	N	N	N	Y	Y	Y	Y
7	<i>Mesoplodon europaeus</i>	N	N	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y	N	N	N
8	<i>Gibbophala macrohynchus</i>	N	Y	N	Y	N	Y	N	N	N	Y	Y	Y	N	Y	N	N	Y
9	<i>Kogia breviceps</i>	Y	Y	N	Y	Y	Y	N	N	N	N	Y	Y	N	Y	Y	Y	Y
10	<i>Kogia breviceps</i>	Y	N	Y	Y	Y	Y	N	N	N	N	N	N	N	N	Y	Y	Y
11	<i>Gibbophala macrohynchus</i>	N	Y	N	Y	N	Y	N	M (R&L)	N	Y	N	N	Y	N	N	N	N
12	<i>Delphinus delphis</i>	Y	N	Y	Y	N	N	N	M (L: 9)	N	Y	N	N	N	N	N	N	Y
13	<i>Delphinus delphis</i>	Y	N	Y	Y	N	N	N	M (L: 6)	N	N	N	N	N	N	N	N	Y
15	<i>Delphinus delphis</i>	N	Y	N	Y	N	N	N	M (L: 3)	N	N	N	N	N	N	N	N	Y
16	<i>Stenella coeruleoalba</i>	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	Y	Y
18	<i>Gibbophala macrohynchus</i>	N	Y	N	Y	N	N	N	Right tympanic and bones of temporal region	N	N	N	N	N	N	N	Y	Y
19	<i>Stenella frontalis</i>	Y	Y	Y	Y	N	N	N	T (2 last) L (3 proximal)	S (5)	N	N	N	N	N	N	N	Y
21	<i>Tursiops truncatus</i>	N	Y	N	Y	N	N	N	N	N	Y	Y	Y	N	N	N	N	Y
22	<i>Gibbophala macrohynchus</i>	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	Y	N	N	N	Y	Y
23	<i>Stenella frontalis</i>	N	Y	N	Y	N	N	N	T (5 last) 5°, 6°, 7°, 8°, 9°, 10°, 11°, 12°	M (L: 3°, 4°, 5°, 6°, 7°, 8°, 9°, 10°, 11°, 12°)	N	Y	N	N	Y	Y	N	Y
24	<i>Gibbophala macrohynchus</i>	N	Y	N	Y	Y	Y	N	N	N	Y	Y	Y	N	N	N	N	N
26	<i>Gibbophala macrohynchus</i>	N	Y	N	Y	Y	Y	N	M (L: 3°, 4°, 5°, 6°, 7°, 8°, 9°)	N	Y	N	N	N	Y	Y	N	Y
27	<i>Ziphius cavirostris</i>	8	2	23	6	24	7	11	2	2	2	12	7	1	3	9	8	15

The cases in which the death was related to an accident during the predation are not included (cases 14, 17, and 20). The table shows the number of the case, the species, and the presence (Y, yes) or the absence (N, no) of intra-interspecific tooth-rake marks; healer rake marks; skin erosion/laceration; vascular changes in the skin; postmortem shark bites; subcutaneous and muscle hematomas; fractures (Cranium, manibles (S: single; M: multiple; R: right; and L: left), vertebrae (T: thoracic and L: lumbar), ribs (the exact number of rib fractures is given in case it was recorded, also the side), and scapula), hemothorax, hemoabdomen, hemopericardium, lung perforation, non-digested food, tracheal edema, and vascular changes and Central Nervous System (CNS).

TABLE 3 | Distance between teeth in four species of small Odontocetes.

Species	Intertooth spacing (mm)	Maximum distance between teeth (mm)
<i>Tursiops truncatus</i>	7–12	15
<i>Globicephala macrorhynchus</i>	20–33	40
<i>Stenella coeruleoalba</i>	4–6	6
<i>Stenella frontalis</i>	5–6	6

Data referred to osteological studies of adult cetaceans stranded in the Canary Islands (34).

Coast

The prevalence of strandings per island during the study period was of 38% in Fuerteventura-Lanzarote (205/540), 31.9% in La Gomera-Tenerife (172/540), 27.6% in Gran Canaria (149/540), and 2.6% in El Hierro-La Palma (14/540). However, we found more animals affected by traumatic intra-interspecific interaction in La Gomera-Tenerife [66.7%; 16/24] (Table 1). None of the animals stranded in the western islands of El Hierro-La Palma were affected by this entity. The prevalence of traumatic intra-interspecific interaction between the different coasts was statistically significantly different ($p = 0.014$) (Table 4 and Figure 3).

Age

The percentage of necropsied animals for each age class were of 43.7% adults (236/540), 29.8% juveniles/subadults (161/540), and 26.3% neonates/calves (142/540). The age class of one animal could not be determined. In contrast, the age class most affected by traumatic intra-interspecific interactions were neonates/calves with 7.7% (11/142), followed by adults with 3.8% (9/236), and juveniles/subadults with 2.5% (4/161) (Table 1). No statistically significant differences ($p = 0.094$) were found between age classes (Table 4).

Body Condition

The prevalence of cetacean's body condition during the study period were 65.3% good/fair (312/540) and 34.7% poor/very poor (166/540). In 62 cetaceans the body condition could not be determined due to advanced decomposition state. This difference was higher in this entity, in which 70.8% (17/24) of affected cetaceans were in good/fair body condition and only 12.5% presented poor/very poor body condition. This difference was found statistically significant ($p = 0.044$) (Table 4).

Other Variables With No Statistical Significance (Sex, Mature, and Temporality)

In our study, no statistically significant differences were found in the prevalence of intra-interspecific interaction entity between animals of different sex ($p = 0.731$) nor sexually mature or immature animals ($p = 0.768$) (Table 4). Finally, regarding the temporality of stranding events, no trend was detected. The yearly average occurrence of intra-interspecific interactions was 1–2 animals per year (24 cases over 18 years).

Traumatic Death Due to an Accident During Predation: Gross and Histological Findings

In three cases interspecific interaction with potential prey resulted in fatalities. A juvenile false killer whale (case 14) in very poor body condition presented a fatal interaction with a stingray. The main necropsy finding was a full-thickness perforating traumatic necrotizing and granulomatous glossitis and stomatitis involving soft palate (Figure 4A), previously briefly reported by Diaz-Delgado et al. (4). In addition, shark bites along the dorsal fin on both sides of the body with a mild inflammatory reaction (the edges of the wound were enlarged and retracted and the exposed tissue was covered by scarce granulation tissue and fibrin), indicating an antemortem interaction was observed (Figure 4B). Histologically, the perforation of the tongue presented cellular debris, bacteria, neutrophils and necrotic changes in skeletal muscle (hypercontraction and hyalinization of myofibers), surrounded by scarce granulation tissue with associated angiogenesis and fibrosis (Figures 4C,D). Severe changes were observed also in the *longissimus dorsi*, with multifocal polyphasic myocyte degeneration and necrosis: segmental myonecrosis associated with the stress stranding syndrome, atrophy due to emaciation, and myositis due to the shark bites (7).

The other cases were two Risso's dolphins (cases 17 and 20) with decompression sickness (44). The first animal (case 17) was an immature male with good body condition that presented multifocal lacerations consistent with a live stranding as well as social intra-interspecific interaction marks. The distal segment of a squid tentacle was observed partially fixed on the mandible skin and ring-associated marks were described on the oral cavity and cervical skin. Two additional 110 cm-long tentacles ran through the esophagus associated with acute hemorrhages on the cranial part. A partially digested large squid *Ommastrephes bartramii* (LeSueur, 1821) was found on the keratinized gastric compartment with abundant dark liquid. Also, non-collapsed lungs with rib marks, marked pleural lymphangiectasia and pulmonary edema filling the trachea were present.

The second animal (case 20) was a mature female with external signs of live stranding in poor body condition, but presented a large undigested squid in the keratinized gastric compartment. Additionally, the left lung was partially collapsed and a 5 cm lung rupture with associated fibrosis and hemorrhage were described on the dorsal aspect. Gross and histologically, both animals presented generalized round to oval intravascular spaces consistent with gas bubbles. Gas analysis of both animals confirmed a systemic decompression sickness (45, 46).

DISCUSSION

Social Traumatic Interactions Between Cetaceans

Gross Findings

In this retrospective study, we focused on 24 cases with severe traumatic lesions highly compatible with intra-interspecific interactions (5, 6, 37). We found that blunt traumas were multifocal, with fractures and bruises in different locations

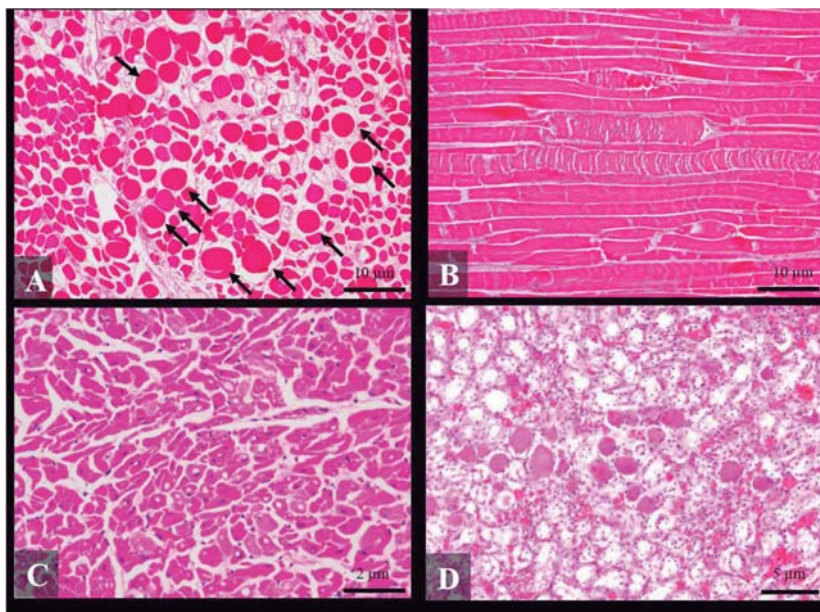


FIGURE 2 | Histological findings of social traumatic intra-interspecific interaction between cetaceans stained routinely with hematoxylin-eosin. **(A)** Transversal section of *rectus abdominis* of an adult short-finned pilot whale with severe multifocal acute degenerative changes (hypercontraction) of muscle fibers (arrows) (case 26) $\times 10$; **(B)** Longitudinal section of the *longissimus dorsi* of an adult Cuvier's beaked whale with severe multifocal segmental myodegeneration of muscle fibers (case 27) $\times 10$; **(C)** Severe multifocal vacuolar degeneration in myocardioocytes of a juvenile striped dolphin (case 5) $\times 40$; and **(D)** Pigmented intratubular casts in the kidney of an adult striped dolphin (case 4) $\times 20$.

affecting both body sides, and in some cases, associated with multiple severe tooth-rake marks. Other findings, such as tracheal edema, undigested food in the stomach, pulmonary perforation, and both, hemothorax and hemoabdomen, were also described.

It is important to emphasize that except for the acute rake marks, none of the lesions described could be considered pathognomonic for this entity, as they can be produced during other traumatic events (i.e., vessel strikes, fishing interactions, or live stranding). Undeniably, severe acute tooth-rake marks are indicative of aggressive interaction with other cetaceans. However, the absence of tooth-rake marks does not rule out this entity. For example, killer whales striking with their snouts produced internal injuries in other cetaceans without causing external wounds (24). Also, blunt-force traumas can be produced by vessel strikes with the hull. However, in vessel strikes, contusions are mostly unidirectional and located on the dorsum (38). Therefore, we should always perform differential diagnoses.

Other traumatic events include fisheries interaction and live strandings. In bycatch cases, the presence of external net marks is a diagnostic key, frequently associated with the presence of undigested food in the stomach or esophagus, red eyes, and disseminated gas bubbles (46). Finally, during active stranding events, multifocal erosions, and lacerations of variable extent can be produced, mainly in ventral parts of the body, flanks, pectoral

fins, tail fluke, and rostrum (4). In our study, we discarded cases in which traumatic etiologies other than inter or intraspecific interactions could not be ruled out.

Histological Findings

There was a high prevalence of acute monophasic degeneration of the skeletal muscle and myocardium in the cases diagnosed with intra- interspecific interaction. In fewer cases we observed pigmentary tubulonephrosis, intracytoplasmic hepatocellular hyaline globules, bronchiolar sphincter contraction, and corticomedullary adrenal hemorrhages. These findings have been previously reported in association with stressful agonal events or in severely polytraumatized animals. For example: segmental myodegeneration and contraction band necrosis have been described before in traumatized stranded cetaceans (7); pigmentary tubulonephrosis, as well as hyaline casts, have been associated with capture myopathy in live strandings (47); and the presence of vacuoles, known as hyaline globules, in the hepatocytes and myocardioocytes have been previously reported in acute stressful deaths (4, 41, 48, 49). Thus, histological determination of agonal changes (in skeletal and cardiac muscle, kidneys, lungs, liver, and adrenal glands) can support gross evidence in cases suspected of traumatic intra-interspecific interaction.

TABLE 4 | Statistical analysis of the stranded and necropsied cetaceans 2000–2017 ($n = 540$), focus on traumatic intra-interspecific interaction between cetaceans ($n = 24$).

	Overall $N = 540$	Other cause of death $N = 408$	Social traumatic interaction $N = 24$	P -value
Species				0.111
<i>Balaenoptera acutorostrata</i>	6 (1.1)	5 (1.2)	0 (0.0)	
<i>Balaenoptera borealis</i>	3 (0.6)	2 (0.5)	0 (0.0)	
<i>Balaenoptera edeni</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Balaenoptera physalus</i>	6 (1.1)	4 (1.0)	0 (0.0)	
<i>Delphinus delphis</i>	55 (10.2)	47 (11.5)	3 (12.5)	
<i>Globicephala macrorhynchus</i>	45 (8.3)	28 (6.9)	8 (33.3)	
<i>Grampus griseus</i>	13 (2.4)	10 (2.5)	0 (0.0)	
<i>Kogia breviceps</i>	29 (5.4)	20 (4.9)	3 (12.5)	
<i>Kogia sima</i>	7 (1.3)	5 (1.2)	0 (0.0)	
<i>Lagenodelphis hosei</i>	4 (0.7)	3 (0.7)	0 (0.0)	
<i>Megaptera novaeangliae</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Mesoplodon bidens</i>	2 (0.4)	1 (0.2)	0 (0.0)	
<i>Mesoplodon densirostris</i>	8 (1.5)	7 (1.7)	0 (0.0)	
<i>Mesoplodon europaeus</i>	11 (2.0)	4 (1.0)	2 (8.3)	
<i>Mesoplodon mirus</i>	1 (0.2)	0 (0.0)	0 (0.0)	
<i>Orcinus orca</i>	1 (0.2)	1 (0.2)	0 (0.0)	
<i>Phocoena phocoena</i>	1 (0.2)	1 (0.2)	0 (0.0)	
<i>Physeter macrocephalus</i>	32 (5.9)	21 (5.1)	0 (0.0)	
<i>Pseudorca crassidens</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Stenella coeruleoalba</i>	105 (19.4)	81 (19.9)	3 (12.5)	
<i>Stenella frontalis</i>	102 (18.9)	83 (20.3)	3 (12.5)	
<i>Stenella longirostris</i>	3 (0.6)	3 (0.7)	0 (0.0)	
<i>Steno bredanensis</i>	22 (4.1)	20 (4.9)	0 (0.0)	
<i>Tursiops truncatus</i>	42 (7.8)	33 (8.1)	1 (4.2)	
<i>Ziphius cavirostris</i>	36 (6.7)	23 (5.6)	1 (4.2)	
Diving behavior				0.003
Shallow diver	356 (65.9)	289 (70.8)	10 (41.7)	
Deep diver	184 (34.1)	119 (29.2)	14 (58.3)	
Coast				0.014
El Hierro y La Palma	14 (2.6)	8 (2.0)	0 (0.0)	
La Gomera y Tenerife	172 (31.9)	131 (32.1)	16 (66.7)	
Gran Canaria	149 (27.6)	113 (27.7)	3 (12.5)	
Fuerteventura y Lanzarote	205 (38.0)	156 (38.2)	5 (20.8)	
Age				0.094
Neonate/calif	142 (26.3)	107 (26.2)	11 (45.8)	
Juvenil/subadult	161 (29.9)	121 (29.7)	4 (16.7)	
Adult	236 (43.8)	180 (44.1)	9 (37.5)	
Body condition				0.044
Poor/very poor	166 (30.7)	145 (35.7)	3 (12.5)	
Good/fair	312 (57.3)	245 (60.2)	17 (68.8)	
Sex				0.731
Female	254 (47.0)	187 (46.4)	12 (50.0)	
Male	273 (50.3)	216 (53.6)	12 (50.0)	
Mature categories				0.768
Immature	261 (48.3)	199 (49.0)	12 (50.0)	
Mature	269 (49.7)	207 (51.0)	11 (43.8)	

Categorical variables are expressed as frequencies and percentages, in brackets, and were compared, as appropriate, using the Chi-square (χ^2) test or the exact Fisher test. Statistical significance was set at $p < 0.05$.

Statistical Analysis

Species

The Canary waters contain one-third of the cetacean species recorded around the world (Banco de Datos de Biodiversidad de Canarias). In our study, the short-finned pilot whale was the most affected species. The south-west coast of Tenerife holds a resident population of short-finned pilot whales in deep waters from 800 to 2000 m (50). Oremland 2010 supports the hypothesis of intraspecific interaction due to sexual competition in this species. High prevalence of mandibular fractures, 54% (27/50), was described in individuals of both sexes [females with 47% (17/36) and males with 71% (10/14)] of two mass stranding events in North and South Carolina (51). In that research, the prevalence of mandibular fractures increased with the length of the animal, suggesting that the animals may use their heads during fights. In our study, the prevalence of mandibular fractures, as well as cranial fractures, was low [25% (2/8)], while multifocal contusions (6/8) associated with hemothorax (4/8) were more prevalent.

The study of tooth-rake marks allowed us to determine fatal interaction with killer whales in three cetacean species, including a Cuvier's Beaked whale, pygmy sperm whale and short-finned pilot whale. Killer whales have been observed preying *Mesoplodon* spp. (28) and feeding on fresh carcasses of Cuvier's beaked whales which they probably killed (52). Also, dwarf sperm whales (*Kogia sima*) have been seen attacked by killer whales in the Bahamas (53). There is also indirect evidence (remnants in the stomach) of killer whales feeding on short-finned pilot whales and pygmy sperm whales, although this evidence did not allow determination of whether the feeding behavior was predation or carrion (24).

In the Canary Islands, killer whales have been sighted in spring and summer, associated with the presence of tuna. Few aggressive encounters between killer whales and short-finned pilot whales have been observed in the Canaries. In one of them, a huge group of short-finned pilot whales was recorded pursuing and deterring a group of killer whales from their territory at the South of La Gomera (<http://www.rtv.es/noticias/video-grabana-un-grupo-de-calderones-persiguiendo-a-una-familia-de-orcas-en-la-189459.aspx#.XcBOq5r7TIV>). On the other hand, a group of killer whales was seen attacking and feeding on two short-finned pilot whales (https://www.antena3.com/noticias/sociedad/un-grupo-de-orcas-atacan-a-dos-calderones-en-tenerife_201807305b5eca210cf267fe6b5e3054.html) in the South of Tenerife. Killer whales have also been observed feeding on two fresh calf carcasses of beaked whales (<https://www.elmundo.es/elmundo/2013/08/02/natura/1375440241.html>), presumably *Mesoplodon* spp. and also on a live pygmy sperm whale (<https://www.youtube.com/watch?v=8Dxkg0n4rRE>) in the Canary Islands.

Tooth-rake marks compatible with bottlenose dolphins were present in two species: short-beaked common dolphin and Atlantic spotted dolphin. Bottlenose dolphins are residents in the Canary Islands (31), and are well-known for interacting aggressively worldwide within these species with different motivations (37). Either way, bottlenose dolphins are well-known

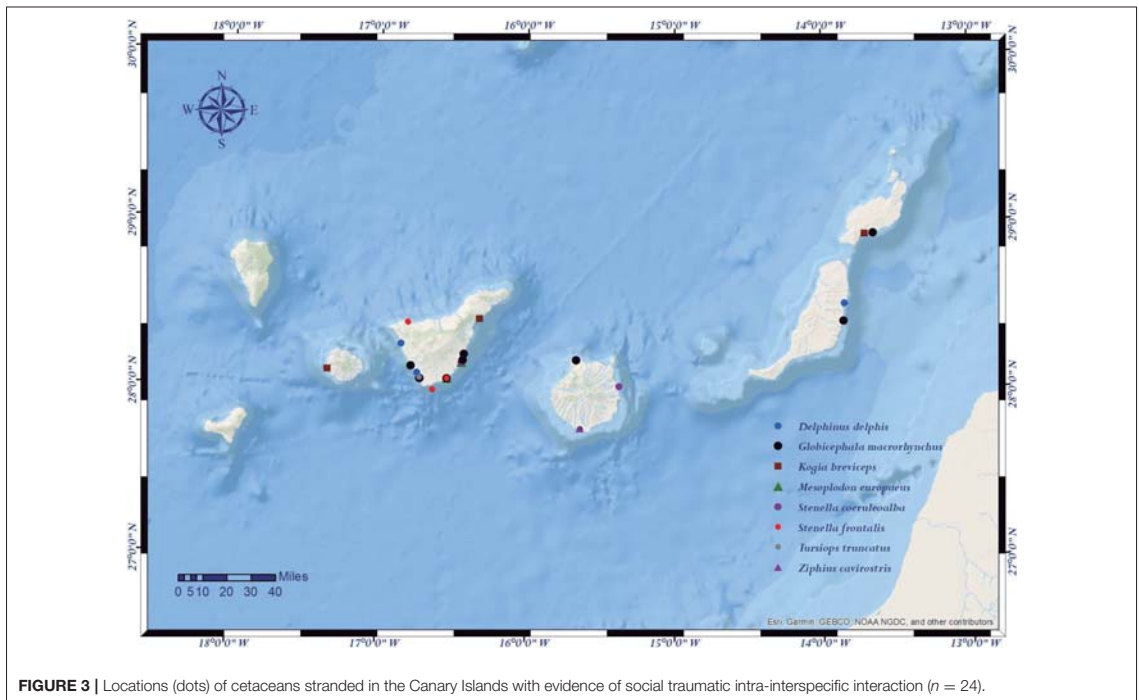


FIGURE 3 | Locations (dots) of cetaceans stranded in the Canary Islands with evidence of social traumatic intra-interspecific interaction ($n = 24$).

for their aggressive interaction with other species (37, 54, 55). Additionally, two females of striped dolphin presented intraspecific tooth-rake marks. One of them (case no 4) presented clear fresh tooth-rake marks surrounding the genital area, which is highly related with sexual aggressive behavior (1).

Summarizing, deep divers were more attacked by killer whales while shallower species were mainly attacked by bottlenose dolphins in our study.

Diving Behavior

Our results show that deep divers are more prone to intra-interspecific interactions than shallow divers unlike consulted references, in which more encounters have been published about shallow-diving species (e.g., 24, 37, 26, 27, 28). On the other hand, the Canary Islands are known for the presence of deep-diving species, at least one-third of the species recorded. Some deep divers require time resting on the surface, predisposing them to vessel strikes (56), but also potentially making them vulnerable to attacks from predatory cetaceans like killer whales.

Coast

The prevalence of intra-interspecific cases was highest on Tenerife and La Gomera coast. Open water observations in the archipelago support that resident populations of bottlenose dolphins and short-finned pilot whales coexist with the Atlantic spotted dolphin, the short-beaked common dolphin, and the rough-toothed dolphins in La Gomera waters (31). Prey competition due to diet overlap has been postulated to

explain cases of lethal interactions between bottlenose dolphins and harbor porpoises (*Phocoena phocoena*) (21). Behavioral observational studies suggest that traumatic interactions between these species were rare in this area, as cetaceans occupying the same living space are separated by their prey specialization (31). However, as evidenced by our results, fatal traumatic interactions do occur, involving especially bottlenose dolphins and killer whales when present.

Age Class

In our study, 50% of the affected individuals were neonates or calves, although this group represented only 25% of the total studied cases. Three affected calves were short-beaked common dolphins and presented rake marks compatible with the bottlenose dolphin. This species is well-known for infanticide (1, 2) and attacking smaller sized cetaceans such as porpoises (57). In this way, infanticide in bottlenose dolphins, in which neonates (1–1.3 m, 12–25 kg) have similar sizes to adult harbor porpoises (0.74–1.66 m) (57), may be explained as fight practice (6).

Additionally, we found three pregnant pygmy sperm whales with intra-interspecific interactions. Interestingly, 85.7% (6/7) of mature female pygmy sperm whales stranded in the Canary Islands were pregnant. The gestation period in this species is about 9.5–11 months and the length at birth around 1 m (58, 59). In Pinedo (60), some cases of stranded females with calves and fetuses were collected. Thus, concurrent lactation and pregnancy in this species is not unusual (58, 59) and therefore a higher

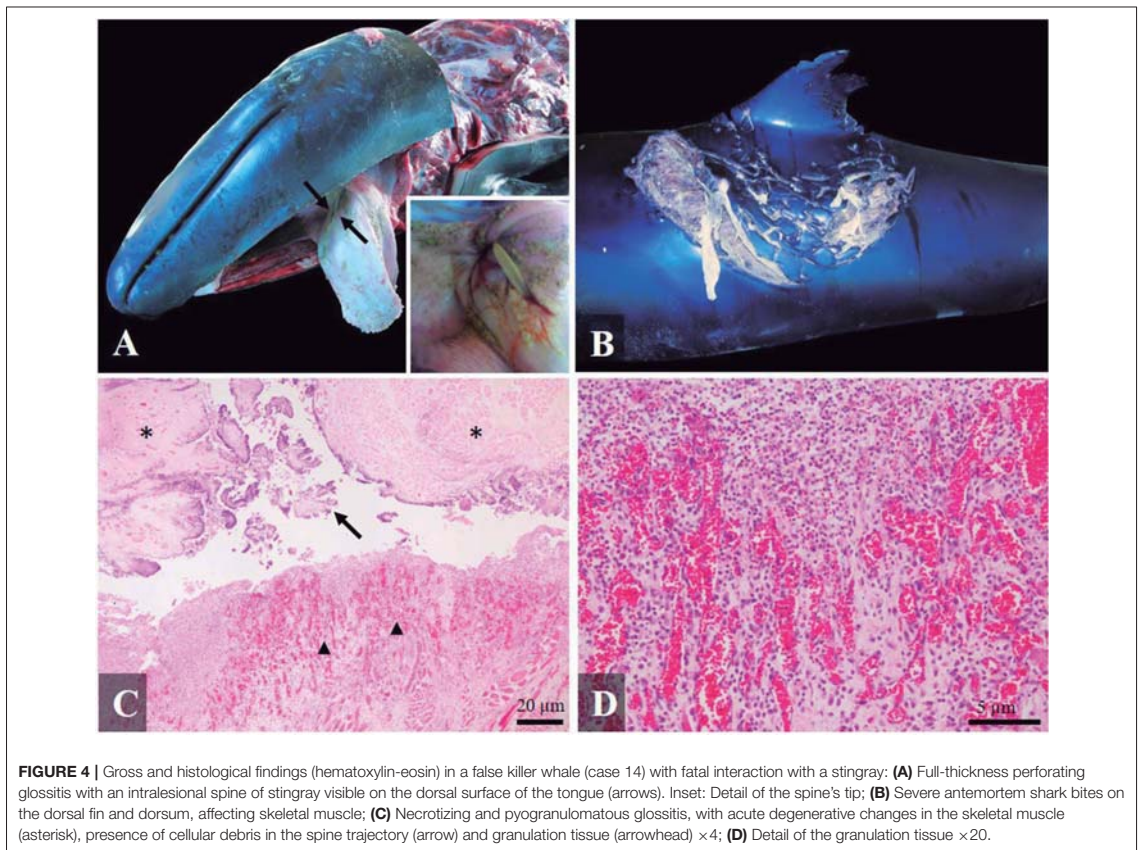


FIGURE 4 | Gross and histological findings (hematoxylin-eosin) in a false killer whale (case 14) with fatal interaction with a stingray: **(A)** Full-thickness perforating glossitis with an intralesional spine of stingray visible on the dorsal surface of the tongue (arrows). Inset: Detail of the spine's tip; **(B)** Severe antemortem shark bites on the dorsal fin and dorsum, affecting skeletal muscle; **(C)** Necrotizing and pyogranulomatous glossitis, with acute degenerative changes in the skeletal muscle (asterisk), presence of cellular debris in the spine trajectory (arrow) and granulation tissue (arrowhead) $\times 4$; **(D)** Detail of the granulation tissue $\times 20$.

percentage of the female population might be pregnant at one time compared to other species.

Body Condition

This variable was statistically significant in this study as most of the cases presented good/fair body condition. However, the ecological and pathological meaning of this result remains unknown as good/fair body condition is a common finding (65%) in cetaceans in this archipelago, based on our stranding data.

Traumatic Death Due to an Accident During Predation

Predation also has its risks: dislocation of the larynx has been reported in bottlenose dolphins due to the ingestion of a black margate (*Anisotremus surinamensis*) (61) and a beheaded sheepshead (*Archosargus probatocephalus*) (62), asphyxia due to obstruction of the airway was reported in long-finned pilot whales (*Globicephala melas*) with a common sole (*Solea solea*) (63) and a European eel (*Anguilla anguilla*) (64), and inflammation of the throat produced by ingested fish species with strong dorsal spines lead to the death of some bottlenose dolphins (65, 66).

In three of our cases, the death of the animals was directly associated with an accident during predation. A false killer whale (case 14) presented an intralesional stingray spine in the tongue causing severe chronic perforating glossitis and stomatitis (4). This animal also presented with severe muscular atrophy due to starvation, muscular degenerative changes due to an active stranding, and antemortem shark bites (7). Fatal interactions between dolphins and stingrays have been well-documented. In bottlenose dolphins, abdominal and lateral chest perforations, organ punctures (in liver, pancreas, esophagus, stomach, heart, lung, and trachea) (66, 67), caudal vena cava perforations (68), and an intestinal concretion with intralesional stingray spines (69) have all been reported. In addition, an accidental finding of a stingray spine was reported in the right scapula of a bottlenose dolphin from South Carolina, USA (70). Also, there is a report of esophageal perforation by a stingray spine in a killer whale (71). To our knowledge this was the first report of a false killer whale fatal predation accident with a stingray.

Finally, two Risso's dolphins (cases 17 and 20) presented fatal interaction with large squids and died from decompression sickness (44). The dolphins presented evidence or

struggling/fighting with the squid. In consulted references, an adult shallow Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) died by suffocation due to fatal octopus ingestion in Bunbury, Western Australia (72). As in case 17, some octopus' arms protruded from the mouth of the dolphin and suckers were firmly adhered to the caudal tongue, pharynx, and the esophageal mucosa, producing red-purple circular umbilicated lesions (72). Also, hyperinflated lungs with marked rib impressions were described. In shallow depths of the same area, adult Indo-Pacific bottlenose dolphins have been seen handling octopus (73). Poorly handled prey items can be fatal (73). In Stephens et al. (72), the "goosebeak" larynx of the dolphin was displaced, compressed ventrally, and obstructed with a remaining tentacle. In our cases 17 and 20, no larynx dislocation was present, but struggling with the squid may have resulted in severe alterations in the diving profile and physiologically induced formation of gas emboli (44).

CONCLUSIONS

This is the first study with a focus on traumatic intra-interspecific interaction between cetaceans in the Canary Islands. The full anatomopathological study is necessary to reach a traumatic intra-interspecific interaction diagnosis and to differentiate it from other traumatic etiologies. We described acute severe tooth-rake marks compatible with killer whales and bottlenose dolphins in five species (pigmy sperm whale, Cuvier's beaked whale, short-finned pilot whale, short-beaked common dolphin, and Atlantic spotted dolphin), and intraspecific aggressive tooth-rake marks in striped dolphin. The aggressor species was identified based on inter-tooth distances. Deep-divers, in good body condition, and/or stranded nearby La Gomera and Tenerife were more affected by social traumatic interaction in this study.

We encourage open water observations and further pathological studies to better understand the origin of this natural behavior, sometimes lethal.

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DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary files.

ETHICS STATEMENT

Required permission for the management of stranded cetaceans was issued by the environmental department of the Canary Islands' Government and the Spanish Ministry of Environment. Experiments on live animals were not performed.

AUTHOR CONTRIBUTIONS

RP-L, YB, and MA: conceptualization. AF, MA, MAn, ES, YB, MT, JD, AX, JD-D, and RP-L: sampling and diagnosis of the cause of death of each animal. PS, RP-L, and YB: data analyses. JD: map editing. RP-L and MR: image editing. RP-L: writing. YB, MA, and MR: supervision. All authors: review and editing.

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3. *Retrospective study of fishery interactions in stranded cetaceans, Canary Islands*



Retrospective Study of Fishery Interactions in Stranded Cetaceans, Canary Islands

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Estimating cetacean interactions with fishery activities is challenging. Bycatch and chronic entanglements are responsible for thousands of cetacean deaths per year globally. This study represents the first systematic approach to the postmortem investigation of fishery interactions in stranded cetaceans in the Canary Islands. We retrospectively studied 586 cases necropsied between January 2000 and December 2018. Of the cases with a known cause of death, 7.4% (32/453) were due to fishery interactions, and the Atlantic spotted dolphin (*Stenella frontalis*) was the most affected species [46.9% (15/32)]. Three types of fishery interactions were recognized by gross findings: bycatch [65.6% (21/32)], chronic entanglements [18.8% (6/32)], and fishermen aggression [15.6% (5/32)]. Among the bycaught cases, we differentiated the dolphins that died because of ingestion of longline hooks [23.8% (5/21)] from those that died because of fishing net entrapments [76.2% (16/21)], including dolphins that presumably died at depth due to peracute underwater entrapment (PUE) [37.5% (6/16)], dolphins that were hauled out alive and suffered additional trauma during handling [43.8% (7/16)], and those that were released alive but became stranded and died because of fishery interactions [18.7% (3/16)]. Gross and histologic findings of animals in each group were presented and compared. The histological approach confirmed gross lesions and excluded other possible causes of death. Cetaceans in good-fair body condition and shallow diving species were significantly more affected by fishery interactions, in agreement with the literature. Low rates of fishery interactions have been described, compared with other regions. However, within the last few years, sightings of entangled live whales, especially the minke whale (*Balaenoptera acutorostrata*) and Bryde's whale (*B. edeni*), have increased. This study contributes to further improvement of the evaluation of different types of fishery interactions and may facilitate the enforcement of future conservation policies to preserve cetacean populations in the Canary Islands.

Keywords: fisherman aggressions, peracute underwater entrapment, entanglement, bycatch, Bryde's whale, Atlantic spotted dolphin, longline hooks, minke whale

INTRODUCTION

Fishery activities are a major threat to cetacean populations globally (1). Bycatch is a major cause of mortality and poses the highest widespread risk (2, 3). Detection of bycatch among cetaceans is challenging, as there are typically no pathognomonic lesions (4, 5).

Bycaught dolphins in gillnets or trawls are often reportedly healthy individuals in good body condition, with pathological findings that are usually consistent with peracute underwater entrapment (PUE) (5). The most common findings in this type of bycatch are net cuts and impressions on the skin (mainly over the head, but also affecting the flippers and body), changes in the lung (edema, multifocal emphysema, and atelectasis), recently ingested food, reddish or bulging eyes, congestion, and disseminated gas bubbles (6). Other bycatch findings include those produced by fishermen, such as gunshots, stabs over the body, or amputations to disentangle the animal from fishing nets, and abdominal cuts to sink the carcasses (5, 7). To identify bycatch as a cause of death, it is essential to rule out other possible causes of death.

Another type of fishery interaction is chronic entanglement with an active net, or with abandoned, lost or otherwise discarded fishing gear, which form part of the marine debris, and cause ongoing “ghost-fishing” for years (8, 9). At least 14 cetacean species have been reportedly entangled, and 97% of the cases were attached to fishing gear (10). Entanglements are considered a global threat, which international entanglement response and monitoring programs urge (11).

The Canary Islands are located in the region of Macaronesia, close to the north-western coast of Africa. With up to 30 cetacean species (Biocan—Banco del Inventario Natural de Canarias¹, seven mysticetes and 23 odontocetes, the Canary Islands hold the greatest cetacean biodiversity among European territories, and its fisheries are mainly artisanal. The Atlantic Center for Cetacean Research has been monitoring the health of free-ranging cetaceans stranded in the Canary Islands over the last 20 years. The three most stranded species are the Atlantic spotted dolphin (*Stenella frontalis*), the short-beaked common dolphin (*Delphinus delphis*), and the striped dolphin (*Stenella coeruleoalba*) (12, 13).

The aim of this study was to retrospectively investigate the prevalence and most common pathological findings of each type of fishery interaction, among stranded cetaceans in the Canary Islands. Our results will likely aid the promotion of adequate conservation policies in the archipelago and improve detection of this anthropic threat.

MATERIALS AND METHODS

Postmortem examinations of 586 cetaceans stranded along the coasts of the Canary Islands were performed from January 2000 to December 2018, following standardized protocols (14). No experiments were performed on live animals. Permission for

the handling of stranded cetaceans was granted by the Spanish Ministry of Environment.

For each necropsied cetacean, the epidemiology of the stranding (i.e., location and date); life history data (i.e., species, growth development, sex, and gonad maturation); body condition; and decomposition code, were systematically recorded. Growth development categories (neonate, calf, juvenile, subadult, and adult) were extrapolated from the osteological characteristics of stranded cetaceans in the Canary Islands (15). Gonad maturation was determined, based on histological gonadal examination (16). Body condition (very poor, poor, fair, or good) was estimated, based on anatomical landmarks (17). The decomposition code (1-very fresh, 2-fresh, 3-moderate autolysis, 4-advanced autolysis, and 5-very advanced autolysis) was determined following the classification of IJsseldijk et al. (18). During the necropsy, lesions were described and photographed. In one animal (case 26), the gas score was determined and gas analysis was performed following standardized protocols (19, 20). Representative tissue samples were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at thickness of 5 μ m, and stained with hematoxylin and eosin for histopathologic analysis.

A conservative approach was adopted to determine fishery interactions, based on previous studies (5–7, 12, 13, 21–24). All stranding cases were reviewed retrospectively, as we looked for individuals with findings that were consistent with fishery interactions, and excluded cases in which other possible traumatic etiologies, such as ship collision, intra-interspecific interactions, or live stranding, could not have been ruled out (25–32). Different types of fishery interactions were determined based on gross findings. Within these various types, histological findings in the skeletal and cardiac muscle, lungs, liver, kidneys, brain, and adrenal glands of the cases with decomposition codes 1–3, were detailed and compared, based on the availability of the samples.

In order to identify factors related to fishery interactions, categorical variables (species, sex, growth development, gonad maturation, body condition, diving behavior, island, and date of stranding) were expressed as frequencies and percentages, and were compared, as appropriate, using the chi-squared (χ^2) test or the Fisher's exact test. Fishing deaths were due to all of the different types of fishery interactions identified: chronic entanglements, fishermen aggressions from the boat, and bycatch (including fishing net entrapments and ingestion of longline hooks). For statistical analyses, some categorical variables were further regrouped as follows: growth development category (neonate/calf, juvenile/subadult, and adult); body condition (very poor/poor and fair/good); and stranding island based on geographical proximities and the presence of high-site fidelity populations [Western Islands (El Hierro and La Palma), La Gomera together with Tenerife, Gran Canaria, and Eastern Islands (Fuerteventura-Lanzarote-La Graciosa)]. All statistical analyses were performed only on those animals with an identifiable cause of death (453/586). Statistical significance was set at $p < 0.05$. Data were analyzed using the R package, version 3.6.1 (33).

¹<https://www.biodiversidadcanarias.es/>

TABLE 1 | Gross findings in stranded cetaceans, which died because of fishery interactions (chronic entanglement, aggression, or bycatch) (n = 32).

		Entanglement (n = 6)		Aggression (n = 5)		Bycatch (n = 21)									
						Hook ingestion (n = 5)		PUE (n = 6)		Aggression during handling (n = 7)		Returned (n = 3)		Total (n = 21)	
Stranding event	Stranding alive	1	17%	0	0%	0	0%	0	0%	0	0%	3	100%	3	14%
	Stranding death	5	83%	5	100%	5	100%	6	100%	7	100%	0	0%	18	86%
	Fishing gears attached	4	67%	0	0%	5	100%	0	0%	0	0%	2	67%	7	33%
Body condition	Poor-very poor	2	33%	0	0%	0	0%	2	33%	0	0%	0	0%	2	10%
	Good-fair	3	50%	5	100%	5	100%	4	67%	6	86%	3	100%	18	86%
Skin	NE	1	17%	0	0%	0	0%	0	0%	1	14%	0	0%	1	5%
	Net impressions over the body	6	100%	0	0%	2	40%	4	67%	3	43%	1	33%	10	48%
	Net cuts in pectoral flippers	0	0%	0	0%	2	40%	4	67%	2	29%	1	33%	9	43%
	Net cuts in head	1	17%	0	0%	3	60%	5	83%	7	100%	2	67%	17	81%
	Net cuts over the body	1	17%	0	0%	3	60%	3	50%	5	71%	3	100%	14	67%
Subcutaneous	Hematoma	2	33%	5	100%	3	60%	0	0%	6	86%	0	0%	9	43%
Skeletal muscle	Hemorrhages	2	33%	5	100%	2	40%	1	17%	6	86%	0	0%	9	43%
Bones	Mandibles fracture	0	0%	0	0%	2	40%	2	33%	2	29%	2	67%	8	38%
	Maxilla fracture	0	0%	0	0%	1	20%	2	33%	1	14%	2	67%	6	29%
	Neurocranium fracture	0	0%	0	0%	0	0%	0	0%	2	29%	0	0%	2	10%
	Tympanic fracture	0	0%	0	0%	0	0%	0	0%	0	0%	1	33%	1	5%
	Vertebrae fracture	0	0%	2	40%	0	0%	0	0%	1	14%	0	0%	1	5%
	Rib fracture	0	0%	0	0%	0	0%	0	0%	0	0%	1	33%	1	5%
	Teeth lost/fractured	0	0%	2	40%	1	20%	3	50%	3	43%	2	67%	9	43%
Digestive tract	Esophagus	1	17%	3	60%	0	0%	3	50%	2	29%	0	0%	5	24%
	Fresh/undigested prey														
	Stomach	1	17%	3	60%	2	40%	3	50%	5	71%	0	0%	10	48%
	Fresh/undigested prey														
	Digested content	0	0%	0	0%	1	20%	1	17%	0	0%	3	100%	5	24%
	Empty/ND	4	67%	1	20%	2	40%	2	33%	1	14%	0	0%	5	24%
Bubbles	Lymphatic vessels	0	0%	1	20%	0	0%	1	17%	2	29%	0	0%	3	14%
	Blood vessels	1	17%	0	0%	1	20%	5	83%	4	57%	1	33%	11	52%
Blood in cavities	Hemothorax	1	17%	3	60%	2	40%	0	0%	1	14%	0	0%	3	14%
	Hemoabdomen	0	0%	0	0%	3	60%	1	17%	1	14%	0	0%	5	24%
	Hemopericardium	0	0%	0	0%	0	0%	0	0%	1	14%	0	0%	1	5%
Lungs	Hyperinflated	1	17%	0	0%	2	40%	2	33%	5	71%	3	100%	12	57%
	Hemorrhagic parenchyma	0	0%	3	60%	0	0%	1	17%	2	29%	1	33%	4	19%
	Subpleural hemorrhage	0	0%	4	80%	0	0%	3	50%	0	0%	0	0%	3	14%
	Tracheal or bronchial edema	2	33%	0	0%	0	0%	0	0%	2	29%	0	0%	2	10%
	Rib impressions	0	0%	0	0%	0	0%	0	0%	0	0%	2	67%	2	10%
	Rupture of the parenchyma	0	0%	3	60%	0	0%	0	0%	1	14%	0	0%	1	5%

(Continued)

TABLE 1 | Continued

		Entanglement (n = 6)		Aggression (n = 5)		Hook ingestion (n = 5)		PUE (n = 6)		Aggression during handling (n = 7)		Returned (n = 3)		Total (n = 21)	
Heart	Hemopericardium	0	0%	0	0%	1	20%	0	0%	0	0%	0	0%	1	5%
	Hemorrhages	0	0%	0	0%	0	0%	0	0%	1	14%	0	0%	1	5%
	Vascular changes in valves	2	33%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Large vessels	Aorta vascular changes	1	17%	0	0%	1	20%	1	17%	4	57%	0	0%	6	29%
	Rete mirabile vascular changes	0	0%	0	0%	0	0%	0	0%	2	29%	0	0%	2	10%
Kidney	Hemorrhages	0	0%	0	0%	0	0%	0	0%	1	14%	0	0%	1	5%
	Retroperitoneal emphysema	1	17%	0	0%	1	20%	2	33%	2	29%	0	0%	5	24%
Lymphatic	Lymph in vessels	0	0%	2	40%	0	0%	3	50%	4	57%	0	0%	7	33%
Brain	Meningeal hemorrhages	0	0%	1	20%	0	0%	0	0%	3	43%	0	0%	3	14%
	Parenchymal hemorrhages	0	0%	0	0%	0	0%	0	0%	1	14%	0	0%	1	5%

Decomposition codes (1–5). For each category, the number and percentage of affected individuals are shown.

The color values correspond to the percentage of a finding or a pathologic feature within each group of studied animals (bycatch, fisherman aggression and chronic entanglement).

RESULTS

A total of 860 cetaceans were stranded along the coasts of the Canary Islands between January 2000 and December 2018. Among them, 586 cetaceans were necropsied. The full anatomopathological study of each case allowed us to identify the most probable cause of death in 453 cases. Of those cases, 32 (7%) cetaceans of seven species died because of the pathological consequences of fishery interactions.

Types of Fishery Interactions

Cases of fishery interactions ($n = 32$) were divided into three categories: bycatch (i.e., longline hook ingestion or fishing net entrapment) ($n = 21$); chronic entanglements ($n = 6$); and fisherman aggressions ($n = 5$) (Supplementary Table 1).

Bycatch

The bycatch group included cetaceans that were presumably entrapped in active fishing gear. In this group ($n = 21$), four species were affected: the Atlantic spotted dolphin ($n = 12$); striped dolphin ($n = 6$); common dolphin ($n = 2$); and Atlantic bottlenose dolphin (*Tursiops truncatus*) ($n = 1$). All growth development categories were affected by bycatch. The adults were the most affected (11/21), followed by the juveniles (6/21), subadults (3/21), and calves (2/21).

This group was further divided into the following subgroups: dolphins that ingested longline hooks (5/21); dolphins with lesions compatible with forced submersion, which presumably died at depth due to PUE (6/21); dolphins that were stranded alive and later died, exhibiting lesions that were consistent with

PUE (3/21); and polytraumatized dolphins that were hauled out alive but suffered additional trauma during handling, including mainly cranioencephalic trauma, and/or perforations produced by fishing equipment (7/21).

Different degrees of chronicity of the lesions were observed; from acute lesions in PUE cases to subacute-chronic lesions in cases of longline hook ingestion. However, some gross findings were common among most cases (Table 1). Almost every bycaught case showed fair to good body condition. In addition, many animals exhibited superficial cutaneous lesions caused by contact with fishing nets, mainly on the rostrum, but also on the flippers, and along the sides of the body, as well as net impressions (Figures 1A,B). Other common findings included diffuse bilateral hyperinflated lungs (Figure 1C, inset upper image), disseminated intravascular gas bubbles in the veins and lymphatic vessels (Figures 1D,E). In fewer cases, undigested food was found in the forestomach (Figure 1C inset lower image), occasionally in the esophagus, and/or associated with the presence of abundant lymph in the lymphatic mesenteric vessels and chyle in the thoracic duct. Lost teeth (Figure 1F), a fractured rostrum and/or maxilla, and retroperitoneal emphysema were also described. Gross findings of each bycatch case are presented in Supplementary Table 2.

Histologically, almost all cases showed clear intravascular spaces compatible with gas and/or fat embolism. Common findings included: mild to moderate multifocal acute degenerative changes in skeletal muscle (i.e., segmental degeneration of the muscular fibers); mild multifocal acute degenerative changes in cardiac muscle (i.e., increased acidophilic cytoplasm of the myocardiocytes, contraction

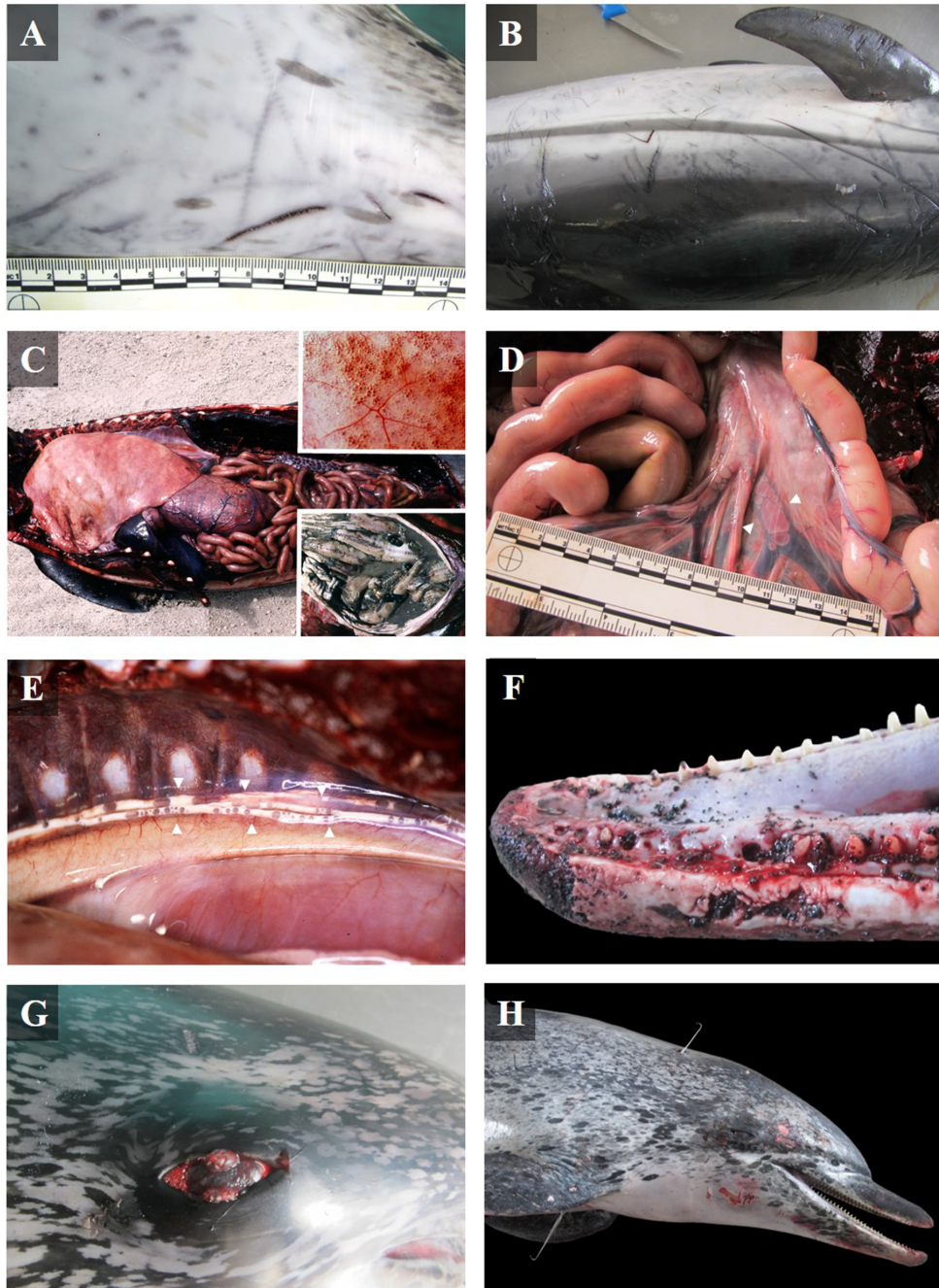


FIGURE 1 | Gross lesions in bycaught dolphins stranded along the Canary Islands. **(A)** Cutaneous impressions presumably produced by a twisted wire net, and **(B)** linear and typical triangular monofilament cuts on an adult Atlantic spotted dolphin (case 29). **(C)** Left lateral view of the thoracic and abdominal cavities of a juvenile *(Continued)*

FIGURE 1 | Atlantic bottlenose dolphin (case 7). Severe hyperinflated lungs with emphysema (inset upper image), and full stomach with undigested food, fresh fish (inset lower image). **(D)** Mesenteric veins with severe multifocal intravascular gas bubbles in an adult common dolphin (case 32). **(E)** Thoracic duct (between arrow points) full of lymph and gas bubbles in an Atlantic spotted dolphin calf (case 1). **(F)** Left lateral view of the rostrum with multifocal fracture and lost teeth in the rostral part of the mandible (case 21). **(G)** Reddish eyes with conjunctival emphysema of an adult Atlantic spotted dolphin, found with an ingested longline hook (case 29). **(H)** Right lateral view of an adult Atlantic spotted dolphin with two perforating thoracic wounds. The tracks of the wounds, marked by two steel skewers, probably produced during aggressive handling (case 21).

band necrosis, and juxtannuclear vacuolization of cardiac cells); multifocal lung changes such as alveolar emphysema, hemorrhages, and alveolar edema; systemic leukocytosis; and multifocal intracytoplasmic hepatocellular hyaline globules. In fewer cases, bronchiolar sphincter contraction, multifocal hyaline casts in distal renal tubules and corticomedullary adrenal hemorrhages, intramuscular hemorrhages, and multifocal pigmentary tubulonephrosis were observed. Regarding the central nervous system (CNS), mild changes manifested as multifocal hemorrhages, perivascular edema, and perivascular cuffs mostly associated with glial nodules were observed in a few cases. A severe non-suppurative meningoencephalitis was present in one case (Table 2). Histological findings of each bycatch case are presented in Supplementary Table 3.

All animals affected by the ingestion of longline hooks ($n = 5$) were adult Atlantic spotted dolphins in fair-good body condition. Ingested hooks had perforated the esophagus (cases 2 and 29), produced fibrinosuppurative pleuritis and pericardial hemorrhages (case 29; Figure 2D), pierced the mandibular fossa (cases 18 and 31), and affected the sublingual soft tissue to produce focal, extensive necrosis and hemorrhage (case 20). Hemothorax, hemoabdomen, and hemopericardium were also observed (Table 1). In one case, reddened eyes were observed (Figure 1G).

Of the six cases showing findings consistent with PUE, four were striped dolphins [one pregnant adult (case 17), two subadults (cases 28 and 30), and one juvenile (case 25)]; one was a Atlantic spotted dolphin calf (case 1); and another, a juvenile Atlantic bottlenose dolphin (case 7). All animals in this category were found dead, and the majority were in good-fair body condition. All had cutaneous lesions associated with contact with fishing nets, as well as disseminated intravascular gas bubbles, cutaneous impressions, lost/fractured teeth, undigested food, hyperinflated lungs with subpleural hemorrhages and hemorrhagic parenchyma, and retroperitoneal emphysema (Table 1).

Three other animals showed lesions compatible with PUE. They were likely caught in nets and released alive back into the ocean. They included a striped dolphin calf (case 6), a juvenile striped dolphin entangled in gear with a fishing ball (case 12), and an adult Atlantic spotted dolphin that appeared to have been entangled in a fishing net (case 23). Supposedly, shortly after release, these animals were stranded alive and subsequently died. Two had fractured bones [mandible and maxilla with lost or fractured teeth, tympanic fracture, and rib fracture], which may have been caused by active stranding (Table 1). Disseminated intravascular gas bubbles were present in case 23 alone. The same animal had bubbles within the posterior chamber of the eye.

Bycaught cases included seven animals that were probably hauled out and raised to the deck alive but suffered different forms of physical trauma during handling. The affected cases included Atlantic spotted dolphins ($n = 5$) [three adults (cases 11, 21, and 22); one subadult (case 27); and one juvenile (case 9)], and common dolphins ($n = 2$) [one adult (case 32) and one juvenile (case 26)]. All were stranded dead, and most were in fair body condition. These animals were polytraumatized and exhibited skin-muscle perforations (Figure 1H) with associated hemorrhages, which affected internal organs in some cases [perforations of the aorta (case 9), trachea and esophagus (case 22)]; neurocranium fractures (2/7) [in the squamous part of the occipital bone (case 27), and in the right occipital condyle (case 32)] with associated hematoma and congestion in the underlying leptomeninges and brain; and fractures of the maxilla and mandible (cases 26 and 32). Case 26 also showed signs of severe trauma to the right caudolateral side of the head with leptomeningeal congestion, severe scoliosis of the peduncle, and an open fracture that affected the caudal vertebrae. Other traumatic findings included hemorrhages on the adventitia of the aorta (cases 9, 11, 22, and 26) and in the rete mirabile (cases 22 and 26); hemothorax (cases 11 and 22); hemoabdomen (case 22); hemopericardium (case 22); and lung perforation (case 11) (Table 1).

Disseminated intravascular gas bubbles were present in four out of seven bycaught animals. One was a juvenile female common dolphin (case 26) that was found stranded dead, refrigerated (4°C) for 24 h, and necropsied while still fresh (decomposition code 2). The gas score and gas analyses were evaluated on this animal. The gas score revealed the presence of occasional small bubbles following careful screening of the subcutaneous veins (gas score 1); abundant presence of gas bubbles in the coronary veins and lumbocaudal venous plexus (gas score 5); and gas bubbles occupying complete sections of the mesenteric veins (gas score 6). Emphysema was present exclusively in the perirrenal subcapsular region.

Gas analyses were performed in mesenteric veins, the right ventricle, aorta, pulmonary artery, and intestinal lumen (Figure 3). Except for samples of the intestine and mesenteric veins, N_2 was the main component of the sampled bubbles [$61.0 \pm 9.2 \mu\text{mol } \%$], followed by CO_2 [$30.0 \pm 16.2 \mu\text{mol } \%$], and O_2 [$8.9 \pm 7.1 \mu\text{mol } \%$]. Both CH_4 and H_2 were absent from these samples. In contrast, gas bubbles from the mesenteric veins contained H_2 [$31.2 \pm 10.2 \mu\text{mol } \%$], in addition to N_2 [$54.8 \pm 3.7 \mu\text{mol } \%$], O_2 [$11.6 \pm 3.2 \mu\text{mol } \%$], and CO_2 [$2.4 \pm 3.3 \mu\text{mol } \%$]. In the intestinal lumen, CO_2 was the main constituent [$78.4 \pm 2 \mu\text{mol } \%$], followed by H_2 [$16.4 \pm 1.3 \mu\text{mol } \%$], N_2 [$4.2 \pm 2.6 \mu\text{mol } \%$], and O_2 [$1 \pm 0.5 \mu\text{mol } \%$].

TABLE 2 | Histological findings in stranded cetaceans, which died because of fishery interactions.

			Bycatch															
			Entanglement		Aggression		Hook ingestion		PUE		Aggression during handling		Returned		Total			
Skeletal muscle	Acute degenerative changes	No	1/5	20%	1/4	25%	1/3	33%	0/5	0%	0/6	0%	0/3	0%	1/17	6%		
		Mild	3/5	60%	1/4	25%	1/3	33%	3/5	60%	2/6	33%	3/3	100%	9/17	53%		
		Moderate	1/5	20%	2/4	50%	1/3	33%	1/5	20%	3/6	50%	0/3	0%	5/17	29%		
		Severe	0/5	0%	0/4	0%	0/3	0%	1/5	20%	1/6	17%	0/3	0%	2/17	12%		
		Atrophy	3/5	60%	1/4	25%	0/3	0%	1/5	20%	2/6	33%	0/3	0%	3/17	18%		
Lungs	Hemorrhages		0/5	0%	1/4	25%	1/3	33%	0/5	0%	4/6	67%	0/3	0%	5/17	29%		
		Alveolar edema	2/5	40%	4/5	80%	3/3	100%	4/6	67%	4/6	67%	0/2	0%	11/17	65%		
		Emphysema	3/5	60%	3/5	60%	2/3	67%	5/6	83%	4/6	67%	2/2	100%	13/17	76%		
		Muscular bronchiolar sphincter contraction	0/5	0%	4/5	80%	2/3	67%	2/6	33%	2/6	33%	0/2	0%	6/17	35%		
Heart	Hemorrhages		1/5	20%	4/5	80%	2/3	67%	4/6	67%	4/6	67%	2/2	100%	12/17	71%		
		Acute degenerative changes	No	0/5	0%	0/4	0%	0/3	0%	1/5	20%	4/6	67%	1/1	100%	6/15	40%	
			Mild	5/5	100%	1/4	25%	1/3	33%	4/5	80%	1/6	17%	0/1	0%	6/15	40%	
			Moderate	0/5	0%	0/4	0%	2/3	67%	0/5	0%	1/6	17%	0/1	0%	3/15	20%	
			Severe	0/5	0%	0/4	0%	0/3	0%	0/5	0%	0/6	0%	0/1	0%	0/15	0%	
Liver	Hemorrhages		2/5	40%	0/4	0%	0/3	0%	0/5	0%	3/6	50%	0/1	0%	3/15	20%		
		Intracytoplasmic hyaline globules	4/5	80%	2/3	67%	2/3	67%	4/4	100%	4/5	80%	1/3	33%	11/15	73%		
Adrenal glands	Hemorrhages		4/5	80%	2/4	50%	1/3	33%	2/4	50%	1/6	17%	1/3	33%	5/16	31%		
		Blood vessels	Leukocytosis	4/5	80%	3/5	60%	3/3	100%	5/6	83%	5/6	83%	2/3	67%	15/18	83%	
			Intravascular coagulation	2/5	40%	0/5	0%	1/3	33%	1/6	17%	5/6	83%	0/3	0%	7/18	39%	
Reproductive system	Gonadal maturity	Mature	Intravascular clear spaces	5/5	100%	5/5	100%	3/3	100%	6/6	100%	5/6	83%	3/3	100%	17/18	94%	
				2/5	40%	3/5	60%	3/3	100%	2/6	33%	5/6	83%	1/3	33%	11/18	61%	
		Kidney	Membranous glomerulonephritis	Immature	3/5	60%	2/5	40%	0/3	0%	4/6	67%	1/6	17%	2/3	67%	7/18	39%
					0/5	0%	2/5	40%	1/3	33%	1/6	17%	1/6	17%	0/3	0%	3/18	17%
Brain	Hemorrhages	Perivascular edema	Hyaline cast	1/5	20%	0/5	0%	2/3	67%	1/6	17%	2/6	33%	0/3	0%	5/18	28%	
			Pigmentary tubulonephrosis	0/5	0%	1/5	20%	0/3	0%	2/6	33%	1/6	17%	1/3	33%	4/18	22%	
				2/5	40%	0/5	0%	0/3	0%	0/6	0%	1/6	17%	0/3	0%	1/18	6%	
				1/4	20%	3/4	75%	1/3	33%	1/6	17%	2/6	33%	1/3	33%	5/18	28%	
				2/4	50%	1/4	25%	2/3	67%	2/6	33%	3/6	50%	1/3	33%	8/18	44%	
				1/4	25%	1/4	25%	0/3	0%	0/6	0%	0/6	0%	1/3	33%	1/18	6%	
				0/4	0%	2/4	50%	0/3	0%	1/6	17%	3/6	50%	0/3	0%	4/18	22%	
Brain	Glia	Nodules		3/4	75%	0/4	0%	1/3	33%	1/6	17%	2/6	33%	0/3	0%	4/18	22%	

For cases with decomposition codes 1–3 (n = 28), histological findings in the skeletal and cardiac muscle, lungs, liver, kidneys, adrenal glands, blood vessels, and brain, were detailed and compared, as well as findings of gonad maturation (M, mature; I, immature) upon the availability of samples.

The color values correspond to the percentage of a finding or a pathologic feature within each group of studied animals (bycatch, fisherman aggression and chronic entanglement).

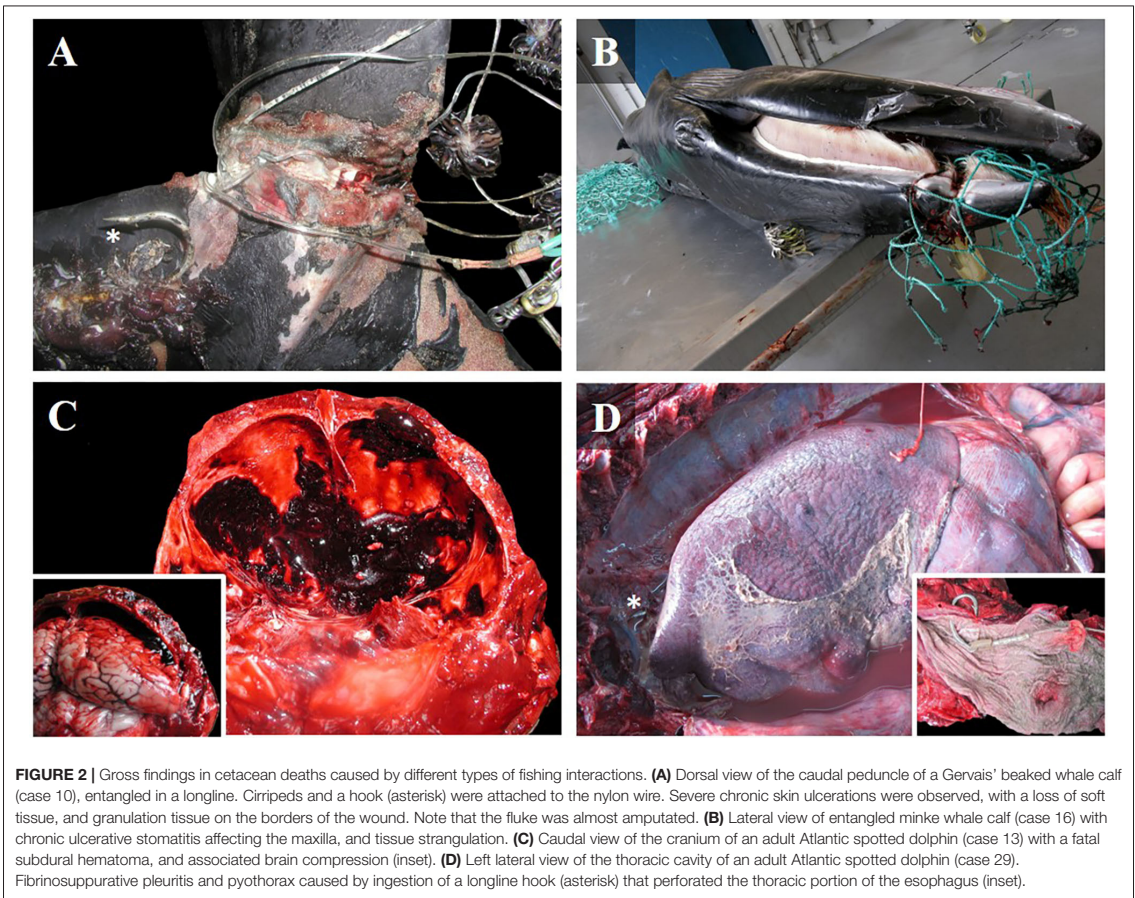


FIGURE 2 | Gross findings in cetacean deaths caused by different types of fishing interactions. **(A)** Dorsal view of the caudal peduncle of a Gervais' beaked whale calf (case 10), entangled in a longline. Cirripeds and a hook (asterisk) were attached to the nylon wire. Severe chronic skin ulcerations were observed, with a loss of soft tissue, and granulation tissue on the borders of the wound. Note that the fluke was almost amputated. **(B)** Lateral view of entangled minke whale calf (case 16) with chronic ulcerative stomatitis affecting the maxilla, and tissue strangulation. **(C)** Caudal view of the cranium of an adult Atlantic spotted dolphin (case 13) with a fatal subdural hematoma, and associated brain compression (inset). **(D)** Left lateral view of the thoracic cavity of an adult Atlantic spotted dolphin (case 29). Fibrinosuppurative pleuritis and pyothorax caused by ingestion of a longline hook (asterisk) that perforated the thoracic portion of the esophagus (inset).

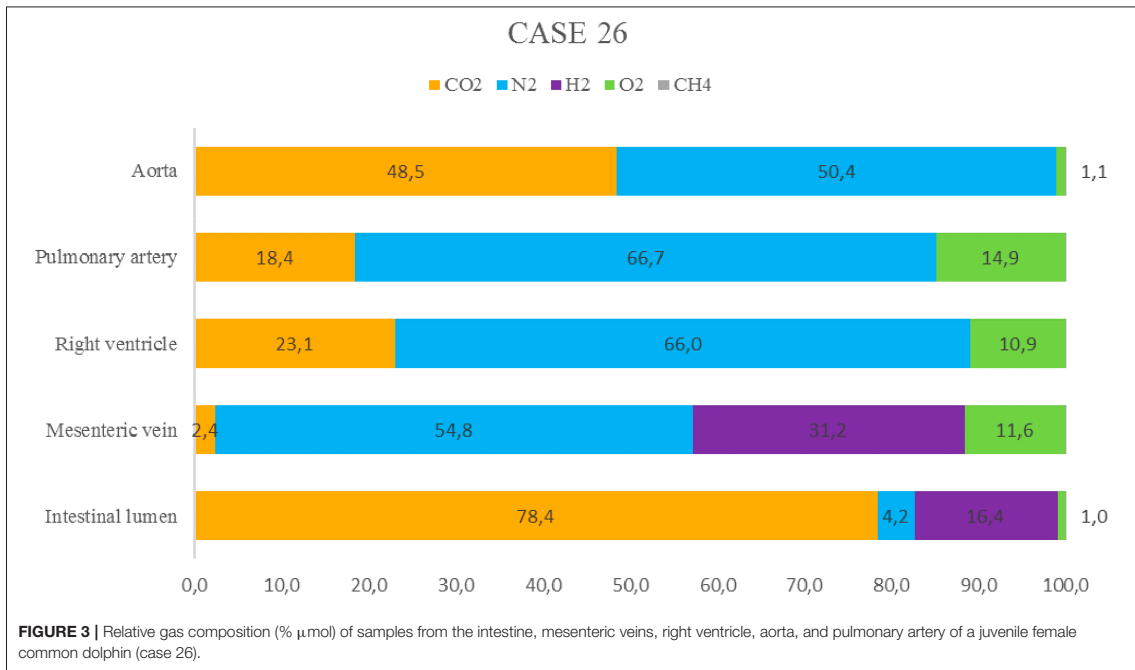
Chronic Entanglement

In this category, we observed six cases with skin wounds that were consistent with chronic entanglement. They included two minke whale calves (*Balaenoptera acutorostrata*) (cases 16 and 19); two short-finned pilot whale calves (*Globicephala macrorhynchus*) (cases 14 and 24); one Gervais' beaked whale calf (*Mesoplodon europaeus*) (case 10); and one subadult Atlantic bottlenose dolphin (case 8). Regarding body condition, three out of six cases showed fair to good body condition (cases 8, 14, and 19), while two were in poor-very poor condition (cases 10 and 16). In case 24, the advanced decomposition (code 5) did not allow us to determine the body condition, nor perform histological evaluation.

All cases exhibited fishing gear impressions, erosions, and/or ulcerative lesions with granulation tissue and fibrosis over the rostrum, flippers, and/or tail. In case 14, the animal was stranded alive with nylon wire attached to a plastic floating bottle. The other five were found dead. Case 8 exhibited rope impressions between the pectoral fins and near the left eye.

In case 10, nylon wire with cirripeds and longline hooks were attached to the caudal peduncle (**Figure 2A**). Case 16 had green monofilament nylon fishing gear attached to the maxilla (**Figure 2B**). Case 19 exhibited symmetrical bilateral ulcers and granulation tissue in the mandibular symphysis, consistent with fishing gear impressions. Case 24 had a thick rope (4 cm in diameter) surrounding the thoracic region. Other pathological findings included tracheal edema, hyperinflated lungs with rib impressions, serous atrophy of pericardial fat, hemorrhage in the adventitia of the thoracic aorta, and retroperitoneal emphysema. Only two cases had undigested food in the stomach (**Table 1**). Gross findings of each case are presented in **Supplementary Table 2**.

Histologically, clear intravascular spaces, compatible with intravascular gas and/or fat, mild to moderate multifocal acute muscular degenerative changes in skeletal muscle, and mild degenerative changes in the cardiac muscle, multifocal myofiber atrophy of the skeletal muscle, multifocal corticomedullary adrenal hemorrhages, intracytoplasmic hepatocellular hyaline



globules, systemic leukocytosis, hemorrhages in multiple organs, intravascular coagulation, multifocal alveolar emphysema, alveolar edema, diffuse hemorrhagic lung parenchyma, multifocal hemorrhages in the cortex of the kidney, and hyaline casts were observed. Regarding the CNS, case 8 showed mild focal non-suppurative meningoencephalitis. Mild multifocal glial nodules and moderate multifocal hemorrhages were occasionally observed (Table 2). Histological findings are presented in Supplementary Table 3.

Fisherman Aggression

In this category ($n = 5$), we observed three Atlantic spotted dolphins [two adults (cases 3 and 13) and one juvenile (case 15)], and two common dolphins [one adult and one calf, possibly relatives as they were stranded on the same date and location (cases 4 and 5)]. All cetaceans in this group showed fair-good body condition, and a full stomach with undigested prey, as well as signs of anthropogenic trauma, such as unique or multifocal stabs inflicted by sharp instruments, mainly in the laterodorsal area affecting the thoracic region (cases 3, 4, 5, and 15) and dorsal side of the head (cases 3 and 13). Net cuts and impressions were not observed on the skin (Table 1). These animals exhibited several lesions associated with incisive trauma, such as vascular changes (i.e., edema, hemorrhage, hematoma) in the skin and muscular tissue, bone fractures [sixth left rib (case 4) and the ninth thoracic vertebrae (case 5)], lung perforations with related focal, extensive hemorrhage, and hemothorax (cases 4, 5, and 15), and hemorrhage in the leptomeninges (case 13; Figure 2C) were

observed (Table 1). Gross findings of each case are presented in Supplementary Table 2.

Histologically, mild to moderate multifocal acute segmental myofiber degeneration of skeletal muscle, diffuse hemorrhages within the affected skin and muscles, mild multifocal acute degenerative changes in cardiac muscle, multifocal alveolar edema, hemorrhages in the lung parenchyma, muscular sphincter contraction of the bronchioles, multifocal emphysema, systemic leukocytosis, intracytoplasmic hepatocellular hyaline globules, multifocal corticomedullary adrenal gland hemorrhages, multifocal membranous glomerulonephritis, and multifocal pigmentary tubulonephrosis were observed. Regarding the CNS, multifocal perivascular edema, perivascular cuffs, and hemorrhages in the brain parenchyma were occasionally observed. Mild multifocal granulomatous encephalitis was present in case 3. Clear intravascular spaces were present in all cases (Table 2). Histological findings are presented in Supplementary Table 3.

Statistical Analysis

Although almost half of the affected animals were Atlantic spotted dolphins [46.9% (15/32)], no statistically significant differences were found in the prevalence of fishery interactions among animals of different species ($p = 0.125$), nor among growth development categories ($p = 0.871$), sex ($p = 0.813$), gonad maturation ($p = 0.704$), or the island of stranding ($p = 0.684$) (Table 3).

TABLE 3 | Statistical analysis of the epidemiological data of studied cetaceans during the period 2000–2018 (n = 586).

	Overall N = 586	Other cause of death N = 421	Fishing interactions N = 32	P-value
Species				0.125
<i>Balaenoptera acutorostrata</i>	6 (1.3)	4 (1.0)	2 (6.2)	
<i>Balaenoptera borealis</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Balaenoptera edeni</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Balaenoptera physalus</i>	4 (0.9)	4 (1.0)	0 (0.0)	
<i>Delphinus delphis</i>	51 (11.3)	47 (11.2)	4 (12.5)	
<i>Globicephala macrorhynchus</i>	36 (7.9)	34 (8.1)	2 (6.2)	
<i>Grampus griseus</i>	11 (2.4)	11 (2.6)	0 (0.0)	
<i>Kogia breviceps</i>	23 (5.1)	23 (5.5)	0 (0.0)	
<i>Kogia sima</i>	6 (1.3)	6 (1.4)	0 (0.0)	
<i>Lagenodelphis hosei</i>	3 (0.7)	3 (0.7)	0 (0.0)	
<i>Megaptera novaeangliae</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Mesoplodon bidens</i>	1 (0.2)	1 (0.2)	0 (0.0)	
<i>Mesoplodon densirostris</i>	7 (1.5)	7 (1.7)	0 (0.0)	
<i>Mesoplodon europaeus</i>	6 (1.3)	5 (1.2)	1 (3.1)	
<i>Orcinus orca</i>	1 (0.2)	1 (0.2)	0 (0.0)	
<i>Phocoena phocoena</i>	1 (0.2)	1 (0.2)	0 (0.0)	
<i>Physeter macrocephalus</i>	22 (4.9)	22 (5.2)	0 (0.0)	
<i>Pseudorca crassidens</i>	2 (0.4)	2 (0.5)	0 (0.0)	
<i>Stenella coeruleoalba</i>	92 (20.3)	86 (20.4)	6 (18.8)	
<i>Stenella frontalis</i>	89 (19.6)	74 (17.6)	15 (46.9)	
<i>Stenella longirostris</i>	3 (0.7)	3 (0.7)	0 (0.0)	
<i>Steno bredanensis</i>	23 (5.1)	23 (5.5)	0 (0.0)	
<i>Tursiops truncatus</i>	36 (7.9)	34 (8.1)	2 (6.2)	
<i>Ziphius cavirostris</i>	24 (5.3)	24 (5.7)	0 (0.0)	
Sex				0.813
Female	205 (45.8)	191 (45.9)	14 (43.8)	
Male	243 (54.2)	225 (54.1)	18 (56.2)	
Growth development categories				0.871
Neonate/calf	131 (28.9)	123 (29.2)	8 (25.0)	
Juvenile/subadult	130 (28.7)	120 (28.5)	10 (31.2)	
Adult	192 (42.4)	178 (42.3)	14 (43.8)	
Coast				0.684
El Hierro y La Palma	8 (1.8)	7 (1.7)	1 (3.1)	
La Gomera y Tenerife	154 (34.0)	141 (33.5)	13 (40.6)	
Gran Canaria	125 (27.6)	116 (27.6)	9 (28.1)	
Fuerteventura y Lanzarote	166 (36.6)	157 (37.3)	9 (28.1)	
Mature categories				0.704
Immature	225 (50.1)	210 (50.4)	15 (46.9)	
Mature	224 (49.9)	207 (49.6)	17 (53.1)	
Body condition				0.004
Poor/very poor	162 (37.8)	158 (39.6)	4 (13.3)	
Good/fair	267 (62.2)	241 (60.4)	26 (86.7)	
Diving behavior				0.008
Shallow diver	317 (70.0)	288 (68.4)	29 (90.6)	
Deep diver	136 (30.0)	133 (31.6)	3 (9.4)	

The pathological cause of death in 453 cases was determined, among which, 32 cases were due to fishery interactions.

Body Condition

Body condition could have been determined in 94.7% of the individuals, with a known cause of death (429/453). Most of the animals [62.2% (267/429)] showed good/fair body condition, while 37.8% (162/429) were in poor/very poor body condition. The body condition of two dolphins, which died as a result of fishery interactions, could not have been determined owing to artifactual loss of tissue (case 9) and the very advanced state of decomposition of the carcass (code 5) (case 24) (**Supplementary Table 1**). Nonetheless, most dolphins showing signs of fishery interactions were in good/fair body condition 86.7% (26/30), compared with dolphins in poor/very poor condition. This difference was statistically significant ($p = 0.004$) (**Table 3**).

Diving Behavior

Shallow-water species were stranded in greater numbers, representing 70% of the animals with a known cause of death (317/453), while deep divers represented 30% of the cases (136/453). Although we observed more shallow than deep divers stranded during the study period, this difference was even larger when the prevalence of shallow [90.6% (29/32)] vs. deep divers [9.4% (3/32)] was compared with the findings of fishery interactions. This difference was statistically significant ($p = 0.008$) (**Table 3**).

Temporality of Stranding Events

The yearly average number of stranding caused by fishery interactions was 1.7 animals (32 cases over 19 years), indicating a low rate of fishery interactions within the geographical area. In 2001 and 2017, a slight increase in the number of cases ($n = 5$ each year) was noted. No fishery interactions were recorded during the years 2003, 2006, 2010, or 2011.

DISCUSSION

The rates of fishery interactions among cetaceans are underreported worldwide (2, 3). In the case of stranded cetaceans, an advanced decomposition code may hinder the determination of the cause of death. In addition, most fishery interaction findings (except gear cuts and impressions) are not pathognomonic (4, 5). Nonetheless, fisheries are considered a major global threat to cetaceans (34). Bycatch especially affects the harbor porpoise (*Phocoena phocoena*), bottlenose dolphin, common dolphin, and striped dolphin [e.g., (1, 35, 36)]. Furthermore, cases of chronic entanglements appear to be on the rise globally (8, 10), and their effects in certain baleen populations such as the minke whale is concerning (34).

In the Canary Islands, fishing is mainly artisanal and multi-specific, and is characterized by the use of small vessels (≤ 15 m in total length) and various types of fishing gear. Larger vessels are also used for tuna, but to a lesser extent². Gillnets are allowed during certain periods in some designated areas, whereas trawling is absolutely forbidden in the archipelago. In addition, longlines and traps are forbidden in El Hierro and Fuerteventura,

as well as in all marine reserves of the archipelago [Annex 1. Decreto 182/2004 de 21 de diciembre, Reglamento de la Ley de Pesca de Canarias]. These conservative policies may explain the low annual rates of fishing-related deaths in cetaceans stranded along the Canary coasts.

Of the seven cetacean species identified, the Atlantic spotted dolphin was the most affected. This species, together with the Atlantic bottlenose dolphin, Gervais' beaked whale, short-finned pilot whale, and striped dolphin are regularly present year-round. Moreover, common dolphins and minke whales are seasonally present (37, 38).

Bycatch

Fishery interactions were likely recent and occurred close to the Canary coast in animals that were stranded alive or found dead in a fresh or very fresh state. Although local artisanal fisheries might have been responsible for these deaths, other fishing activities in international waters and illegal fishing cannot be ruled out. Until now, no pathognomonic clinical findings have been identified for bycaught animals (4). However, the greater the number of compatible lesions identified in a case, the more consistent the identification of bycatch (5, 39).

In this study, the ingestion of longline hooks affected Atlantic spotted dolphins alone ($n = 5$). Although this interaction with longline fisheries is well-known worldwide (40, 41), its related pathologies have been poorly reported. In this study, we identified two cases of Atlantic spotted dolphins with hooks that pierced the mandibles. To the best of our knowledge, this is also the first report of esophageal perforation with fibrinosuppurative pleuritis caused by hook ingestion in a dolphin species (**Figure 2D**). Although the literature reflects more information about larynx strangulation with longline fishing gear (42, 43), this type of lesions was not observed in the present study.

Different necropsy findings might be observed in cases of fishing net entrapment, depending on the fishery and the affected cetacean species (5, 21, 22, 44). Our results are consistent with those of Bernaldo de Quirós et al. (6), who reported external net marks, evidence of recent feeding (fresh undigested gastric contents), and disseminated intravascular gas bubbles.

In contrast with the findings of Bernaldo de Quirós et al. (6), only one case had reddish eyes (case 29), and another case had bubbles within the posterior chamber of the eye (case 23). In the present study, hyperinflated and hemorrhagic lungs were also identified, with occasional froth and rib impressions, corresponding histologically with areas of marked emphysema and alveolar edema. These findings agreed with those of Moore et al. (5). However, Bernaldo de Quirós et al. (6) found that froth in the airways and other lung changes (i.e., wet, heavy edema, congestion, and hemorrhage) were statistically poor indicators of bycatch.

In the present study, case 26 was one example of a bycaught dolphin with gas embolism. This animal exhibited a large number of gas bubbles (gas score of 18), consistent with the findings of Bernaldo de Quirós et al. (24). In addition, the composition of the gas bubbles was consistent with gases produced by compression and decompression, in which nitrogen is the main component,

²<https://www.gobiernodecanarias.org/pesca/>

and CO₂ can sometimes be present at high concentrations (20). Hydrogen, a marker of putrefaction, was found in the mesenteric veins alone, which is consistent with the findings of Bernaldo de Quirós et al. (24).

Pathological findings in cases of fishing net entrapment often suggest some degree of physical struggle associated with varying degrees of muscular exertion (5). In bycaught dolphins, the adrenocortical response and hyperthermia (45) induce injury to the skeletal muscle, similar to that described in live stranded cetaceans (26, 27, 31). Acute degenerative changes have also been observed in severely polytraumatized free-ranging stranded cetaceans, such as in cases of ship strikes (23, 28) and fatal social traumatic intra-interspecific interactions (23, 32). Previous studies have reported cardiac changes (29, 46, 47), as well as intracytoplasmic hepatocellular hyaline globules (13, 27, 48) in agonal situations. Intravascular coagulation, mostly present in bycaught dolphins that endured aggression during handling, has been described in domestic animals with extensive tissue destruction (49). We also observed a few cases with mild inflammation of the CNS. These findings are consistent with the presence of concomitant infections.

Chronic Entanglement

It is difficult to know the actual number of entangled cetaceans even if the number of entangled individuals within a specific population is known, as the same animal can become entangled multiple times (50), and entangled carcasses tend to sink (8). Even with the limitations of the available data, the number of reported entanglements during the last decade is three times higher than that reported during the 1990s, and 97% of those cases were entangled in fishing gear (10).

In the Atlantic Ocean, at least half of the mysticetes' deaths are caused by fishing gear (51). Minke whales particularly, appear to be less likely to survive entanglements than larger whales (52). The first description of an entangled minke whale in the Canary Islands was reported in 1993 in Morro Jable-Fuerteventura. This stranded animal was found dead, with a fishing net in the rostrum (53). In recent years (2012–2020), entanglements have included seven minke whales, six Bryde's whales (*B. edeni*), one humpback whale (*Megaptera novaeangliae*)³, two rough-toothed dolphins (*Steno bredanensis*), and one member of the Delphinidae family, sighted close to the Canary coasts (personal communication with the Canary Islands stranding network). Among these, three minke whales and two Bryde's whales were disentangled⁴. Whether those whales survived remains unknown, as no follow-up investigations were conducted after the disentanglement. Consistent with the findings of the present study, we consider chronic entanglements a potential threat to cetacean populations in the Canary Islands, especially minke and Bryde's whales.

Entangled mysticetes are commonly found with ropes and nets within the oral cavity, or surrounding the flippers, or tail, and entangled odontocetes are usually found with recreational fishing gear, longlines, and fishing lures (5). Even if the gear

becomes detached, the scars of healed wounds may remain (54). In chronic entanglements, open and unhealed wounds could lead to septicemia and even death (5). Although no microbiological studies have been conducted on entangled cases in the present study, histological evaluation of the cases revealed intravascular coagulation, leukocytosis (mostly neutrophilic), and multiorgan hemorrhages in most of the affected animals.

In addition, those cases in poor body condition showed atrophy of the skeletal muscle. Case 19 also exhibited severe serous atrophy of pericardial fat, which indicates a catabolic condition. In the present study, only one animal was stranded alive and most cases showed mild degenerative changes in myocardiocytes. The occurrence of cardiac failure in cases of chronic entanglement should be further investigated. Similarly, the presence of hepatocellular hyaline globules in almost all cases was remarkable, previously described in agonal situations of stranded cetaceans (27, 48).

Fisherman Aggression

The predominance of artisanal fisheries in the Canary Islands suggests daily direct contact between cetaceans and fishermen, who are usually in small vessels a few meters above. Our results showed lethal trauma on the dorsal side of the dolphins, which is consistent with the fishermen's position just above, on the water's surface. Stabs and contusions affected mainly the thoracic cavity (with associated hemothorax, bone fractures, lung perforations), and/or the cephalic region (head contusions with associated brain hemorrhages). No other common findings were observed between these cases and the bycatch cases (e.g., net cuts/impressions on the skin, hyperinflated lungs, intravascular disseminated gas bubbles), nor evidence of being brought to deck. Although this category has been previously described, few cases have been reported (7, 55), because the most frequent aggressions inflicted by fishermen (amputations, stabs, and perforations) occur onboard, when animals are trapped in gillnets or trawlnets (5). Another form of human aggression is gunshot wounds (56), which were not observed in the present study. Histological findings, such as severe focal, extensive hemorrhages in the skin and muscles, and hemorrhagic lungs, agreed with the gross findings, which indicated that the various cases of trauma were inflicted while the animals were still alive. Histological findings were associated with agonal perimortem changes in dolphins, as well as systemic leukocytosis, due to open wounds.

Statistical Analysis

Body Condition

The body condition index is a good indirect indicator of the nutritional status of cetaceans (17). Previous studies have described bycaught dolphins in good body condition (4), but this is common in cetacean deaths due to the fatal trauma of ship strikes (5, 7, 12, 13), and social traumatic intra-interspecific interactions (32).

Our results indicate that individuals with good nutritional status may be more susceptible to adverse fishery interactions (especially bycatch), compared with animals exhibiting catabolic metabolism due to energy demanding pathologies (i.e., severe

³<https://twitter.com/GranCanariaCab/status/1209437122345734145>

⁴https://www.eldiario.es/canariasahora/sociedad/Liberada-canarias-ballena-atrapada-marana_0_76292969.html

infections, parasitism, or neoplasia). In contrast, chronic entangled cetaceans typically appear in poor body condition (5, 7). Our results showed only a few necropsied cases of entanglement, only half of which showed poor-very poor body condition. However, observational records along the Canary coasts have confirmed the presence of entangled whales in poor body condition (personal communication with the Canary Islands stranding network).

Diving Behavior

In the present study, shallow diving species were affected by fishery interactions to a significantly greater extent than deep diving species. These findings are consistent with those of previous studies, in which the majority of the affected cetaceans were shallow-water species (5, 10, 40). In the Canary Islands, artisanal fishing activities are mainly pelagic and superficial (0–150 m depth)⁵, which is consistent with the swimming patterns of shallow species.

Nonetheless, it is important to highlight the fact that deep divers, especially sperm whales (*Physeter macrocephalus*), have been reported worldwide with considerable amounts of ingested marine debris, including fishing gear (10, 57, 58). The Canary waters contain a great variety of deep divers, which are known to be affected by marine debris (59). We highly recommend more detailed descriptions of ingested foreign bodies, to better understand whether they originate from fishery activities.

Temporality of Stranding Events

Fortunately, our geographical area showed relatively low numbers of deaths due to fishing activities. This might be related to the sustainable artisanal fisheries and restrictive laws in the Canary waters. Similarly, in the Azores of Portugal, bycatch rates are low, with no evidence of any increase over the last 15 years (60). Artisanal fisheries cause a considerable number of deaths worldwide, even though these numbers are typically lower than those caused by industrial fisheries (1, 61).

In recent years, although sightings of live chronic entangled cetaceans have increased along the archipelago, necropsied cases remain scarce. The widespread use of social media to report the consequences of pollution in marine ecosystems has helped us to learn more about these interactions.

CONCLUSIONS

This is the first retrospective study of fishery interactions in stranded cetaceans along the Canary Islands. The determination of different types of fishery interactions as the cause of death was based on a 20-year investigation of stranded cetaceans. We described the most relevant gross and histologic findings in each type of interaction. Three types of fishery interactions were observed according to gross findings, including chronic entanglement, fisherman

aggression, and bycatch (longline hook ingestion and fishing net entrapment).

We found that the Atlantic spotted dolphin, regularly seen in our geographical area, was the most affected species. We also found that cetaceans in good-fair body condition and shallow-water species were significantly more affected. The low prevalence of fishery interactions in this region could be attributed to the broad protective legislation applicable to the Canary waters and the most prevalent type of fisheries, artisanal fisheries. However, there is increasing concern about chronic entanglements, especially in minke and Bryde's whales. We encourage continuous pathological studies on stranded cetaceans, to monitor fishing-related deaths and their consequences in cetacean populations. The findings of this study might contribute to the implementation of appropriate conservation policies in the Canary Islands.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

RP-L, YB, MArb, AF, and ES: conceptualization. RP-L, AF, ES, JD, JD-D, SS, A, NG-Á, DZ, AX, MR, IF-J, FC, PD-S, SS-G, NC, CS-S, MArr, YB, and MArb: sampling and diagnosis of the cause of death of each animal. PS, RP-L, and YB: data analyses. RP-L and MR: image editing. RP-L: writing. YB, MArb, MR, AF, and ES: supervision. All authors: review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2020.567258/full#supplementary-material>

⁵<http://www.grancanariapescaenred.com/sobre-nosotros/como-pecamos/>

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Conclusions



Conclusions

First: From January 2000 to December 2015, 7.7% of the cetaceans necropsied in the Canary Islands presented foreign bodies in the digestive tract. The affected individuals belonged to 15 species of cetaceans, both mysticetes and odontocetes.

Second: In this period, 2.8% of necropsied cetaceans died as consequence of pathologies (gastric impaction/obstruction, gastric perforation and/or severe ulcerative gastritis) caused by the ingestion of foreign bodies. Most of the objects found were plastics (80.6%).

Third: The statistical study indicated that poor body condition and deep diving behaviour were predisposing or risk factors for foreign body ingestion, whereas the adult age was a protective factor.

Fourth: From January 2000 to December 2017, 4.4% of cetaceans necropsied in the Canary Islands died as consequence of an intra-interspecific traumatic interaction with other cetacean/s. Of the 8 species included in this group, the most affected was the short-finned pilot whale.

Fifth: The study of the skin lesions and the determination of the distance between the teeth, allowed us to identify interspecific aggressions such as killer whale/s on Cuvier's beaked whale, pygmy sperm whale, and pilot whale, and Atlantic bottlenose dolphin/s aggressions on common dolphin and spotted dolphin. Likewise, it allowed us to identify intraspecific aggressions between individuals of striped dolphin.

Sixth: The deep divers, specimens in good body condition and stranded individuals in La Gomera and Tenerife were statistically the most affected by fatal encounters between cetaceans.

Seventh: From January 2000 to December 2017, 0.6% of the cetaceans necropsied in the Canary Islands died due to a fatal interaction with their potential prey. Two species of cetaceans were affected, the Risso's dolphin and the false killer whale.

Eighth: From January 2000 to December 2018, 5.5% of cetaceans necropsied in the Canary Islands died because of an interaction with fishing activities. Of the 7 species included in this group, the most affected was the Atlantic spotted dolphin.

Ninth: The injuries identified in this group of cetaceans were caused (from highest to lowest prevalence) by accidental capture or "bycatch", persistent entanglement in fishing devices (including remains of nets) and aggressions by fishermen. Recently, an increase in the number of sightings of mysticetes swimming entangled near the coast has been detected, mainly minke whales and tropical fin whales.

Tenth: The shallow divers and specimens in optimal body condition were statistically the most affected by interaction with fishing activities.

Resumen extendido del Proyecto de Tesis Doctoral



Resumen extendido del Proyecto de Tesis Doctoral

1. Introducción

Las Islas Canarias, situadas en el área geográfica de la Macaronesia, presentan unas características oceanográficas únicas que propician la presencia de una gran biodiversidad marina. Este archipiélago es un punto estratégico para el estudio de los cetáceos en Europa. En sus aguas se han descrito hasta 30 especies (Biocan - Banco del Inventario Natural de Canarias⁸), en concreto 7 especies de misticetos y 23 de odontocetos. Algunas de estas especies se pueden avistar a lo largo de todo el año, aunque con distinta frecuencia: el delfín de dientes rugosos (*Steno bredanensis*), el delfín listado (*Stenella coeruleoalba*), el delfín moteado del Atlántico (*Stenella frontalis*), el delfín mular (*Tursiops truncatus*), el cachalote común (*Physeter macrocephalus*), el cachalote pigmeo (*Kogia breviceps*), el calderón gris (*Grampus griseus*), el calderón tropical (*Globicephala macrorhynchus*), el rorcual tropical (*Balaenoptera edeni*), el zifio de Blainville (*Mesoplodon densirostris*), el zifio de Cuvier (*Ziphius cavirostris*) y el zifio de Gervais (*Mesoplodon europaeus*) (Comunicación interna, Red de Varamientos de las Islas Canarias).

Los cetáceos son considerados especies centinela de la salud de los diversos ecosistemas que habitan. Su larga esperanza de vida, sus hábitos residentes o estacionales, el hecho de encontrarse en un nivel trófico alto compartiendo recursos alimenticios con el ser humano y que cuenten con abundante tejido graso que favorece la bioacumulación y biomagnificación de compuestos tóxicos lipofílicos, entre otros, hace que los cetáceos estén expuestos a factores de riesgo comparables a las poblaciones humanas con las que comparten ecosistema (Bossart 2011).

Las poblaciones de cetáceos hacen frente a amenazas de origen antrópico y de origen natural. Dentro de las amenazas de origen humano encontramos aquellas que pueden provocar la muerte de los individuos directamente, como las actividades pesqueras, las colisiones con embarcaciones, la ingestión de cuerpos extraños y las maniobras militares con uso de sónar. De manera indirecta, las poblaciones de cetáceos sufren las consecuencias de las actividades que se llevan a cabo en el medio marino (p. ej., la escasez de recursos alimenticios debido a la sobrepesca, la contaminación acústica producida por las distintas actividades humanas y los efectos tóxicos derivados de la exposición a contaminantes). Existen además otros factores asociados a la acción del hombre que afectan al medio marino, como los derivados del cambio

climático, que por su amplitud son capaces de afectar de muy diversas formas a los cetáceos. Por otra parte, la actividad del avistamiento de cetáceos produce un impacto sobre las poblaciones, además de otras actividades náuticas.

Los efectos a largo plazo del deterioro del estado sanitario del medio acuático y las consecuencias para la salud de los cetáceos son preocupantes a la vez que indeterminadas. Sin embargo, se sabe que los factores estresantes crónicos pueden afectar a la respuesta inmune de los individuos frente a los agentes patógenos naturales. Por ejemplo, en las epizootias por Morbillivirus que han causado grandes mortandades en cetáceos y otros mamíferos marinos, se han detectado niveles altos de contaminantes (Aguilar y Borrell 1994). Además, la contaminación ambiental se ha relacionado con neoplasias y disminución de la tasa reproductiva en cetáceos de todo el mundo (Martineau y cols. 1999). Así mismo, el contacto de poblaciones humanas con animales salvajes como los cetáceos puede producir la transmisión de agentes infecciosos, como *Brucella ceti* y *Toxoplasma gondii*, algunos de ellos zoonóticos (Bossart 2011). Para finalizar, los cetáceos hacen frente no solo a agentes infecciosos propios de su medio o costeros, sino también a agentes biológicos transportados a grandes distancias por objetos flotantes a la deriva (como los microplásticos y otras basuras marinas) o por otras especies acuáticas. Por todo ello, los efectos indirectos de las actividades humanas sobre el medio acuático y la salud de los cetáceos son difíciles de delimitar.

El Centro Atlántico de Investigación de Cetáceos colabora con la red de varamientos de cetáceos de las Islas Canarias desde 1992 hasta la actualidad. Este centro ha realizado necropsias a casi mil cetáceos varados de veinticinco especies. Gracias a estos estudios se ha podido determinar que los cetáceos en Canarias mueren en su mayor parte por causas de origen natural, si bien existe un porcentaje menor de muertes que son ocasionadas por interacciones con actividades pesqueras, colisiones con embarcaciones e interacción con desechos marinos (Arbelo 2007, Díaz-Delgado et al. 2015). Desde que se aprobó la moratoria para el uso de sónar en las aguas Canarias en el año 2004 no se han detectado más cetáceos varados con lesiones compatibles a un embolismo gaseoso-graso asociado al uso de sónares (Fernández y cols. 2013).

Anualmente aparecen animales con lesiones traumáticas y otras patologías relacionadas cuyo origen resulta difícil de determinar. Esto podría estar indicando una interacción de origen antrópico o natural. La presente tesis doctoral pretende mejorar el diagnóstico del origen de las lesiones traumáticas y, por tanto, el diagnóstico de las causas de muerte en cetáceos varados.

2. Objetivos

El objetivo general de este proyecto de tesis doctoral es:

Identificar los hallazgos patológicos (lesiones) asociados a causa/s de muerte traumática en las especies de cetáceos varados en las Islas Canarias.

Para alcanzar el objetivo general, se propusieron tres objetivos específicos:

1. Identificar los hallazgos patológicos asociados a la ingestión de cuerpos extraños y su prevalencia en las especies de cetáceos varados en las Islas Canarias (publicación 1).
2. Identificar los hallazgos patológicos asociados a interacciones traumáticas intra-interespecíficas letales y su prevalencia en las especies de cetáceos varados en las Islas Canarias (publicación 2).
3. Identificar los hallazgos patológicos asociados a interacciones letales con actividades pesqueras y su prevalencia en las en las especies cetáceos varados en las Islas Canarias (publicación 3).

3. Material y métodos

Los materiales y la metodología de trabajo empleados para los estudios derivados del proyecto de tesis han sido comunes. Es por ello que, para resumirlos, se han unificado en este apartado destacando las diferencias más notables.

Durante el periodo de estudio, 465 cetáceos vararon y fueron necropsiados siguiendo el protocolo de Kuiken y García-Hartmann (1991). Los diversos tejidos fueron sistemáticamente observados y muestreados. Las lesiones externas e internas se describieron, fotografiaron y muestrearon. Las muestras fueron fijadas en formol al 10%, embebidas en parafina, seccionadas a 5 μ m y teñidas con hematoxilina y eosina para el estudio histológico de las mismas. En el desarrollo de la presente tesis doctoral y previamente a la misma, la doctoranda ha participado activamente en las necropsias realizadas por el personal especializado del Centro Atlántico de Investigación de Cetáceos, con consentimiento del Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente. No se realizó ningún experimento con animales vivos.

Fruto de este proyecto de tesis se han publicado tres estudios retrospectivos sobre tres causas de muerte o entidades patológicas de origen traumático en cetáceos varados en las Islas Canarias: las patologías asociadas a la ingestión de cuerpos extraños (publicación 1),

las interacciones traumáticas intra-interespecíficas (publicación 2) y la interacción con pesca (publicación 3). Dichos estudios retrospectivos abarcaron distintos periodos:

- Primera publicación: entre enero de 2000 y diciembre de 2015. Se necropsiaron un total de 465 cetáceos varados y 36 presentaron cuerpos extraños.
- Segunda publicación: entre enero de 2000 y diciembre de 2017. Se necropsiaron 540 cetáceos y 27 murieron como consecuencia de una interacción traumática intra-interespecífica.
- Tercera publicación: entre enero de 2000 y diciembre de 2018. Se necropsiaron 586 cetáceos y 32 murieron como consecuencia de una interacción con actividades pesqueras.

En estos estudios retrospectivos se recogieron los datos epidemiológicos de cada cetáceo varado (fecha, lugar y tipo de varamiento), así como las variables biológicas (especie, desarrollo físico, sexo, madurez sexual), la condición corporal, el grado de descomposición del animal y la entidad patológica. Para ciertas variables se realizó una categorización:

- Desarrollo físico: Según la longitud total del individuo (Perrin y cols. 2009) y el estudio histológico de las gónadas (Geraci y Lounsbury 2005). En las publicaciones 2 y 3 se utilizaron además las características osteológicas estudiadas por Tejedor (2016). Esta categoría se subdividió en: neonato, cría, juvenil, subadulto y adulto.
- Condición corporal: Fue estimada según la conformación física externa de los cetáceos siguiendo los criterios de Joblon y cols. (2014). Esta categoría se subdividió en muy pobre, pobre, moderada y buena condición corporal.
- El grado de conservación: En la publicación 1 se determinó siguiendo el protocolo de Kuiken y García-Hartmann (1991), adaptando el código 1 de un cetáceo varado vivo, a un cetáceo muerto durante la asistencia o eutanasiado (muy fresco). Este cambio ya se refleja en el protocolo de IJsseldijk y cols. (2019), referenciado en las publicaciones 2 y 3.

En las tres publicaciones, se catalogaron las especies de buceadores profundos como aquellas que alcanzan profundidades de más de 500 m en busca de alimento (*Kogia* spp, *Physeter macrocephalus*, *Ziphius cavirostris*, *Mesoplodon* spp, *Globicephala macrorhynchus* y *Grampus griseus*) (Gannier 1998, Astruc y Beaubrun 2005, Aguilar de Soto 2006, Tyack y cols. 2006, Watwood y cols. 2006, West y cols. 2009)

Todos los informes de necropsia de los cetáceos varados correspondientes a los periodos anteriormente citados se revisaron sistemáticamente. Para ello, se tuvo acceso además al registro fotográfico de los casos y a las muestras histológicas para el estudio de las mismas en caso de que fuera necesario para descartar o confirmar algunas lesiones. Los casos de necropsia en los que la causa de muerte fuera desconocida, es decir, no hubiera evidencias suficientes que permitieran el diagnóstico de una causa probable de muerte, se catalogaron como entidad patológica no determinada.

Las causas de muerte de los animales de estudio se agruparon en las entidades patológicas descritas por Arbelo y cols. (2013) como:

- Patologías de origen natural o no directamente antropogénicas: Interacción traumática intra-interespecífica, patología natural asociada a una pérdida significativa de la condición corporal, patología natural asociada a una condición corporal moderada/buena, patología neonatal-perinatal y varamiento activo.
- Patologías de origen antrópico: Interacción con pesca, patología asociada a la ingestión de cuerpos extraños, colisión con embarcación y maniobras militares asociadas al uso de sónar.

En las publicaciones 2 y 3, los resultados del estudio histológico de ciertos tejidos y órganos (p. ej., músculo esquelético, músculo cardiaco, pulmón, hígado, riñón, glándula adrenal, encéfalo) fueron revisados y comparados con el fin de encontrar lesiones relacionadas con estrés.

En la publicación 3, se realizó el estudio cuantitativo del embolismo gaseoso (gas score) y la determinación de la composición del gas en un individuo (caso 26) siguiendo protocolos estandarizados (Bernaldo de Quirós 2011, 2013).

En todas las publicaciones, se empleó el estudio estadístico para identificar significancia estadística entre las variables y la entidad patológica del estudio. Las variables categóricas (p. ej., especie, comportamiento de buceo, edad, sexo, madurez sexual, condición corporal, localización del varamiento) fueron expresadas en frecuencias y porcentajes y comparadas adecuadamente usando el test de Chi cuadrado (χ^2) o el test exacto de Fisher. La significancia estadística se fijó en $p < 0,05$. En la publicación 1, con motivo de identificar factores que mantuvieran una asociación independiente con la entidad, se realizó un análisis de regresión logística multivariable. Las variables que mostraron una asociación significativa con la entidad en el análisis univariable fueron introducidas en el análisis multivariable. Después se seleccionaron las variables basándose en la enumeración de un algoritmo completo y en el criterio de la información de Bayes. Los

modelos se resumieron en coeficientes (SE), p-valores (prueba de razón de verosimilitud) y proporción de probabilidades (odds ratio), los cuales fueron estimados por intervalos de confianza al 95%. Los datos de todos los análisis fueron tratados con el paquete de R, en la versión 3.3.1. para la publicación 1 y 2, y en la versión 3.6.1 para la publicación 3.

Por motivos estadísticos, en los tres estudios se reagruparon los animales estudiados en las siguientes categorías:

- Desarrollo físico: neonato-cría, juvenil-subadulto y adulto.
- Condición corporal: muy pobre-pobre y moderada-buena.
- Localización de varamiento: En la publicación 1 se reagruparon las islas por proximidad geográfica en islas del Este (Fuerteventura, Lanzarote y La Graciosa), islas del Oeste (El Hierro, La Gomera y La Palma) y Tenerife y Gran Canaria por separado. En la publicación 2 y 3, se reagruparon las islas basándonos además en la presencia de poblaciones de cetáceos residentes en islas del Este (Fuerteventura, Lanzarote y La Graciosa), islas del Oeste (El Hierro, La Palma), La Gomera y Tenerife juntas y Gran Canaria por separado.

El estudio anatomopatológico completo de los cetáceos varados en el archipiélago canario ha desvelado la presencia de diversas lesiones traumáticas que se corresponden con entidades patológicas de distinta naturaleza:

- Traumatismos no antropogénicos: varamiento activo e interacción traumática intra-interespecífica.
- Traumatismos antropogénicos: colisión con embarcación, interacción con pesca, patologías asociadas a la ingestión de cuerpos extraños.

4. Resultados y Discusión: Publicaciones

A continuación, se exponen y discuten de manera resumida los principales resultados obtenidos del estudio detallado de las tres publicaciones del proyecto de tesis doctoral.

4.1. *Patologías asociadas a la ingestión de cuerpos extraños (Publicación 1)*

Los desechos marinos presentan múltiples orígenes. Se trata de vertidos de basura ilegales o accidentales provenientes de tierra o mar. Dentro de estos se incluyen los descartes de redes de pesca, que producen “ghost fishing” o pesca fantasma durante años. Gran parte de los desechos marinos están formados por compuestos sintéticos,

generalmente plásticos, que se distribuyen a lo largo de toda la columna de agua, así como en la superficie marina, depositándose en los lechos marinos, e incluso formando parte del hielo polar (Obbard y cols. 2014, Woodall y cols. 2014, Cózar y cols. 2014, Fossi y cols. 2018). La Corriente de Canarias arrastra los desechos marinos hasta el archipiélago canario, proveniente del Atlántico Norte (Baztan y cols. 2014). La ingestión de cuerpos extraños (CEs) se ha descrito en especies de cetáceos de todo el mundo (p. ej., Derraik 2002, Baulch y Perry 2014). Estudios anteriores en cetáceos varados en el archipiélago revelaron una baja prevalencia de cetáceos que han muerto por ingestión de desechos marinos (Arbelo y cols. 2013, Díaz-Delgado y cols. 2018).

Como resultado de la presente tesis doctoral se publicó un estudio retrospectivo de los cetáceos varados que presentaban CEs entre enero de 2000 y diciembre de 2015, así como de las patologías asociadas a la presencia de CEs. En ese periodo, de los 465 cetáceos necropsiados, 36 animales presentaron CEs. Lo que representa el 7,7% (36/465) de los cetáceos necropsiados en ese periodo de estudio. Se trata de individuos de 15 especies: 6 cachalotes, 5 delfines moteados del Atlántico, 5 zifios de Cuvier, 4 calderones grises, 3 delfines de dientes rugosos, 3 delfines listados, 2 cachalotes pigmeos, 2 zifios de Blainville, 1 zifio de Gervais, 1 delfín común (*Delphinus delphis*), 1 delfín de Fraser (*Lagenodelphis hosei*), 1 delfín mular, 1 rorcual aliblanco (*Balaenoptera acutorostrata*), 1 rorcual común (*Balaenoptera physalus*) y 1 zifio de Sowerby (*Mesoplodon bidens*). La mitad de las especies de cetáceos descritas en Canarias se vieron afectadas por la ingestión de CEs, lo que puede significar un problema con los desechos marinos en estas aguas.

En el 80,6% (29/36) de los casos, los CEs estaban constituidos por objetos de plástico, en su mayoría bolsas de plástico, pero también por tapones, hilos de nylon y objetos cilíndricos. Este dato coincide con el hecho de que la mayoría de los objetos que forman parte de los desechos marinos son plásticos (Cózar y cols. 2014). En una revisión de más de 460 casos de cetáceos varados, la mayor parte de los CEs encontrados eran plásticos (Baulch y Perry 2014). Dos misticetos (un rorcual común y un rorcual aliblanco) fueron incluidos en esta categoría al verse afectado el tracto digestivo con enredamientos.

Las lesiones encontradas fueron: gastritis ulcerativa con presencia de sangre en la luz gástrica (14/36; 38,9%), impactación estomacal (9/36; 25,0%), perforación gastro-intestinal (3/36; 8,3%), glositis ulcerativa (2/36; 5,6%), estomatitis (2/36; 5,6%), lesiones en la mucosa ya cicatrizadas (2/36; 5,6%) y petequias (1/36; 2,8%). Estas lesiones han sido descritas anteriormente en cetáceos que habían ingerido CEs (p.ej., Walker y Coe 1989, Laist 1997, Abollo y cols. 1998, Stamper y cols. 2006, Unger y cols. 2017). En diez animales no se encontraron lesiones asociadas a la presencia de CEs. Este hecho ha sido relacionado con una ingestión reciente del CE durante el varamiento en

estudios anteriores (Denuncio y cols. 2011, Mazzariol y cols. 2011, Unger y cols. 2016).

En este estudio, trece individuos murieron a causa de la gravedad de las lesiones asociadas a la presencia del CE (13/36), concretamente por impactación (11/36; 30,6%) o perforación gastrointestinal (2/36; 5,6%). Esto representa un 2,8% del total de individuos estudiados (13/465). El resto de los cetáceos que presentaron CEs murieron como consecuencia de otras patologías.

Este es el primer estudio en cetáceos varados que emplea estadística para determinar la significancia estadística de varias variables respecto a la presencia de CEs y la muerte por patologías asociadas a la presencia de CEs. Como resultado de la misma se detectó que la mayor parte de los individuos afectados eran odontocetos (34/36; 94,4%). Se encontraron diferencias significativas entre la variable especie y la presencia de CEs ($p < 0,001$). Respecto al total de individuos varados y necropsiados por especie, el calderón gris presenta la mayor prevalencia de CEs (4/12; 33,3%), seguido del cachalote (6/28; 21,4%) y las especies del género *Mesoplodon* (4/19; 21,1%).

Se encontraron diferencias significativas entre la variable tipo de buceo y la presencia de CEs ($p = 0,004$). En este estudio, más de la mitad de los cetáceos que presentaron CEs eran buceadores profundos (21/36; 58,3%). Este dato coincide con otros estudios en los que los buceadores profundos son las especies más afectadas (p. ej., Jacobsen y cols. 2010, de Stephanis y cols. 2013, Baulch y Perry 2014, Unger y cols. 2016). El estudio estadístico indicó que el comportamiento de buceo profundo es un factor de riesgo para la presencia de CEs (OR=3,330; 95%CI=1,470; 7,546).

Se detectaron diferencias estadísticas cuasi significativas ($p = 0,053$) entre la categoría de desarrollo físico (edad) y la presencia de CEs. La mayor prevalencia de CEs se encontró en individuos juveniles/subadultos (15/36; 41,7%), seguidos de las crías (12/36; 33,3%), siendo menor en adultos (9/36; 25,0%). También se detectó una diferencia significativa entre esta variable y la patología asociada a la presencia de CEs ($p = 0,024$), siendo la prevalencia más alta de muertes en individuos juveniles/subadultos (7/13; 53,9%). La edad adulta mostró una asociación independiente con la presencia de CEs y se consideró un factor de protección frente a ésta (OR= 0,233; 95%CI= 0,084; 0,641).

También se detectaron diferencias estadísticas cuasi significativas ($p = 0,067$) entre la variable de madurez sexual y la presencia de CEs [inmaduros (23/36; 63,9%); maduros (13/36; 36,1%)]. Además, se detectaron diferencias significativas en cuanto a la patología por CEs ($p = 0,043$), ya que la mayoría de los individuos que murieron por cuerpos extraños eran inmaduros sexualmente (10/13; 76,9%). Hasta la fecha, no se tenía constancia de estudios previos sobre prevalencia de CEs según la madurez sexual.

Para finalizar, la mayor parte de los individuos que habían ingerido CEs presentaron una condición corporal deficiente (20/36; 55,6%). Respecto al total de necropsiados, el 30,1% de los cetáceos estudiados presentaron una condición corporal pobre o muy pobre. Esta diferencia resultó ser significativa ($p=0,002$). Además, se determinó que una condición corporal deficiente es un factor de riesgo para la presencia de CEs (OR= 4,080; 95% CI=1,794; 9,278). El hallazgo de cuerpos extraños en cetáceos varados con una condición corporal pobre o muy pobre ha sido descrita con anterioridad en cetáceos (p. ej., Secchi y Zarzur, 1999). Si bien, hasta la fecha se desconoce si los cetáceos con CEs presentan una baja condición corporal como causa o como consecuencia de la ingestión de éstos.

4.2. Interacciones traumáticas intra-interespecíficas (Publicación 2)

Las interacciones intra-interespecíficas forman parte de la conducta social entre cetáceos con individuos de la misma y distintas especies de su hábitat. Cuando estas interacciones se vuelven agresivas las lesiones traumáticas derivadas de estos encuentros pueden llevar a los individuos agredidos a la muerte (p. ej., Dunn y cols. 2002, Parsons y cols. 2003, Scott y cols. 2005).

Como resultado de este proyecto de tesis, se publicó un estudio retrospectivo sobre la prevalencia y las patologías asociadas a las interacciones traumáticas intra-interespecíficas en cetáceos varados entre enero de 2000 y diciembre de 2017. En ese periodo un total de 27 cetáceos de los 540 estudiados murieron a causa de lesiones severas resultado de una interacción agresiva intra-interespecífica. Este dato representa el 6,3% (27/432) de los cetáceos con causa de muerte conocida en dicho periodo y un 5% (27/540) del total de necropsiados. De ellos, 24 murieron a causa de una interacción traumática entre cetáceos de la misma o diferente especie y otros 3 animales murieron como consecuencia de un accidente durante la depredación. Los individuos afectados pertenecían a 10 especies: 8 calderones tropicales, 3 delfines listados, 3 cachalotes pigmeos, 3 delfines moteados del Atlántico, 3 delfines comunes, 2 zifios de Gervais, 2 calderones grises, 1 delfín mular, 1 falsa orca (*Pseudorca crassidens*) y 1 zifio de Cuvier.

En el 95,8% (23/24) de los casos de agresión con otro/s cetáceo/s, se apreciaron lesiones lineales, paralelas, no severas y poco profundas, en la mayoría de los casos cicatrizadas, conocidas como “rake marks” o marcas en rastrillo. Estas lesiones son producidas por encuentros sociales entre cetáceos, en los dientes de un animal se ponen en contacto con la epidermis del otro. Cuando estas lesiones profundizan, asociadas a un carácter más agresivo en el comportamiento, pueden llegar a ulcerar la piel e incluso exponer planos profundos (Arbelo y cols. 2013). En el 7% (10/24) de los individuos de esta categoría, se apreciaron marcas de dientes recientes y severas producidas por encuentros agresivos. Estas marcas fueron compatibles con interacciones agresivas

con orcas en dos hembras gestantes de cachalote pigmeo, una cría de calderón tropical y un adulto de zifio de Cuvier. En estos animales se apreciaron marcas en rastrillo con una separación interdental de 28–43mm, superior a la distancia interdental registrada de otros delfínidos varados como los calderones tropicales (Tejedor 2016), así como marcas multifocales redondeadas con la misma distancia interdental. Otros cuatro animales, tres crías de delfín común y un adulto de delfín moteado del Atlántico, presentaron lesiones en rastrillo severas con una separación de 7–12mm compatible con la distancia interdental de un individuo adulto de delfín mular (Tejedor 2016). También, se encontraron lesiones cutáneas compatibles con una agresión intraspecífica en dos hembras de delfín listado, mayoritariamente afectando a la zona genital y cabeza. El 29,2% de los casos (7/24), presentaron mordidas de tiburón postmortem, que se manifiestan como marcas incisivas semi-circulares paralelas entre sí y distribuidas de manera multifocal sin cambios vasculares ni inflamatorios asociados en el tejido circundante, mayoritariamente distribuidas en la parte dorsal y ventral del pedúnculo cercanas al área perianal.

Todos los cetáceos que murieron por encuentros agresivos con otro cetáceo presentaron cambios vasculares. El 62,5% (15/24) de los animales presentaron hemorragias y/o congestión en el sistema nervioso central, el 54,2% (13/24) presentaron hematomas subcutáneos, el 50% (12/24) presentaron hemotórax, el 29,2% (7/24) hemoabdomen y el 4,2% (1/24) hemopericardio. La mitad de los animales diagnosticados con trauma intra-interespecífico (12/24) presentaron fracturas que afectaban a varios huesos, siendo bilaterales en 5 casos. Estas lesiones han sido descritas en anteriores ocasiones en cetáceos que han sufrido traumas intra-interespecíficos (Ross y Wilson 1996, Jepson y Baker 1998). Otros hallazgos macroscópicos fueron: la presencia de contenido digestivo no digerido en el estómago (37,5%; 9/24), edema traqueal (33,3%; 8/24) y perforación pulmonar (12,5%; 3/24).

Es importante destacar que a excepción de las marcas en rastrillo el resto de hallazgos macroscópicos citados no son lesiones características de esta entidad patológica, ya que se han descrito en cetáceos asociadas a muy diversas causas (Arbelo y cols. 2013, Díaz-Delgado y cols. 2018).

El estudio histológico de las muestras permitió observar lesiones asociadas a un cuadro de estrés agudo, como son la degeneración muscular aguda del músculo esquelético, en el 70,8% (17/24) de los casos (degeneración segmentaria, aumento de la eosinofilia del sarcoplasma e hipercontracción de las fibras musculares). En el músculo cardiaco se apreciaron cambios como vacuolizaciones yuxtancleares y aumento de la acidofilia del citoplasma en el 66,7% (14/21) de los casos. Estos cambios en el músculo esquelético y cardiaco se han descrito con anterioridad en animales afectados por esta entidad patológica, pero también por otras causas como interacción con pesca (p. ej., Sierra y cols.

2017, Díaz-Delgado y cols. 2018, Cámara y cols. 2019). En los riñones se observó tubulonefrosis pigmentaria con cilindros hialinos en túbulos renales distales en el 36, 8% (7/19) de los casos, hallazgo descrito previamente en varamientos activos (p. ej., Herráez y cols. 2007, 2013). Otras lesiones comunes fueron la presencia de glóbulos hialinos intracitoplasmáticos en los hepatocitos [31,6% (6/19)], así como hemorragias en la unión cortico-medular de la glándula adrenal [21,4% (3/14)] y contracción de los esfínteres bronquiolares [17,3% (4/23)], indicativos de estrés agudo en cetáceos (Díaz-Delgado y cols. 2018).

Este es el primer estudio en cetáceos varados que emplea estadística para determinar la significancia estadística de varias variables respecto a la entidad patológica de interacción traumática intra-interespecífica con otro/s cetáceo/s. En total, ocho especies de cetáceos presentaron lesiones consistentes con interacción traumática intra-interespecífica. La especie más afectada fue el calderón tropical [33,3% (8/24)]. La prevalencia de esta entidad respecto a la variable especies no mostró significancia estadística ($p=0,111$).

En este estudio se necropsiaron un menor número de cetáceos de buceo profundo [34,1%; (184/540)] que de buceo superficial [65,9%; (356/540)]. Sin embargo, más de la mitad de los cetáceos muertos por esta entidad eran buceadores profundos [58,3%; 14/24]. Si comparamos el número de ejemplares afectados en cada grupo, se observa que el 7,6% (14/184) de buceadores profundos presenta esta entidad patológica, frente al 2,8% (10/356) de los ejemplares de buceo superficial. Esta diferencia resultó ser estadísticamente significativa ($p=0,003$).

El 70,8% (17/24) de los animales afectados presentaron condición corporal moderada-buena y el 12,5% (3/24) muy pobre-pobre. Esta diferencia resultó ser estadísticamente significativa ($p=0,044$). No se tiene constancia de trabajos previos que estudiaran la condición corporal en individuos afectados por este tipo de interacciones.

En cuanto al lugar de varamiento, la mayor parte de los cetáceos afectados por esta entidad vararon en las islas de La Gomera-Tenerife [66,7%; 16/24]. La diferente prevalencia en los lugares de varamiento resultó estadísticamente significativa ($p=0,014$). Teniendo en cuenta que la especie más afectada es el calderón tropical, especie habitualmente avistada en la franja marina que separa ambas islas, cabe esperar que la gran mayoría de las interacciones fatales en este archipiélago se produzcan en dicha población.

Para finalizar, 3 cetáceos murieron como consecuencia de un accidente durante la depredación. Un juvenil de falsa orca que presentaba una condición corporal muy pobre y había sufrido una interacción fatal con un pez elasmobranquio del orden Rajiforme (rayas o pastinacas) y dos calderones grises que sufrieron un síndrome descompresivo como consecuencia de la lucha con su presa, un calamar de grandes dimensiones. En el primer caso, el aguijón de la cola había

producido una perforación en la lengua y se encontraba anclado al paladar blando. Este caso fue descrito brevemente por Díaz-Delgado y cols. (2018). En el caso de los calderones grises, publicado por Fernández y cols. (2017), ambos animales aparecieron varados con signos claros de lucha con un calamar de grandes dimensiones recién ingerido alojado en el esófago y primer compartimento estomacal. Presentaban marcas de tentáculos en la cavidad oral y piel de la región mandibular y cervical, así como hemorragias en el esófago. Los hallazgos macroscópicos e histológicos de ambos animales confirmaron la presencia de dilataciones intravasculares consistentes con la presencia de gas. El análisis de los gases de ambos animales confirmó el diagnóstico de un síndrome descompresivo.

4.3. *Interacción con pesca (Publicación 3)*

En la actualidad, la interacción con pesca es la actividad humana que directamente ocasiona más muertes en cetáceos de todo el mundo (Reeves y cols. 2013). Sin embargo, se estima que la cantidad de casos detectados anualmente está infravalorada (Young y Iudicello 2007, Dolman y Moore 2017) por varios motivos. Por una parte, el diagnóstico de cetáceos que han caído accidentalmente en redes de pesca, conocidos como casos de “bycatch” o captura accidental, resulta difícil, al no existir lesiones específicamente asociadas a esta situación, a excepción de las lesiones cutáneas causadas por el contacto con las redes, que no tienen por qué aparecer en todos los casos (Kuiken 1996, Moore y cols. 2013). Por otra, gran parte de los cetáceos que interaccionan con redes activas y se liberan, o que quedan atrapados en redes fantasma a la deriva, pueden quedar enredados en más de una ocasión a lo largo de su vida (Knowlton y cols. 2012), en ocasiones ocasionando secuelas. Además, debido al peso de las redes, sus cadáveres se hunden con más facilidad (Laist 1997). Para finalizar existen muy pocos datos acerca de las interacciones traumáticas por parte de los pescadores a los cetáceos que se aproximan o caen en las distintas artes de pesca.

En la tercera publicación de esta tesis doctoral, se estudiaron retrospectivamente 586 cetáceos necropsiados entre enero de 2000 y diciembre de 2018. En dicho periodo, 32 animales murieron como consecuencia de una interacción con pesca. Este dato representa el 7,1% (32/453) de los cetáceos necropsiados con causa de muerte conocida y un 5,5 (32/586) del total de cetáceos necropsiados. Los individuos afectados pertenecían a 7 especies de cetáceos: 15 delfines moteados del Atlántico, 6 delfines listados, 4 delfines comunes, 2 delfines mulares, 2 calderones tropicales, 2 rorcuales aliblancos y 1 zifio de Gervais. Los casos fueron divididos en tres categorías: captura accidental o “bycatch” (casos de ingestión de anzuelo o enredamiento en redes de pesca activas) (n=21), enredo persistente con artilugios de pesca (incluyendo restos de redes)

(n=6) y muerte traumática por agresiones por parte de los pescadores (n=5).

En la categoría de captura accidental se incluyeron individuos que presentaron lesiones compatibles con haber quedado enredados en redes de pesca activas (n=21). De ellos, la mayor parte fueron adultos (11/21; 52,4%), seguidos de ejemplares juveniles (6/21; 28,6%), subadultos (3/21; 14,3%) y crías (2/21; 9,5%). Este grupo se subdividió en animales que habían ingerido anzuelos de palangre (5/21); delfines que presentaron lesiones compatibles con una inmersión forzada, que murieron presumiblemente en profundidad, situación conocida como “peracute underwater entrapment” (PUE) (Moore y cols. 2013) (6/21); delfines que vararon vivos que murieron como consecuencia de lesiones consistentes con PUE (3/21); y delfines politraumatizados que fueron izados a bordo y sufrieron traumas adicionales durante el manejo producidos por artilugios de pesca (7/21).

Se observaron diversos grados de cronicidad, desde lesiones agudas propias de PUE a lesiones subagudas-crónicas en los casos de ingestión de anzuelo. La mayor parte de los individuos capturados accidentalmente mostraron una buena condición corporal. Fueron frecuentes las lesiones cutáneas superficiales causadas por el contacto directo con las redes de pesca, mayoritariamente en la zona rostral de la cabeza, pero también en las aletas y rodeando al cuerpo, así como impresiones de redes. Otros hallazgos comunes fueron: pulmones hiperinsuflados de manera bilateral y difusa, así como la presencia multifocal de espacios intravasculares compatibles con gas en el interior de venas y vasos linfáticos. En un porcentaje inferior de casos, se apreció contenido alimenticio no digerido en estómago, ocasionalmente en el esófago, asociado a la presencia de linfa en los vasos mesentéricos y en el conducto torácico. También se describieron casos de pérdida de dientes, mandíbula y/o maxilar fracturados y enfisema retroperitoneal. Estas lesiones habían sido descritas previamente en individuos capturados accidentalmente en otras localizaciones (Moore y cols. 2013, Bernaldo de Quirós y cols. 2018).

En la bibliografía consultada, existen pocas referencias sobre cetáceos que han ingerido anzuelos (p. ej., Díaz-Delgado et al. 2018), por lo que los resultados expuestos en esta tesis aportan más datos sobre las lesiones derivadas de la ingestión de anzuelos. En dos animales el anzuelo perforó el esófago, en uno de ellos provocó una pleuritis fibrinosupurativa, piotórax y hemorragias pericárdicas. Otros dos animales presentaron un único anzuelo perforando la mandíbula, concretamente a nivel de la fosa mandibular. En un caso el anzuelo perforó el tejido blando sublingual produciendo necrosis y hemorragias focales localmente extensivas. En algunos de estos casos se observó hemotórax, hemoabdomen y hemopericardio.

Siete cetáceos presentaron lesiones compatibles con haber sido capturados, subidos a cubierta y traumatizados durante el manejo. En la bibliografía consultada hay escasas referencias a las patologías derivadas de estas agresiones por parte de los pescadores (Moore y cols. 2013). Todos los individuos se encontraron vararon muertos, con perforaciones en piel y músculo, afectando a órganos internos (perforación en la aorta, tráquea y esófago). Dos de ellos presentaron fracturas de neurocráneo (parte escamosa del hueso occipital y el cóndilo derecho del hueso occipital) con hematomas y hemorragia en las leptomeninges y el cerebro y fracturas del maxilar y la mandíbula. Un animal presentó un traumatismo severo en la región caudolateral derecha de la cabeza, con congestión en las leptomeninges, así como una escoliosis severa, afectando a las vértebras lumbares y caudales y una fractura abierta de una vértebra caudal. Además, se describieron hemorragias en la túnica adventicia de la aorta y en la rete mirabile, hemotórax, hemoabdomen, hemopericardio y perforación pulmonar. En una hembra juvenil de delfín común se pudo cuantificar la cantidad de gas intravascular que presentaba y la composición del mismo. Los resultados de dichos análisis indicaron la presencia de embolismo gaseoso, debido a la existencia de abundantes burbujas intravasculares en diversas localizaciones, compuestas principalmente por N₂ [61,0 ± 9,2 μmol %].

Tres animales presentaron lesiones compatibles con una captura accidental, pero vararon vivos, posiblemente debido a que la interacción tuvo lugar cerca de la costa. En estos animales además se observaron lesiones compatibles con varamiento activo.

Histológicamente, la mayoría de los casos presentaron espacios intravasculares compatibles con embolismo grasoso/gaseoso, leve-moderada presencia multifocal de cambios degenerativos agudos en el músculo esquelético, leves cambios degenerativos agudos en músculo cardíaco, enfisema, hemorragias y edema alveolar, leucocitosis sistémica y glóbulos hialinos intracitoplasmáticos en los hepatocitos. En un menor número de casos se apreciaron esfínteres bronquiolares contraídos, presencia de cilindros hialinos en túbulos renales distales, hemorragias en la unión cortico-medular de las glándulas adrenales, hemorragias intramusculares y tubulonefrosis pigmentaria. En el sistema nervioso central (SNC), se apreciaron en algunos casos cambios leves como hemorragias multifocales, edema y manguitos perivasculares asociados a la presencia de nódulos gliales. Todas estas lesiones no se observan únicamente asociadas a esta entidad patológica, ya que aparecen en individuos que han muerto por otras causas de muerte (p. ej., Kuiken 1996, Arbelo y cols. 2013, Herráez y cols. 2013, Sierra y cols. 2017, Díaz-Delgado y cols. 2018).

Además, en este estudio se describieron los hallazgos postmortem de seis cetáceos con heridas cutáneas compatibles con un enredo persistente en artilugios pesqueros. Se trataba de impresiones,

erosiones o lesiones ulcerativas causadas por redes, asociadas a la presencia de tejido de granulación y/o fibrosis en las zonas afectadas (rostro, aletas o pedúnculo). En los últimos años (2012-2020) se han notificado un creciente número de cetáceos avistados nadando enredados en artes de pesca próximos a las costas canarias. Concretamente siete rorcuales aliblanco, seis rorcuales tropicales, una ballena jorobada, dos delfines de dientes rugosos y un delfínido de especie no identificada. El creciente uso de las redes sociales ha permitido la rápida detección de estos animales y la actuación de la red de varamiento de cetáceos del archipiélago. En total, se ha podido desenmallar tres rorcuales aliblanco y dos rorcuales tropicales. Sin embargo, se desconoce la tasa de supervivencia de los mismos, ya que no se ha realizado ningún seguimiento de los cetáceos liberados. El aumento de la detección de cetáceos enredados vivos en las costas canarias, que casi triplica a la de los animales varados muertos, es preocupante. Estas cifras deben ser consideradas a la hora de elaborar o implementar planes de conservación para las distintas especies de cetáceos de las aguas canarias.

Finalmente, citar las lesiones observadas en los cinco cetáceos que murieron por lesiones compatibles con agresiones por parte de pescadores desde la cubierta de los barcos, escasamente reportadas en la bibliografía consultada (Moore y cols. 2013). Estos delfines presentaron signos de traumatismo antropogénico, como una o varias incisiones producidas por objetos afilados, principalmente en la parte dorsal del tórax y de la cabeza. Los operarios de pesca artesanal pueden acceder a la parte dorsal de los animales que nadan próximos a la embarcación, ya que se encuentra a pocos metros de la superficie del agua. En estos animales no se observaron lesiones cutáneas derivadas del contacto con redes de pesca. Todos los individuos presentaban una buena o moderada condición corporal. La mayoría aparecieron con contenido alimenticio sin digerir en el esófago y estómago. Se apreciaron cambios vasculares (p. ej., edema, hemorragia y hematoma) relacionados con la piel y tejido muscular traumatizado, así como fracturas costales y vertebrales, perforaciones pulmonares con hemorragias focales localmente extensas, hemotórax y hemorragias en las leptomeninges.

Para finalizar, se exponen los datos más representativos del estudio estadístico realizado con los datos epidemiológicos de los animales afectados. La mayor parte de los cetáceos muertos por una interacción con pesca [86,7%; (26/30)] presentaron una condición corporal óptima. Esta diferencia resultó estadísticamente significativa ($p=0,004$). Además, en la mayoría de las especies afectadas eran de buceo superficial [90,6% (29/32)]. Este resultado fue también estadísticamente significativo ($p=0,008$).

Tabla resumen de los resultados de los tres estudios retrospectivos del Proyecto de Tesis Doctoral

Entidad Patológica	Preval.	Especies más afectadas	Condición Corporal	Principales hallazgos macroscópicos	Posibles hallazgos microscópicos
Ingestión de cuerpos extraños (CE)	7,7% (36/465) [2000-2015]	Buceadores profundos (cachalotes, zifios y calderones grises) y superficiales	Deficiente	<ul style="list-style-type: none"> - Gastritis ulcerativa - Impactación estomacal - Perforación gastro-intestinal - Glositis y estomatitis ulcerativa - En algunos casos ausencia de lesiones asociadas al CE 	<ul style="list-style-type: none"> - Cambios vasculares e inflamatorios asociados a la presencia del CE
Interacción con pesca	5,5% (32/586) [2000-2018]	Buceadores superficiales (delfín moteado del Atlántico, delfín listado, delfín común y misticetos)	Óptima	<ul style="list-style-type: none"> - Bycatch/capturas accidentales: marcas cutáneas de redes (cortes e impresiones), pulmones hiperinsuflados, contenido alimenticio fresco, pérdida de dientes, linfa en conducto torácico y vasos mesentéricos, embolismo gaseoso (PUE), ingestión de anzuelos (perforaciones, desgarros, hemorragias, piotórax, sepsis), agresiones durante el manejo (lesiones punzantes, rupturas orgánicas, hemorragias, fracturas de neurocráneo, amputaciones) 	<ul style="list-style-type: none"> - Lesiones cutáneas agudas-crónicas (hemorragias, tejido de granulación, fibrosis) - Músculo esquelético: hipercontracción, hiperacidofilia y necrosis segmentaria - Músculo cardíaco: hiperacidofilia y degeneración vacuolar - Riñón: tubulonefrosis pigmentaria - Hígado: glóbulos hialinos en hepatocitos - Pulmón: enfisema, hemorragias, edema alveolar - Leucocitosis sistémica - Sistema cardiovascular: Embolismo gaseoso (PUE)
			Óptima	<ul style="list-style-type: none"> - Agresiones desde barco: fracturas y hematomas a nivel dorsal, contenido alimenticio fresco, ausencia marcas de redes 	
			Deficiente	<ul style="list-style-type: none"> - Enredamiento crónico: lesiones cutáneas crónico-activas, necrótico-ulcerativas o cicatrizadas 	
Interacción traumática intra-interespecífica entre cetáceos	4,4% (24/540) [2000-2017]	Buceadores profundos (calderón tropical)	Óptima	<ul style="list-style-type: none"> - Erosiones y/o ulceraciones cutáneas lineales y paralelas (marcas en rastrillo) - Hematomas subcutáneos - Hemorragias en cavidades y en SNC - Traumatismos contusos - Fracturas óseas - Ruptura pleural y del parénquima pulmonar - Edema traqueal - Perforación de la pared abdominal. - Presas sin digerir en esófago y estómago 	<ul style="list-style-type: none"> - Músculo esquelético: hipercontracción, hiperacidofilia y necrosis segmentaria - Músculo cardíaco: hiperacidofilia y degeneración vacuolar - Riñón: tubulonefrosis pigmentaria - Hígado: glóbulos hialinos en hepatocitos - Pulmón: émbolos grasos en traumatismos severos
Interacción traumática interespecífica con la presa potencial	0,6% (3/540) [2000-2017]	Buceadores profundos (calderón gris, falsa orca) y superficial (delfín mular)	Óptima	<ul style="list-style-type: none"> - Presas sin digerir en esófago y estómago - Lesiones traumáticas en de las mucosas del tracto digestivo (perforaciones, erosiones y úlceras) derivadas del forcejeo con la presa - CE perforando el tracto digestivo (espinas) 	<ul style="list-style-type: none"> - Lesiones ulcerativo-necróticas o perforativas (espinas) con cambios inflamatorios asociados - Sistema cardiovascular: Embolismo gaseoso (cambios en el patrón de buceo)

5. Conclusiones

Primera: El 7,7% de los cetáceos necropsiados en las Islas Canarias, desde enero del año 2000 a diciembre del 2015, presentaron cuerpos extraños en el tracto digestivo. Los individuos afectados pertenecían a 15 especies de cetáceos, tanto misticetos como odontocetos.

Segunda: En este periodo, el 2,8% de los cetáceos necropsiados murieron como consecuencia de las patologías (impactación/obstrucción gástrica, perforación gástrica y/o gastritis ulcerativa grave) provocadas por la ingestión de cuerpos extraños. Mayoritariamente, los objetos encontrados eran plásticos (80,6%).

Tercero: El estudio estadístico realizado indicó que las especies con hábitos de buceo profundo y/o una pobre condición corporal constituyen factores predisponentes o de riesgo para la ingestión de cuerpos extraños, en cambio, la edad adulta, constituye un factor de protección.

Cuarta: El 4,4% de los cetáceos necropsiados en las Islas Canarias, desde enero del año 2000 a diciembre de 2017, murieron como consecuencia de una interacción traumática intra-interespecífica con otro/s cetáceo/s. De las 8 especies de cetáceos incluidas en este grupo, la más afectada fue el calderón tropical.

Quinta: El estudio de las lesiones cutáneas y la determinación de la distancia interdental en las mismas, permitió definir agresiones interespecíficas como la de orca/s sobre zifio de Cuvier, cachalote pigmeo y calderón tropical, y agresiones de delfín/es mular/es sobre delfín común y delfín moteado. Igualmente, permitió identificar agresiones intraespecíficas entre individuos de delfín listado.

Sexta: Los buceadores profundos, ejemplares en buena condición corporal e individuos varados en La Gomera y Tenerife fueron estadísticamente los más afectados por encuentros fatales entre cetáceos.

Séptima: El 0,6% de los cetáceos necropsiados en las Islas Canarias, desde enero del año 2000 a diciembre de 2017, murieron a causa de una interacción fatal con su presa potencial. Dos especies de cetáceos resultaron afectadas, el calderón gris y la falsa orca.

Octava: El 5,5% en cetáceos necropsiados en las Islas Canarias, desde enero del año 2000 a diciembre de 2018, murieron como consecuencia de una interacción con actividades pesqueras. De las 7 especies de cetáceos incluidas en este grupo, la más afectada fue el delfín moteado del Atlántico.

Novena: Las lesiones identificadas en este grupo de cetáceos fueron causadas (de mayor a menor prevalencia) por captura accidental o “bycatch”, enredos persistentes en artilugios pesqueros (incluyendo restos de redes) y agresiones por parte de los pescadores. Recientemente, se ha detectado un incremento en el número de avistamiento de misticetos nadando enredados próximos a costa, mayoritariamente rorcuales aliblancos y rorcuales tropicales.

Décima: Los buceadores superficiales y ejemplares en condición corporal óptima fueron estadísticamente los más afectados por interacción con actividades pesqueras.

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Legislation

- Decreto 178/2000, 6 septiembre, por el que se regulan las actividades de observación de cetáceos
- Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive)
- Council Directive 92/43/EEC of 21 May 1992
 - Annex II Animal and Plant Species of Community Interest Whose Conservation Requires the Designation of Special Areas of Conservation
 - Annex IV Animal and Plant Species of Community Interest in Need of Strict Protection
- Orden ARM/2417/2011, de 30 de agosto, por la que se declaran zonas especiales de conservación los lugares de importancia comunitaria marinos de la región biogeográfica Macaronésica de la Red Natura 2000 y se aprueban sus correspondientes medidas de conservación
- Decreto 182/2004, de 21 de diciembre, por el que se aprueba el Reglamento de la Ley de Pesca de Canarias
 - Anexo I. Zonas prohibidas o habilitadas para el uso de determinadas artes
- Ley 17/2003, de 10 de abril, de Pesca de Canarias
- Ley 3/2001, de 26 de marzo, de Pesca Marítima del Estado
- Orden APA/1200/2020, de 16 de diciembre, por la que se establecen medidas de mitigación y mejora del conocimiento científico para reducir las capturas accidentales de cetáceos durante las actividades
- Real Decreto 556/2011, de 20 de abril, para el desarrollo del Inventario español del Patrimonio Natural y de la Biodiversidad
- Real Decreto 1727/2007, de 21 de diciembre, por el que se establecen medidas de protección de los cetáceos
- Ley 42/2007, de 13 de diciembre, del Patrimonio Natural y de la Biodiversidad
- Decreto 20/2014, de 20 de marzo, por el que se modifican los anexos de la Ley 4/2010, de 4 de junio, del Catálogo Canario de Especies

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