

Retrospective study of fishery interactions in stranded cetaceans in the Canary Islands (2019-2023)

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1.INTRODUCTION

Marine ecosystems are a type of aquatic environment characterized by the presence of saltwater as their main component. Marine ecosystems include oceans, seas and coasts that constitute some of the most biodiverse habitats in the world, where mammals from different families and species coexist in biological balance.

The Canary Islands archipelago lies in the Atlantic Ocean, in the Macaronesia region. It is made up of 8 volcanic islands, known for offering mild temperatures and a variety of exceptional natural attractions.

In the deep ocean waters of the Canary Islands, important communities of cetaceans frequently transit, making the islands privileged worldwide locations for observing these animals in the wild. The reason for this high biodiversity lies in their geographical and oceanographic characteristics, as well as the presence of high depths very close to the coast, which is highly suitable for the life of these animals. Additionally, they often have not-extreme temperature ranges, allowing the passage of animals from both cold and temperate waters.

The Canary Islands are home to 31 species of cetaceans, including 24 toothed whales (odontocetes) and 7 baleen whales (mysticetes). Among the toothed whales, members of the Delphinidae family have been recorded, such as the short-beaked common dolphin (Delphinus delphis), bottlenose dolphin (Tursiops truncatus), striped dolphin (Stenella coeruleoalba), Atlantic spotted dolphin (Stenella frontalis), longsnouted spinner dolphin (Stenella longirostris), Fraser's dolphin (Lagenodelphis Risso's dolphin (Grampus griseus), rough-toothed dolphin (Steno hosei), bredanensis), short-finned pilot whale (Globicephala macrorhynchus), long-finned pilot whale (Globicephala melas), melon-headed whale (Peponocephala electra), the false killer whale (Pseudorca crassidens), pygmy killer whale (Feresa attenuata) and the orca (Orcinus orca); a member of the Phocoenidae family, the harbor porpoise (*Phocoena Phocoena*); the two members of the Kogiidae family, pygmy sperm whale (Kogia breviceps) and dwarf sperm whale (Kogia sima); the only member of the Physeteridae family, the sperm whale (*Physeter macrocephalus*); members of the Ziphiidae family such as Cuvier's beaked whale (Ziphius cavirostris), Blainville's beaked whale (Mesoplodon densirostris), Gervais' beaked whale (Mesoplodon europaeus), Sowerby's beaked whale (Mesoplodon bidens), True's beaked whale

(*Mesoplodon mirus*), and northern bottlenose whale (*Hyperoodon ampullatus*). Among the baleen whales, members of the Balaenopteridae family have been reported, such as the blue whale (*Balaenoptera musculus*), fin whale (*Balaenoptera physalus*), sei whale (*Balaenoptera borealis*), Bryde's whale (*Balaenoptera edeni*), minke whale (*Balaenoptera acutorostrata*), and humpback whale (*Megaptera novaeangliae*); also two reports of a member of the Balaenidae, the North Atlantic right whale (*Eubalaena glacialis*) (Sociedad para el Estudio de los Cetáceos en el Archipelago Canario-SECAC).

Confirmed resident populations of cetaceans in the Canary Islands include the bottlenose dolphin and the short-finned pilot whale, other species reported in the waters of the Canary Islands all year round are Risso's dolphin, sperm whale, Blainville's beaked whale, Gervais' beakes whale, Cuvier's beaked whale and Atlantic spotted dolphin, while seasonal visiting species include striped dolphin, common dolphin, rough-toothed dolphin and Bryde's whale among others (Rodriguez-Juancá *et al.*, 2023).

Vulnerable species in IUCN list in the area include the short-finned pilot whale, the bottlenose dolphin, the blue whale, sperm whale and the fin whale.

Regarding endangered species, the North Atlantic right whale (*Eubalaena glacialis*) stands out.

The systematic study of the different cetacean populations can allow for the description, recognition, and prevention of adverse scenarios that may affect the populations and the ecosystems they are part of. For this reason. anatomopathological, histological and molecular studies carried on stranded carcasses are a fundamental tool for obtaining important information regarding conditions, diseases, and pathologies of stranded cetaceans (Bossart, 2011), that may explain some anthropogenic and natural threats.

The Canary Islands stranding network register any stranded cetacean, its biological characteristics and pathological findings. The most stranded species is the Atlantic spotted dolphin, followed by striped dolphin and short-beaked common dolphin.

Anatomopathological studies on stranded cetaceans allowed to discern between anthropogenic and natural threats for different cetacean's populations. Regarding anthropogenic impact, ship collision (due to the highly need of transportation of goods and people between the islands) and ingestion of marine debris, as well as the discharge or urban, agricultural, and industrial pollutants have been reported in stranded cetaceans. Additionally, the increasing demand for cetacean sightings has a negative effect on cetacean's life if it is not well done (Arbelo, 2007).

Globally, the most important threat for cetaceans' population is fishing activities (Reeves *et al.*, 2013). It is estimated that at least 300.000 cetaceans die each year in fisheries (Elliot *et al.*, 2023). However, in the Canary Islands it caused relatively low deaths (Puig *et al.*, 2020). But it is important to know and continuedly review the incidence associated to this threat in order to find increases or changes in the trend.

Incidental capture is the leading cause of human-induced mortality in cetaceans worldwide and is one of the most significant challenges for their conservation (Ambien *et al.*, 2023). Furthermore, it has far-reaching consequences for the well-being of cetaceans that are injured, stressed, or experience the loss of conspecifics. Pathological data indicate that the majority of incidentally captured cetaceans die due to suffocation (Dolman & Moore, 2017). Methods for estimating incidental capture rates include remote electronic monitoring, voluntary reporting by fishermen, and the presence of onboard observers (IJsseldijk *et al.*, 2021).

Cetaceans are vulnerable to injuries from fishing activities due to different types of fishing interactions: incidental capture (bycatch) getting hooked trapped or entangled underwater, aggression from fishermen onboard, and chronic entanglements (ghost fishing) (Wade *et al.*, 2021; Elliot *et al.*, 2023). A recent study reports that a minimum of 27 cetacean species have been recorded entangled in fishing gear, with more than 30% of the incidents affecting threatened, vulnerable, or endangered species (Tulloch *et al.*, 2020).

In the case of bycatch, cetaceans exhibit findings caused by underwater acute entrapment (PUE) (Moore, 2013). The challenge of identifying PUE stems from nonspecific injuries associated with drowning and the unique physiological adaptations of marine mammals that hold their breath (de Quirós *et al.*, 2018).

The most common pathological findings in incidental capture include bulging or reddened eyes, the presence of scattered gas bubbles, external cuts on the skin due to entanglement (mainly on the head), fresh ingested prey, congestion in multiple organs and lung changes such as edema, atelectasis, and multifocal emphysema (Puig *et al.*, 2020). External injuries such as amputation and maxillary, mandibular and/or dental fractures (Dolman & Brakes, 2018) can also be observed, as well as linear impressions or incisions on the head, neck, appendages, mouth, and body caused by a net (de Quirós *et al.*, 2018). Findings such as amputations, stab wounds on the body and gunshot injuries are often caused by fishermen (Puig *et al.*, 2020).

Another type is chronic entanglements, in which lost, abandoned, or discarded fishing gear has a significant impact on cetaceans and other marine species is known as "ghost fishing". Sometimes, fishing nets are broken by large cetaceans, producing same lesions of chronic entanglements. Entangled animals swim injured, sometimes with the gear still attached, and their predatory behaviour is also affected. These animals used to die after a long time even though they are not counted in incidental capture statistics (Reeves *et al.*, 2013).

2.OBJETIVES

The objectives of the present study are:

1. To retrospectively review and compilate the anatomopathological and histologic evidences of stranded cetaceans that presented lesions compatible with fishing interactions in the Canary Islands archipelago between the years 2019-2023.

2. To compare the prevalence of cases compatible with fishing interactions during the study period with the existing literature in this geographic area and neighbouring regions, statistically analyzing if there is any deviation in the historical trend in recent years.

3.MATERIALS AND METHODS

A retrospective study was conducted, recruiting necropsy reports of stranded cetaceans in the Canary archipelago between January 2019 and December 2023, included.

During this period, as well as previously and nowadays, postmortem examinations were conducted following international protocols (Kuiken, 1991; IJsseldijk *et al.,* 2019).

Necropsies were performed, including sampling for histological study and observation of lesions, which were described and photographed. Representative samples were collected from major organs such as brain, cochlea, gastro-intestinal tract, kidneys, liver, lung, spleen, skin, adrenal glands, lymph nodes, pancreas, skeletal muscle, cardiac muscle and reproductive tract. The representative samples were processed routinely, being fixed in 10% neutral buffered formalin, and embedded in paraffin. Additionally, they were sectioned at a thickness of 5 micrometers and stained with hematoxylin and eosin (H&E) for histopathological analysis.

Additionally, samples are collected for complementary studies, including microbiology, molecular studies, parasitology, and toxicology.

For microbiological studies, samples are obtained from the lungs, brain, cerebrospinal fluid (CSF), heart, kidneys, liver, spleen, reproductive tract, blood clots, and any organ or tissue with macroscopic lesions suggestive of a microbiological etiology. These samples should be collected using aseptic techniques and kept refrigerated at temperatures between 0 and 4°C, ideally processed within 24 hours of collection. If processing within this timeframe is not possible, the samples should be frozen at -20°C to preserve the potential for the growth of demanding microorganisms.

For molecular studies, tissue samples are extracted from the lungs, liver, spleen, brain, lymph nodes, and kidneys. If these samples are sent to the laboratory within 24 hours after collection, they can be stored refrigerated at temperatures between 0 and 4°C. Otherwise, they should be frozen at -80°C. If ultra-low temperature freezing

is not possible, the samples can be placed in RNA later and stored, cooled, or frozen at -20°C.

Samples intended for parasitological studies are collected from the stomach contents, intestines, lungs, liver, subcutaneous tissue, renal vasculature and kidneys, cochlea/Eustachian tube/surrounding tissue, faeces, and cardiac and skeletal muscles. These samples can be kept submerged in a Petri dish with water for immediate analysis, stored in 70% ethanol or 10% glycerin alcohol, or frozen at -20°C.

For toxicological studies, samples are obtained from fat, muscle, liver, kidneys, and brain. These samples should ideally be wrapped in aluminum foil before being stored in plastic containers, and duplicate samples should be taken to analyze persistent organic pollutants (POPs), plastic additives, trace elements, and/or fatty acids. Additionally, milk samples should be collected from lactating females and stored in glass containers before POP analysis. For trac element analysis, samples from the brain, muscle, liver, and kidneys should be stored in stainless steel or plastic containers. Heavy metal analysis can also be performed on bone and/or blood samples. If a fetus is too small, it can be wrapped along with its placenta in aluminum foil for POP analysis. All samples should be frozen at -20°C until analysis. To measure lipid percentages in the fat layer (DCC1-2), samples should be stored at -80°C in liquid nitrogen or RNA later for real-time PCR (RT).

For statistical analysis, data regarding species, age, sex, reproductive status, body condition, location, stranding date, and diving behavior were collected. Age categories were based on total body length and gonadal development (fetus/neonate/calf, juvenile/subadult and adult). Reproductive status was classified as immature or mature based on gonadal histological examination. Body condition was rated as very poor, poor, air, or good considering the prominence of vertebral and spinous processes and ribs, the amount of fat deposition, and the mass of epaxial musculature. The preservation code of the carcass was classified as very fresh, fresh, moderate autolysis, advanced autolysis, or severely advanced autolysis according to the classification by IJsseldijk *et al.*, 2019. Diving behavior was noted as shallow or deep, depending on the main depth of diving referenced in the bibliography for each species.

4.RESULTS

Between January 2019 and December 2023, 167 stranded cetaceans were found on the coasts of the Canary Islands. After performing necropsies on each specimen, the most probable cause of death was identified in most cases. Out of these cases, 12 cetaceans from 3 species presented gross evidence or injuries related to fisheries interaction; histologically, other possible causes of death were ruled out. Statistical analysis of the main variables of the study is shown below.

4.1. Epidemiological analysis

4.1.1. Affected cetacean species

During this study period, the cetaceans affected by fishing interactions belonged to the following species: Atlantic spotted dolphin (8/12-66.67%), striped dolphin (3/12-25%), and short-beaked common dolphin (1/12-8.33%) (**Figures 1 and 2**). Thus, it was determined that in this region the most affected species was the Atlantic spotted dolphin.



Figure 1: Graphical representation of the prevalence by species of the total stranded cetaceans in the Canary Islands due to fishing interactions during the period from January 2019 to December 2023.





Figure 2: Images of the affected cetaceans. **(A)** Striped dolphin specimen affected by PUE (Case 5). **(B)** Atlantic spotted dolphin specimen affected by hook ingestion (Case 6). **(C)** Atlantic spotted dolphin specimen affected by aggression (Case 8). **(D)** Specimen of Atlantic spotted dolphin affected by hook ingestion (Case 10). **(E)** Atlantic spotted dolphin specimen affected by hook ingestion (Case 11). **(F)** Specimen of Atlantic spotted dolphin affected by PUE (Case 12).

4.1.2. Year of stranding

The average of fishing interaction cases per year was distributed as follows: 2019 (3/12-25%), 2020 (2/12-16.67%), 2021 (2/12- 16.67%), 2022 (4/12-33.33%) and 2023 (1/12-8.33%). The results can be seen in **Figure 3**.



Figure 3: Graphical representation of the prevalence of cases compatible with fishing interaction during the period from January 2019 to December 2023.

The highest prevalence was detected in 2022 (4 cases), followed by 2019 (3 cases), with 2023 being the year with the lowest number of affected individuals (1 case).

4.1.3. Location

The stranding cases were distributed among the islands as follows: Tenerife (7/12-58.33%), Fuerteventura (3/12-25%), and Gran Canaria (2/12-16.67%).

It is important to note that in some islands there were not stranded animals with lesions compatible with fishing interaction. These islands included El Hierro, La Palma, La Gomera, Lanzarote, and La Graciosa.

The results are illustrated in Figure 4.



Figure 4: Graphical representation of the prevalence by island of cases compatible with fishing interaction during the period from January 2019 to December 2023.

The island with the highest prevalence was Tenerife. The affected species on this island were the Atlantic spotted dolphin and the striped dolphin.

The second island with a greater number of cases was Fuerteventura. Species found on this island included the three affected.

The third and final island where affected animals were found was Gran Canaria, where two species were identified, the striped dolphin and Atlantic spotted dolphin.

4.1.4. <u>Sex</u>

The incidence of lesions due to fishing interactions was not similar between males and females, as 66.67% were males (8/12) and 33.33% females (4/12). These results indicate that a higher number of males were found compared to females.

The prevalence can be seen in Figure 5.



Figure 5: Graphical representation of the prevalence by sex of cases compatible with fishing interaction during the period from January 2019 to December 2023.

4.1.5. <u>Age</u>

The distribution according to age categories of the studied cetaceans, considering physical development, was as follows: 75 % of the affected individuals were adults (9/12), 16.67% were juveniles/subadults (2/12), and 8.33% were undetermined individuals (1/12). Thus, it is noted that no neonates or calves were found. These results indicate that the majority of affected specimens were adults compared to the other categories. The results are illustrated in **Figure 6**.



Figure 6: Graphical representation of the prevalence by age class of cases compatible with fishing interaction during the period from January 2019 to December 2023.

4.1.6. Sexual maturity

Regarding sexual maturity, and related with gonadal development, 7 animals were found to be mature (58.33%), 2 immature (16.67%), and 3 specimens could not be evaluated (25%) due to advanced decomposition code or evisceration. These results indicate that the majority of affected animals were sexually mature as can be seen in **Figure 7.**



Figure 7: Graphical representation of the prevalence by sexual maturity of cases compatible with fishing interaction during the period from January 2019 to December 2023.

4.1.7. Body condition

Body condition could be determined in 83.33% of cases with fishery interactions (10/12). It was classified as very poor-poor (2/12-16.67%) and fair-good (8/12-66.66%). However, there were 2 cases that could not be evaluated (16.67%), due to advanced decomposition status. Most animals showed a fair-good body condition. The prevalence can be seen in **Figure 8**.



Figure 8: Graphical representation of the prevalence by body condition of cases compatible with fishing interaction during the period from January 2019 to December 2023.

4.1.8. Diving

All stranded cetaceans collected for this study belonged to Delphinidae species that are classified as shallow divers.

4.1.9. Temporal Pattern of Stranding Events

The average annual number of stranded cetaceans related to fishing interactions was 3 animals (12 cases in 4 years). The year with the highest number of strandings was 2022 (n=4), followed by 2019 (n=3).

According to the seasons shown in **Figure 9**, a total of 9/12 cases (75%) occurred in spring, 1/12 cases (8.33%) in summer, 1/12 cases (8.33) in autumn, and 1/12 cases (8.33%) in winter. Additionally, the month of March had the highest incidence of cases (5/12-41.66%), followed by April (3/12-25%).



Figure 9: Graphical representation of the prevalence by season of cases compatible with fishing interaction during the period from January 2019 to December 2023.

4.1.10. Concomitant infectious diseases

During the necropsies, 91.67% of cases (11/12) were found to have parasitic infestation, including nematodes (Crassicauda spp and Anisakis spp.) Trematodes (*Brachycladidae* family, *Pholeter* sp., and *Nasitrema* sp.), Cirripeds (*Xenobalanus* sp. and *Conchoderma* spp.), as well as the presence of the cestodes *Monorygma grimaldii* and *Phyllobothrium delphini*.

Additionally, in 16.67% of cases (2/12), pathological findings indicative of an infectious process of unknow etiology were observed.

4.2. Anatomopathological results

4.2.1. Gross findings

Most of the stranded animals were found dead (9/12), of which 6 individuals were found entangled in fishing gears (Cases 3, 6, 7, 9, 10 and 11).

Cases of interactions with fishing activities were classified into three groups: bycatch [such as ingestion of longline hooks (Cases 3, 6, 7, 9, 10 and 11) or entanglement in fishing nets (Cases 1, 2, 4, 5 and 12)] and 1 aggression by fishermen (Case 8). No cases related to chronic entanglements were found (*Table 1*).



Table 1 | Gross finding in stranded cetaceans that die due to fishery interactions (aggression or bycatch) (n=12).

			By	catch (n=1	1)
		Aggression (n=1)	Hook ingestion (n=6)	PUE (n=5)	Total (n=11)
	Stranding alive	0	2	1	3
Stranding event	Stranding dead	1	4	4	8
	Fishing gears attached	0	6	0	6
	Poor- very poor	0	1	1	2
Body condition	Good-fair	1	3	4	7
	NE	0	2	0	2
	Net impressions over the body	0	1	5	6
Skin	Net cuts in pectoral flippers	0	1	0	1
	Net cuts in head	0	2	4	6
	Net cuts over the body	1	2	4	6
Subcutaneous	Hematoma	0	0	2	2
Skeletal muscle	Hemorrhages	1	2	2	4
	Mandibles fracture	0	0	3	3
	Maxilla fracture	0	0	2	2
	Neurocranium fracture	0	0	0	0
Bones	Tympanic fracture	0	0	0	0
	Vertebrae fracture	0	0	1	1
	Rib fracture	0	0	1	1
	Teeth lost/fractured	0	2	4	6
	Esophagus Fresh/undigested prey	0	0	0	0
Digestive tract	Stomach Fresh/undigested prey	0	2	1	3
	Digested content	0	3	2	5

				/	
	Empty/ND	1	1	2	3
Bubbles	Lymphatic vessels	0	0	1	1
Dubbics	Blood vessels	0	1	2	3
	Hemothorax	1	4	1	5
Blood in cavities	Hemoabdomen	0	2	1	3
	Hemopericardium	0	2	0	2
	Hyperinflated	0	2	1	3
	Hemorrhagic parenchyma	1	1	3	4
	Subpleural hemorrhage	0	0	0	0
Lungs	Tracheal or bronchial edema	0	1	1	2
	Rib impressions	0	0	1	1
	Rupture of the parenchyma	1	1	1	2
	Hemopericardium	0	2	0	2
Heart	Hemorrhages	0	0	0	0
	Vascular changes in valves	0	1	1	2
	Aorta vascular changes	1	2	1	3
Large vessels	Rete mirabile vascular changes	1	1	2	3
	Hemorrhages	0	0	1	1
Kidney	Retroperitoneal emphysema	0	1	0	1
Lymphatic	Lymph in vessels	0	0	1	1
Brain	Meningeal hemorrhages	0	0	0	0
2.um	Parenchymal hemorrhages	0	0	0	0

<u>Bycatch</u>

In this group (n=11), the three cetacean species were affected: Atlantic spotted dolphin (n=7), striped dolphin (n=3), and common short-beaked dolphin (n=1). Adults were most affected in this category (n=8), followed by juveniles and subadults (n=2).



Within the incidental bycatch group, cetaceans presumably entrapped in active fishing gears were included.

The incidental bycatch was further subdivided into the following subgroups: dolphins that ingested longline hooks (6/11-54.54%); animals with injuries indicating forced submersion, likely dying at depth due to peracute underwater entanglement (PUE) (5/11-45.45%).

Different degrees of chronicity of the lesions were observed: subacute-chronic lesions in individuals affected by hook ingestion and acute lesions in cases of PUE. However, in most cetaceans affected by incidental bycatch, common macroscopic findings were identified (*Table 1*). A large number showed superficial skin lesions and marks consistent with net abrasion on the face, fins, and along the rest of the body (Figure 10A and 10B). Additionally, loss and fractures of dental pieces were observed (Figure 10C). They also presented diffusely hyperinflated lungs (Figure 10D). Furthermore, they exhibited scattered intravascular gas bubbles in veins and lymphatic vessels (Figures 10E and 10F). In some cases, the presence of undigested content in the stomach was observed (Figure 10G), as well as hemoabdomen (Figure 10H).







Figure 10 | Severe injuries in incidentally captured dolphins stranded in the Canary Islands. (A) Cut in pectoral fin presumably caused by a net and (B) impresions on a short-beaked common dolphin (Case 3). (C) Right lateral view of the rostrum with multifocal fractures and loss of teeth on the right maxila and mandible (Case 5). (D) Left lateral view of the thoracic cavity showing severe diffuse hyperinflated lungs (Case 7). (E) Mesenteric veins with severe multifocal intravascular gas bubbles (Case 7). (F) Thoracic duct filled with lymph and presence of gas bubbles (Caso 2). (G) Undigested content in stomach (Caso 7). (H) Lateral view of abdominal cavity with hemoabdomen (Case 10).

Of the 6 individuals affected by longline hook ingestion, 5 cases were Atlantic spotted dolphins [4 adults (Cases 6, 7, 9, and 10) and Case 11 whose sexual maturity could not be assessed] and 1 was a short-beaked common dolphin [adult specimen (Case 3)]. Most cases were found dead but in fair to good body condition. In some animals, the hook had pierced and torn the trachea and esophagus (Cases 6,7,9,10 and 11; Figure 11A), causing mandibular trauma due to hook presence (Case 3), fibrinosuppurative pleuritis (Case 9), multifocal moderate to severe intrabronchial and intra-alveolar hemorrhages (Cases 6 and 7), pulmonary perforation (Case 6), and linear transmural perforation of the thoracic aorta (Case 7). Additionally, other specimen exhibited a hook perforated in jaw (Case 3; Figure 11B). Hemopericardium, hemothorax, and hemoperitoneum were also observed (*Table 1*).

The individuals affected by PUE (n=5) belonged to the following species: Atlantic spotted dolphin (*Stenella frontalis*) [1 juvenile-subadult (Case 12), 1 adult (Case 4)] and striped dolphin (*Stenella coeruleoalba*) [1 juvenile-subadult (Case 1), 2 adults (Cases 2 and 5)]. Most affected individuals were found dead with fair to good body condition. They exhibited vertebral and rib fractures (**Case 12; Figure 11C**) and mandibular and maxillary fractures (**Cases 1, 4 and 5; Figure 11D**), as well as net impressions (**Case 12**). Another notable finding was the presence of gas bubbles in veins and lymphatic vessels (**Cases 2 and 5**). Severe skin lesions related to fishing net contact were also found (**Cases 1, 2, 4, 5 and 12**). Less frequently, hemothorax and hemoperitoneum were detected (**Case 12**). In one case (**Case 5**), sectioning of pectoral fins and caudal peduncle was observed.





Figure 11 | Macroscopic findings on deceased cetaceans caused by different types of fishing interactions. (A) Lateral view of trachea and esophagus perforation associated with a hook presence in an Atlantic spotted dolphin (Case 6). (B) Left jaw perforated by a hook in a short-beaked common dolphin (Case 3). (C) Left lateral view of complete fracture and dislocation of the thoracic spine, with fractures of transverse processes in an Atlantic spotted dolphin (Case 12). (D) Severe comminuted multiple fractures of the mandible with hematoma association in a striped dolphin (Case 1).

Aggressions by fishermen

In this category, an adult of Atlantic spotted dolphin (**Case 8**) was included. This animal was found stranded dead in fair to good body condition. It showed puncture-like skin perforations on the side near the dorsal fin, as well as puncture-like lacerations (**Figure 12A**). As a consequence of these injuries, perforations and hemorrhages in musculoskeletal tissues were observed (**Figures 12B and 12C**), along with hepatic capsular lacerations (**Figure 12D**), laceration and hemorrhage in the pleura of the lung (**Figure 12E**), and right hemothorax (**Figure 12F**). Vascular changes were also noted in the rete mirabile (**Figure 12G**) and thoracic aorta (**Figure 12H**). In this case, no cuts or net impressions were observed on the skin, but vascular changes were evident. Additionally, there was little content in the digestive tract.









Figure 12 | Macroscopic findings on an adult Atlantic spotted dolphin caused by fishermen aggression. (A) Puncture-like wound on the right flank. (B) Deep and complete perforation in the musculature of the left flank. (C) Hemorrhages in the musculature on the left flank. (D) Moderate acute focal hepatic capsular laceration. (E) Laceration and hemorrhage in the parietal pleura of the right lung. (F) Right hemothorax in the thoracic cavity. (G) Severe rupture and hemorrhage at the cranial level of the rete mirabile. (H) Markedly reddish coloration in the caudal portion of the thoracic aorta.

4.2.2. Microscopic findings

Table 2 | Microscopic finding in stranded cetaceans that die due to fishery interactions (aggression, or bycatch) (n=12).

				Bycatch (n=11)			
			Aggression (n= 1)	Hook ingestion (n= 6)	PUE (n=5)	Total (n= 11)	
	Acute degenerative changes	No	0	0	1	1	
		Mild	0	3	2	5	
Skeletal muscle		Moderate	1	2	1	3	
Skeletal masele		Severe	0	0	1	1	
	Atrophy		0	0	1	1	
	Hemorrhages		1	0	1	1	
	Alveolar edema		1	4	4	8	
	Emphysema		0	3	5	8	
Lungs	Muscular bronchiolar sphincter contraction		1	2	1	3	
	Hemorrhages	es	1	2	2	4	

					/	
	Acute degenerative changes	No	0	2	2	4
		Mild	1	2	3	5
Heart		Moderate	0	0	0	0
		Severe	0	0	0	0
	Hemorrhages		0	2	2	4
Liver	Intracytoplasmic hyaline globules		0	0	2	2
Adrenal glands	Hemorrhag	ges	0	1	3	4
	Leukocytosis		1	4	4	8
Blood vessels	Intravascular coagulation		0	3	2	5
	Intravascular clear spaces		1	3	4	7
Reproductive	Gonadal maturity	Mature	1	4	3	7
System		Immature	0	0	2	2
	Membranous glomerulonephritis		0	1	2	3
Kidney	Hyaline cast		1	3	1	4
	Pigmentary tubulonephrosis		0	0	1	1
	Hemorrages		0	0	0	0
	Perivascular edema		0	2	3	5
	Hemorrhages		0	1	2	3
Brain	Meningitis/encephalitis		0	0	1	1
	Perivascular cuffs		0	1	2	3
	Glial nodules		1	3	2	5

<u>Bycatch</u>

Of the 11 cases, there was one (**Case 11**) that could not be evaluated due to advanced autolysis. Additionally, in another case (**Case 10**), only two parameters from those listed in the table (**Table 2**) could be assessed because it also presented advanced autolysis.

The most common histological findings were mild multifocal acute degenerative changes in skeletal muscle (hypercontraction, hypereosinophilia and, segmental necrosis) and signs of regeneration (central nuclei in a row) (Figure 13A); multifocal pulmonary lesions (such as alveolar edema, atelectasis, emphysema, bronchiolar sphincter contraction, and hemorrhages) (Figure 13B). Additionally, in most cases, mild degenerative changes in cardiac muscle fibers and multifocal hemorrhages were observed (Figure 13C). The presence of intravascular coagulation (Figure 13D), leukocytosis, and clear intravascular spaces compatible with gas was also detected (Figure 13E). Regarding the kidneys, membranous glomerulonephritis (i.e., moderate multifocal thickening of the glomerular basement membrane) was observed, as well as multifocal hyaline casts (Figure 13F) and subcapsular gas. In the central nervous system (CNS), multifocal perivascular edema, multifocal hemorrhages, perivascular cuffs (i.e., multifocal presence of intravascular inflammatory infiltrates), and multifocal gliosis/satelitosis were found (Figure 13G). In fewer cases, intracytoplasmic hepatocellular hyaline globules (Cases 1 and 2; Figure 13H) and multifocal adrenal hemorrhages (Cases 2, 4, 6 and 12) were detected. In Case 12, meningitis and multifocal encephalitis were observed. The histological findings for each case are presented in **Table 2**.





Figure 13 | Microscopic findings on cetaceans that died due to bycatch. (A) Hypercontracted muscle fiber with aligned nuclei and increased eosinophilia in a Atlantic spotted dolphin (Case 9) (H&Ex20).
(B) Bronchiolar edema with submucosal inflammatory infiltrate in an Atlantic spotted dolphin (Case 12) (H&Ex10). (C) Epicardial hemorrhages in an Atlantic spotted dolphin (Case 6) (H&Ex10). (D) Intravascular coagulation and leukocytosis in rete mirabile of a short-beaked common dolphin (Case 3) (H&Ex4). (E) Pulmonary vessel with presence of gas in an Atlantic spotted dolphin (Case 12) (H&Ex10). (F) Satellitosis and perivascular edema in the brain of a striped dolphin (Case 7) (H&Ex20).
(G) Leptomeningitis and mononuclear infiltrate in an Atlantic spotted dolphin (Case 7) (H&Ex4). (H) Intracytoplasmic bile pigments and hyaline globules in a striped dolphin (Case 2) (H&Ex40).



In cases related to hook ingestion (**Cases 6, 7, 9, 10 and 11**) where perforation and tearing of the trachea and esophagus were found, various lesions were observed. In the esophagus, severe multifocal hemorrhages were noted (**Figure 14A**), along with acute degeneration of muscle fibers, multifocal leukocytosis in the mucosa, and diffuse inflammatory infiltrate (**Figure 14B**). Additionally, inflammatory changes and areas of necrosis in the submucosa were detected. Regarding the trachea, multifocal hemorrhages and diffuse suppurative infiltrate were found (**Figure 14C**). Two specimens (**Cases 6 and 7**) showed pulmonary content compatible with aspirated elements in the bronchial lumens (**Figure 14E**), as well as intraalveolar macrophages and the presence of bacteria in the vascular and bronchiolar lumens.



Figure 14 | Microscopic findings in cetaceans that died due to hook ingestion in an Atlantic spotted dolphin (Case 6). **(A)** Severe multifocal hemorrhage in the esophagus (H&Ex4). **(B)** Inflammatory infiltrate in the esophagus submucosa (H&Ex40). **(C)** Multifocal hemorrhage and diffuse suppurative infiltrate in the trachea (H&Ex20). **(D)** Content compatible with aspirated elements in the bronchial lumen (H&Ex4).

Aggressions by fishermen

In the skeletal muscle fibers, moderate acute degenerative changes were observed, including hypercontraction, hypereosinophilia, and segmental necrosis, alongside signs of regeneration (centrally located nuclei in rows). Additionally, multifocal moderate hemorrhages, congestion, and edema were detected in the intercostal muscles. Pulmonary alterations were also found, including moderate alveolar edema, bronchial sphincter contraction, extensive focal hemorrhage, and the presence of a neutrophilic infiltrate lymphoplasmacytic and indicative of suppurative bronchopneumonia. Mild acute degenerative changes (hypereosinophilia) were detected in the cardiac muscle, along with multifocal large-size interstitial dilatations. Multifocal hyaline cylinders were also present in the kidneys, systemic leukocytosis, and a histologically mature reproductive system with the presence of albicans bodies and follicles in various stages of developments. Concerning the central nervous system (CNS), mild multifocal satelitosis was observed, along with intravascular dilatations in the meninges, consistent with gas.

The histological findings are presented in **Table 2**.

5.DISCUSSION

Every year, a significant number of cetaceans die due to bycatch in fishing gear (Dolman & Moore, 2017). Thus, fishing activities pose a serious threat to the conservation of various populations and species of marine mammals (Read *et al.,* 2006).

This study provides an update on the data regarding fishing interactions with stranded cetaceans in the Canary Islands since the last review conducted by Puig-Lozano *et al.*, (2020).

Epidemiological information on strandings

During the study period from January 2019 to December 2023, 167 cetacean strandings were recorded on the coasts of the Canary Islands, of which 12 specimens showed evidence related to fishing interactions, representing 7.18% of the cases. This prevalence is similar to the referenced by Puig-Lozano *et al.*, (2020), who detected a prevalence of 7.4%. Furthermore, the affected cetaceans belonged

to three different species. Among the 12 stranded specimens, the Atlantic spotted dolphin was the most affected species, representing 66.67% of the cases, with adults showing a higher prevalence (75%). In the study by Puig-Lozano *et al.*, (2020), adults were also the most affected, but with a lower prevalence (43.8%).

Regarding the distribution by islands, Tenerife recorded the highest number of cases (58.33%), followed by Fuerteventura (25%) and Gran Canaria (16.67%). These dates are consistent with the findings of Puig-Lozano *et al.*, (2020), where Tenerife also recorded the highest number of cases (40.6%). However, in the previous study, Fuerteventura and Gran Canaria showed similar prevalence, unlike the observations in the current study.

Additionally, there was a higher prevalence in males (66.67%) compared to females. In terms of body condition, 66.66% of the specimens displayed a moderate/good condition. These results are similar to previous studies.

Of the 12 specimens, 6 had fishing gear attached, and 91.67% of the cases showed concomitant infectious and/or parasitic diseases.

Concerning the presence of concomitant diseases, this study identified several groups of parasites, including cestodes, nematodes, and trematodes. As noted by Bossart (2011), the presence of these concomitant diseases is common in free-ranging cetaceans and may be linked to complex factors such as climate change, toxin exposure, and immunosuppression, among others. These factors make coastal marine mammals particularly vulnerable to infestations.

In the previous retrospective study (Puig-Lozano et *al.*, 2020), six specimens were identified as affected by chronic entanglement. However, in the present study, no cases affected by this condition were found.

Compared to other Spanish regions, the Canary Islands report a lower number of strandings compared to Galicia, the region with the highest number in Spain, where an average of one dolphin is stranded each day (Aracil *et al.*, 2020).

<u>Bycatch</u>

Bycatch is considered the primary global threat to cetaceans (Elliot *et al.*, 2023) and has significant implications for their welfare (Dolman & Brakes, 2018). The most common pathological findings observed in our study are consistent with those of Puig-Lozano *et al.*, (2020), who reported the presence of dispersed gas bubbles, external skin cuts from entanglement, freshly ingested prey, and reddened eyes.

The ingestion of longline hook had the highest incidence in the Atlantic spotted dolphin, representing 83.33% of the cases (5/6), with adults being the most affected. However, in the study by Alcaraz *et al.*, (2023), this species represented 88% of the cases. Additionally, in the present study, one case of a short-beaked common dolphin with a hook perforating the jaw was identified. Another significant finding was observed in five cases, where perforations and tears of the trachea and esophagus were present, which was also reported by Alcaraz *et al.*, (2023). In these cases, the presence of the hook caused hemorrhages and severe acute muscle fiber degeneration, as well as areas of necrosis; two of the cases showed aspirated elements in the bronchial lumen, representing 33.33% of the total cases affected by hook ingestion.

The most frequent histological findings in individuals affected by hook ingestion were alveolar edema and leukocytosis. In the study by Alcaraz *et al.*, (2023), alveolar edema was also the most common finding, thus this a consistence observed lesion but highly inespecific between both studies. Furthermore, to the best of our knowledge, this is the second reported case of esophageal perforation with fibrinous-suppurative pleuritis caused by hook ingestion in a dolphin species (Case 9). The hooks found vary in type and can measure up to 6 cm in length. The characteristics of most of the hooks resembled those used by tuna fisheries from the waters of the Canary Islands.

The species most affected by PUE was the striped dolphin, with adults having the highest prevalence. Despite they are non-specific lesions, as reported by Moore *et al.*, (2013), gas bubbles were observed in veins and lymphatic vessels associated with drowning due to their physiological adaptations to diving. Additionally, skin lesions on the head, snout, and fins due to contact with nets, as well as linear cuts, were found, consistent with the findings reported by different authors (Arbelo *et al.*,

2013; Diaz-Delgado *et al.*, 2018). Tooth fractures and wears from biting the fishing net were also noted.

According to van der Hoop *et al.*, (2013), fractures are commonly found in by-caught cetaceans. In this study, fractures in the jaw and maxilla (cases 1, 4, and 5) were observed. In one case (case 12), fracture and dislocation of the thoracic spine and ribs were identified. Additionally, lungs with a shiny surface and slightly crepitant texture were found, attributed to oxygen deprivation (hypoxia), as indicated by van der Hoop *et al.*, (2013). The presence of foam in the respiratory tract was also observed in our study. However, Bernaldo de Quirós *et al.*, (2018) noted that this finding is not a good indicator of bycatch. Van der Hoop *et al.*, (2013) also mentioned evidence of physical trauma during the release of cetaceans from a net. In this context, our study documented the amputation of fins in case 5.

Previous studies have reported the presence of intracellular hepatocellular hyaline globules in specimens affected by PUE (Godinho, 2010). This condition can occur when individuals are exposed to processes of stress and death by asphyxiation. In the present study, two cases with this condition were identified. However, the most frequent finding in individuals affected by bycatch was marked pulmonary emphysema (hyperinflated lungs), which is indicative of bycatch by not specific to underwater entrapment (Jamison, 2016).

Although there are many reports that marine mammals caught incidentally often present recently ingested gastric content (Kuiken, 1996; Bernaldo de Quirós *et al.,* 2018), this was not a significant finding in our study. Most of the specimens (72.73% of the cases) had an empty stomach or already digested content.

Aggressions by fishermen

In the marine environment, humans and cetaceans are direct competitors for the same resources, positioning us at the same level in the food chain (Feliu, 2018). This competition results in the interactions these animals have with fisheries (Bearzi, 2002).

In our study, the aggressions affected only one Atlantic spotted dolphin, which exhibited injuries caused by a sharp fishing gear, resulting in perforations and

hemorrhages. However, no common findings of bycatch, such as cuts and net impressions or hyperinflated lungs, were found.

The contusions and stabbings were mainly concentrated in the thoracic cavity, involving deep tissues with the presence of hemorrhages, hemothorax, lung perforation, and hepatic capsular laceration. Histological findings, such as focally extensive hemorrhages, acute degenerative changes, and hemorrhages in the lungs, were related to the injuries found, indicating that these traumatic wounds occurred while the animal was still alive.

6.CONCLUSIONS

This is a retrospective analysis examining fishing interactions in stranded cetaceans in the Canary Islands in the last five years. The findings revealed two types of fishing interactions: aggression from fishermen and incidental capture (hook ingestion and PUE). However, unlike previous studies, no cases of chronic entanglement were detected.

This study updates the trends of fishing interaction in the Canary Islands, showing results similar to those of previous years, although the type of interaction appears to have changed, with an increase in cases affected by hook ingestion. Adult animals were more affected in this retrospective study compared to previous studies.

Additionally, different types of fishing hooks were identified, suggesting an increased interaction with the tuna fisheries in the Canary Islands. This aspect has been poorly researched and requires further investigation in future studies.

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Table with the data of all cetaceans included in the study

CASE AND CET	MORPHOLOGICAL DIAGNOSIS
NUMBER	
	- Skeleton: Severe comminuted multiple fracture of mandible and maxilla, with associated bematoma
0.054	- Lung: Moderate multifocal lymphoplasmacytic bronchointerstitial pneumonia with
CASE 1	multifocal edema and moderate multifocal emphysema.
(CET 983)	 Liver: Periportal and subcapsular lymphoplasmacytic hepatitis with mild multifocal ductal hyperplasia
	- Kidney: Mild multifocal lymphoplasmacytic nephritis. Mild multifocal glomerulosclerosis
	with multifocal areas of fibrosis.
	- Dermis: Mild multifocal panniculitis.
	- Multiple organs: Gas embolism, hemorrhages, and congestion.
	- Teeth: Cutting and loss of dental pieces, with associated hemorrhages.
	- Lung: Severe generalized pulmonary emphysema. Moderate multifocal
CASE 2	- Liver: Centrolobular congestion and presence of hyaline globules.
(CET 990)	- Prostate: Moderate-severe chronic-active pyogranulomatous and necrotizing
	prostatitis, with intralesional nematodes and parasitic eggs.
	pancreatitis.
	- Stomach: Full of food content. Mild-moderate multifocal pyogranulomatous gastritis
	with intralesional trematode parasites (<i>Pholetel</i> sp).
CASE 3	 Mandibular trauma caused by a hook. Net or fishing line marks on the shout and fin
(CFT 1045)	- Alveolar and bronchial edema.
(0=1 1010)	- Multiorgan intravascular leukocytosis.
	- Mandible: Comminuted fracture of the left hemimandible with extensive focal
	- Lung: Multifocal subacute suppurative and histiocytic fibrinonecrotizing
	bronchopneumonia, moderate to severe, with occasional multinucleated giant cells and
	pneumonia, moderate. Moderate multifocal pulmonary emphysema and edema.
	- Skin: Moderate multifocal ulcerative and fibrinonecrotic dermatitis with hemorrhages,
	degeneration of small and medium-caliber vascular structures, and intralesional
CASE 4	coccoid bacteria.
(CET 4422)	- Gingiva: Moderate to severe subacute multifocal ulcerative, suppurative, and necrohemorrhadic dindivitis with intralesional coccoid bacteria
(00011122)	- Tongue: Suppurative and lymphoplasmacytic fibrinonecrotic glossitis with intralesional
	coccoid bacteria and multifocal intravascular thrombosis.
	lymphadenitis.
	- Pancreas: Moderate chronic multifocal lymphoplasmacytic and eosinophilic pancreatic
	cholangitis with intracanalicular trematode parasites, epithelial hyperplasia, marked
	- Liver: Moderate to severe multifocal ischemic hepatic necrosis. Multifocal periportal
	lymphoplasmacytic hepatitis, mild to moderate.



	- Intestine: Moderate chronic multifocal-coalescing lymphoplasmacytic and eosinophilic
	eggs.
	- Heart: Mild chronic focal lymphoplasmacytic myocarditis.
CASE 5 (CET 1128)	 Mandible: Multiple fractures of the left and right mandible, maxillary bone, and teeth with associated hemorrhages and separation of the mandibular symphysis. Lung: Chronic moderate multifocal lymphoplasmacytic bronchointerstitial pneumonia and bronchitis, with occasional massive mineralization obliterating a bronchial lumen. Moderate multifocal necrotizing pyogranulomatous bronchopneumonia with intralesional nematode parasites, intrabronchial hemorrhages, and associated dystrophic mineralization. Mild to moderate multifocal interstitial and subpleural fibrosis. Moderate multifocal pulmonary emphysema. Kidney: Mild to moderate chronic multifocal lymphoplasmacytic nephritis. Liver: Moderate chronic periportal lymphoplasmacytic hepatitis. Pharyngeal Tonsil: Moderate chronic-active multifocal suppurative and histiocytic tonsillitis with apparent nematode parasites localized in pharyngeal crypts. Moderate multifocal interstitial fibrosis.
	 Esophagus: Transmural, linear, focally extensive perforation with severe vascular and inflammatory changes and involvement of surrounding tissues, associated with the
CASE 6	presence of a hook.
(CET 1158)	- Irachea: Multifocal perforations with severe vascular and inflammatory changes, associated with the presence of a hook.
	- Lung: Moderate pulmonary perforation and hemorrhages associated with the
	Economy Linear transmural perference with severe vessular and inflammatory
	changes involving peripheral tissues, associated with the presence of a hook.
	- Trachea: Focally extensive perforation with severe vascular and inflammatory changes
CASE 7	- Thoracic aorta: Linear transmural perforation with severe vascular and inflammatory
(CET 1161)	changes involving peripheral tissues, associated with the presence of a hook Thoracic cavity: Severe hemothorax
、	- Lung: Marked diffuse emphysema. Moderate to severe multifocal hemorrhages. Mild
	multifocal atelectasis.
	- CNS. Moderate to severe non-supportative meningoencephantis and meningomyentis.
	 Tegument: On the right side, caudal to the pectoral fin, a wound of puncture appearance is observed. On the left side, caudal to the pectoral fin, in the ventral thorax region, two wounds of puncture appearance are observed. Another wound of puncture appearance is noted on the left side, caudal to the dorsal fin. Additionally, in the more dorsal region, cranial to the dorsal fin, two other lacerations of puncture aspect are observed. Musculaskalatal system: Doep complete perferations of the left flack musculature.
CASE 8	 inusculoskeletal system: Deep complete perforations of the left flank musculature (complete oblique perforation of superficial muscles, extending to complete perforation
(CET 4047)	of intercostal muscles) and diaphragm. Moderate acute multifocal degenerative
(CET(217))	lymphoplasmacytic myositis in the rectus abdominis muscle.
	 Thoracic cavity: Bilateral pneumothorax. Right hemothorax. Moderate mediastinal hemorrhage.
	 Lungs: Severe generalized compensatory atelectasis and emphysema. Moderate generalized suppurative bronchopneumonia with intralesional parasites. Moderate multifocal lymphoplasmacytic interstitial bronchopneumonia in left lung. Liver: Moderate acute focal hepatic capsular laceration. Mild periportal



	lymphoplasmacytic hepatitis. Mild multifocal periportal fibrosis.
	- Kidneys: Focal piogranulomatous interstitial nephritis with intralesional parasites.
	- Pancreas: Moderate ductal lymphoplasmacytic pancreatitis with intralesional
	parasites.
	- Others: Multiorgan parasitism.
CASE 9 (CET 1220)	 Esophagus: Transmural perforation due to hook. Severe focal extensive hemorrhage. Severe multifocal generalized myositis. Severe multifocal segmental necrosis. Lung: Moderate-severe acute necrosuppurative bronchopneumonia. Moderate multifocal lymphoplasmacytic bronchitis. Moderate multifocal fibrinosuppurative pleuritis. Severe generalized alveolar edema. Pulmonary lymph node: Moderate-severe multifocal necrosuppurative lymphadenitis. Thoracic cavity: Severe bilateral hydro/hemothorax. Musculoskeletal system: Mild lymphocytic and histiocytic myositis with sarcolemma degeneration and moderate myocytic necrosis in the longissimus dorsi muscle. Mild multifocal segmental necrosis in the rectus abdominis muscle. Laryngeal tonsil: Moderate-severe necrosuppurative tonsillitis. Rete mirabile: Severe multifocal fibrinosuppurative interstitial inflammation. Integumentary system: Moderate extensive focal lymphohistiocytic panniculitis. Moderate multifocal segmental serous adipocyte atrophy.
CASE 10	- Esophagus: Transmural, linear, focal extensive perforation associated with the
(CET 1228)	 presence of a hook. Trachea: Multifocal perforations associated with the presence of a hook.
CASE 11	- Esophageal perforation due to a sharp foreign body (hook).
(CET 1229)	- Bilateral hydro-hemothorax.
CASE 12 (CET 1273)	 Bone Skeleton: Complete fracture and dislocation of the thoracic spine, with fractures of right and left transverse processes, spinous processes. Multiple left rib fractures. Lung: Severe multifocal intrabronchial and intralveolar hemorrhages. Severe multifocal alveolar overdistension. Moderate multifocal interstitial lymphoplasmacytic bronchopneumonia. Longissimus dorsi muscle and intercostal musculature: Severe acute multifocal degenerative changes. Moderate multifocal microhemorrhages. Central Nervous System: Moderate multifocal microhemorrhages. Mild multifocal meningitis, encephalitis, and choroiditis. Moderate presence of edema and ballooning astrocytes. Heart: Mild to moderate acute multifocal degenerative changes. Kidney: Mild multifocal membranous glomerulopathy. Mild granulomatous interstitial nephritis.