UNIVERSITÀ DEGLI STUDI DI PADOVA

Department of Comparative Biomedicine and Food Science

First Cycle Degree (B.Sc.)
in Animal Care

Spatio-temporal trends and human-interaction evidence on marine mammals and sea turtles stranded in the northeastern Aegean Sea, Greece

Supervisor:
Prof. Cinzia Centelleghe

Co-supervisors:
Dr. Guido Pietroluongo
Dr. Beatriz Tintoré

Submitted by:
Elena Zuglian
Student n.
1224108

ACADEMIC YEAR 2022/2023

Table of Contents

1. Summary

The anthropogenic impacts on marine wildlife populations are growing worldwide, causing an alarming rise in animal-human interactions. The current report relies on monitoring indicator species, such as sea turtles, pinnipeds, and cetaceans, to assess marine ecosystem health in the northeastern Aegean Sea. A total of 113 specimens from 5 species of marine mammals, including dolphins and monk seals, and 2 species of sea turtles, found stranded along the coastlines of Samos, Lipsi, Ikaria, and Agathonisi Islands, Greece, between 2017 and 2022 were analyzed for spatial and temporal patterns and trends, as well as for evidence of human interactions.

These findings highlight the power of stranding records to detect potentially vulnerable population groups in time and space. This knowledge is vital for conservation purposes, as it can guide the implementation of protection measures such as establishing time-area-specific limits to potentially harmful human activities, aiming to reduce the number and intensity of human-wildlife conflicts.

2. Introduction

2.1. Stranding events

Stranding events refer to marine animals found alive on the shore or in shallow waters, which are unable to return to the water under their power, and those found dead on the beach or floating in the water. A stranding episode can involve a single or multiple animals (McGovern et al., 2016). It may occur due to natural factors, such as age and diseases, or human-related factors, such as bycatch, marine traffic, environmental degradation, and pollution. Causes are often a combination of natural and anthropogenic factors.

The most frequently recorded stranding events worldwide are single dead individuals (Norman et al., 2004); the cause of death is generally related to pathological occurrences such as disease, illness, parasitism, or injury (McGovern et al., 2016).

When a stranding involves 2 or more individuals of the same species, that are not mother and calf, a serial of strandings along the same shoreline, or strandings recorded on the same short time period, it is defined as mass stranding (Geraci et al., 2005) and unusual mortality event (UME). Even though the cause of this kind of events is poorly understood, it seems mainly associated with navigational errors due to natural circumstances such as geomagnetic anomalies, climate, and oceanographic variations, or anthropogenic activities, especially the use of active sonar in naval exercises and seismic surveys (McGovern et al., 2016).

Even though marine mammals and sea turtles are threatened globally, with almost a quarter of these species on the verge of extinction, their strandings are not such an impact in themselves but rather a visible symptom of natural or human-induced processes. This is especially true because of the position of these animals in food webs, as marine mammals are top predators, and their long generation time makes them particularly sensitive to perturbations in ecosystems (Truchon et al., 2013). Furthermore, since these nektonic species are highly mobile and spend most of their lives below the surface, even the most basic population metrics are difficult to detect and generally unknown (IJsseldijk et al., 2020; Pikesley et al., 2012).

For this reason, the systematic gathering of data on stranded megafauna represents a cost-effective real-time bio-indicator of environmental health and an efficient method for scientists to understand mortality factors, as well as the primary source of information on all aspects of their ecology and biology (Belmahi et al., 2020; Truchon et al., 2013). In fact, the efficiency of a monitoring plan depends on three challenging aspects: (I) ecological relevance, (II) statistical credibility, and (III) cost-effectiveness (Ijsseldijk et al., 2020).

When integrated over broad spatial-temporal extents, stranding data can provide information about occurrence patterns, population structure, distribution, seasonal abundance, species diversity, anthropogenic threats, disease prevalence, life history, and diet ecology. All this information leads to a better understanding of marine mammal and sea turtle populations and the identification of oceanographic changes originating from climatic variability and anthropogenic activities.

Therefore, it is beneficial to set effective monitoring actions at both local and global scales that help in the development of conservation and management plans within protected areas (Belmahi et al., 2020; Ijsseldijk et al., 2020; McGovern et al., 2016; Truchon et al., 2013; Tomás et al., 2008).

Knowledge of population demographics and distribution makes it easier to understand the severity of anthropogenic stressors on populations and how to mitigate human activities practically (Ijsseldijk et al., 2020).

2.2. Stranding evidence in the Mediterranean Sea

The Aegean Sea is an arm of the Mediterranean Sea between the Greek peninsula on the west and Asia Minor on the east. It represents one of the main marine ecosystems of Europe, supporting 7% of all existing marine species, including 12 marine mammal species and 3 sea turtle species (Archipelagos, 2023).

According to Milani et al. (2017), a national sighting and stranding network was established in Greece in 1991 until December 2008. After that year, stranding data have been collected locally by NGOs and local authorities with no standard regulatory criteria. Between 1991 and 2008, 1.392 strandings were reported in Greek waters. Since 2009, the number of total strandings remains unknown, but the literature provides several studies that aim to identify the mortality trends and causes in the Mediterranean Sea and Greek Seas.

Anthropogenic activity, environmental and biotic factors directly affect many species' survival, as they can influence spatial and temporal patterns and post-mortem drift (Truchon et al., 2013; Pikesley et al., 2012). Even though the causes of stranding are several and usually species-specific, factors like bathymetry, seabed topography, water temperature, seasonality, and currents are often significant variables associated with cetacean distribution that could influence their spatial-temporal stranding patterns (Pikesley et al., 2012).

Other environmental components affecting stranding dynamics are discontinuities in the geomagnetic fields, which human activities might influence (McGovern et al., 2016).

Nowadays, researchers seem to agree that the incidental capture or entanglement in fishing gear, known as bycatch, of sea turtles and other marine megafauna is one of the most severe threats affecting the Mediterranean Sea (Casale, 2008; Tomás et al., 2008). According to Casale (2008), the associated mortality is estimated at 150.000 for sea turtles and 50.000 for marine mammals per year. Tomás et al. (2008) state that the Greek/Turkey fishery is probably responsible for more than 20.000 captures of sea turtles per year. Even though every interaction with fishing gear is not lethal, it is a clear symptom that the interaction with fishery practices leads to short-term and long-term adverse effects, such as behavioral changes, physical stress, and injury (Miliou et al., 2018).

Besides bycatch, fishermen often intentionally kill marine animals because they are seen as competitors or for consumption (Pietroluongo et al., 2022; Casale, 2008). The monk seal population of Greece and Turkey is an example of this.

Furthermore, due to the inadequate regulation of fishing practices, the marine ecosystem has been impacted by the overexploitation of fishery resources and marine traffic. Therefore, habitat degradation and competition due to resource depletion between marine animals and between marine animals and fisheries are estimated to increase if effective conservation measures are not taken (Miliou et al., 2018).

Other human-related aspects impacting the marine fauna are represented by pollution and economic activities, especially tourism. Aquaculture, agriculture, industries, economic and military activities are majorly responsible for chemical and noise pollution (Margaritoulis et al., 2003). Marine litter, above all plastic, represents a main threat as it can be ingested or the animal can get trapped in it, which could affect the individual's ability to feed, to reproduce, as well as its health (Pietroluongo et al., 2022). Likewise, the coastal anthropic disturbance deriving from tourism activities presents a risk, especially for sea turtles, as they are primarily responsible for nesting site degradation (Geldiay et al., 1982).

2.3. *Marine mammals and sea turtles inhabiting the Aegean Sea*

The Aegean Sea ecosystem is an important feeding area for numerous marine mammal and sea turtle populations, including endangered species such as the Mediterranean Monk Seal (*Monachus monachus*), a critically endangered species according to the IUCN Red List.

2.3.1. Cetaceans

The Cetacean infraorder includes whales, dolphins and porpoises. In the Greek Seas, there are 7 regularly present Odontocetes species and 1 regularly present Mysticetes species (Pardalou & Tsikliras, 2020; Bearzi et al., 2008; Frantzis et al., 2003) (Figure 2.1).

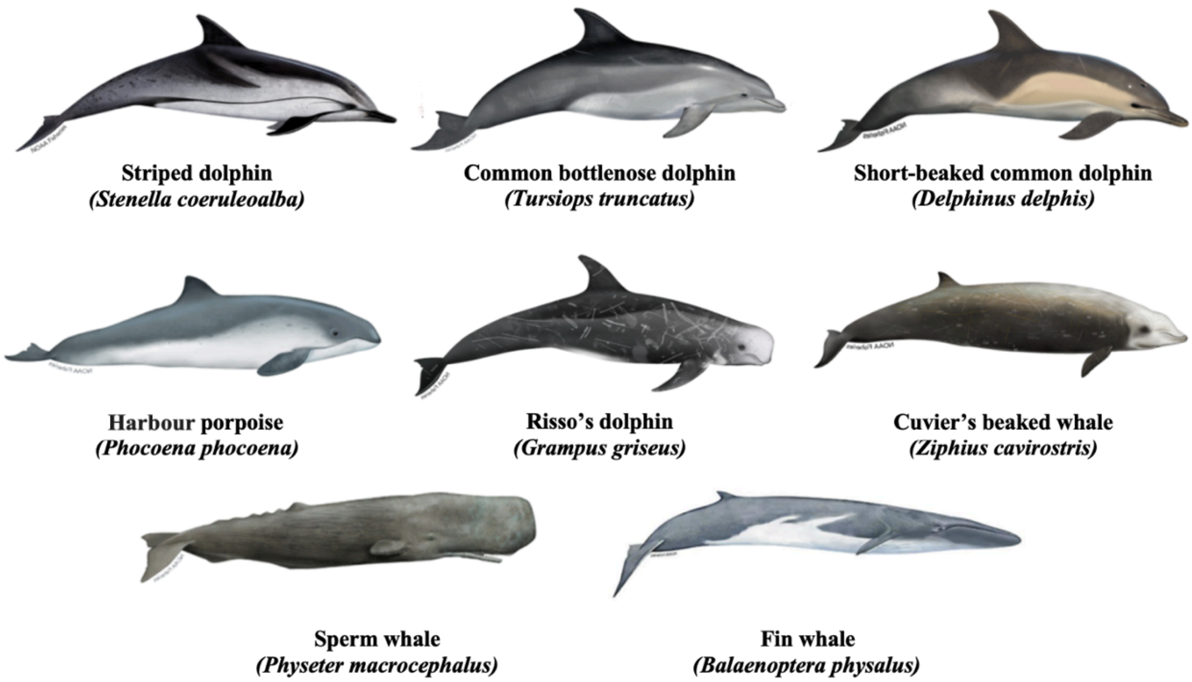


Figure 2.1. Cetacean species commonly occurring in the Aegean Sea – illustrations by NOAA Fisheries.

In addition to these, other species are restricted to relatively small areas or have been sighted or stranded infrequently, for instance, the long-finned pilot whale (*Globicephala melas*) and the false killer whale (*Pseudorca crassidens*) (Frantzis et al., 2003).

In this study, stranding data regard only four of these species, which are the striped dolphin (*Stenella coeruleoalba*), the common bottlenose dolphin (*Tursiops truncatus*), the short-beaked common dolphin (*Delphinus delphis*), and the harbor porpoise (*Phocoena phocoena*).

Striped dolphin – Stenella coeruleoalba

The striped dolphin presents a harmonious physique of dark-grey coloration on the back, which fades to lighter colors towards the venter, and peculiar dark stripes on both sides: from the eye to the pectoral fin and from the eye to the anus.

It is acknowledged as the smallest *Odontocetus* of the Mediterranean Sea, with a mean length in adult specimens of 2.1 m and a maximum length of 2.6 m.

S. coeruleoalba is the most abundant cetacean in the Greek Sea. It generally resides in pelagic and deep waters, but it can also be sighted close to the Greek coast due to steep depressions of the continental shelf (Frantzis et al., 2003).

The population size varies from 20 to dozens of individuals, with 1:1 sex ratio (Kasuya, 1999); striped dolphins are very social animals. Differences in pod dimension are mainly related to prey distribution and availability.

In terms of conservation status, the Mediterranean subpopulation of *S. coeruleoalba* is considered of "Least Concern" (LC) according to the IUCN Red List of threatened species, as *"given the estimated population of over 2 million individuals worldwide, despite mortality due to direct and incidental takes in many parts of the world, there is no evidence of a major global decline that would warrant listing in a category of threat."* (Bearzi et al., 2022).

The main threats concerning this species are related to fishing activities, particularly bycatch, intentional killing, and pollution.

Common bottlenose dolphin – Tursiops truncatus

This species is characterized by a sturdy body of faded grey coloration with paler ventral areas which, depending on the age, social and non-social interactions, can be pinkish or whitish with several dots; dorsal cape and flank stripes. The head presents a well-pronounced melon and short rostrum (Bearzi et al., 2008). The mean length in adult specimens is 3 m, but they can reach up to 4.5 m.

The bottlenose dolphin is one of the most frequently observed cetaceans in the Mediterranean. It is mainly found in coastal areas surrounding islands and archipelagos in Greek waters, but movements into and across pelagic waters are frequent. It tends to reside in specific areas with an average movement of 120 km, even if it is a migratory species able to travel more than 400 km (Bearzi *et al.*, 2008; Frantzis *et al.*, 2003). The extension and population size are variable as they are influenced directly and indirectly by several factors, including geophysical factors (e.g., water depth), prey distribution and availability, social interactions, human-related threats, and predation risk.

Furthermore, the size of bottlenose dolphin groups varies according to age, sex, sexual maturity, breeding success, and social hierarchy. Most encounters have been with pods of a maximum of ten individuals, including specimens of both sexes (Bearzi *et al.*, 2008).
Behavioral and communication patterns in this species and other marine mammal species can significantly differ according to the habitat in which they live. A main driving force in shaping their behavior is feeding habits, as diet and foraging are influenced by the surrounding ecosystem, the season, and interspecific interactions (Bearzi *et al.*, 2008).
In terms of conservation status, the Mediterranean subpopulation of *T. truncatus* is considered of "Least Concern" (LC) according to the IUCN Red List of threatened species, as the full justification states that *"Although there are many threats operating on local populations, the species is widespread and abundant, and none of these threats is believed to be resulting in a major global population decline."* (Hammond et al., 2014).
However, in the Greek Seas, bottlenose dolphins are exposed to a wide variety of threats, especially human-related threats such as bycatch, intentional killing, and environmental degradation due to ecosystem disruption, overfishing, acoustic, and water pollution (Ijsseldijk et al., 2020; Pardalou & Tsikliras, 2020; McGovern et al., 2016; Truchon et all. 2013; Pikesley et al., 2012; Bearzi *et al.*, 2008; Tomás et al., 2008).

Short-beaked common dolphin – Delphinus delphis

The *D. delphis* appears slender and tapered in shape, dark grey on the back and whitish on the venter, with a characteristic pattern on both sides consisting of a beige front ring and a grey back ring. It ranges from 1.5 m to 2.7 m in length (Frantzis et al., 2003).
Common dolphins reside mainly at the level of the continental shelf and continental slope (Bearzi et al., 2003). In the Aegean Sea, they are sighted in shallow and coastal areas in groups of 20-30 individuals, according to prey availability and seasonality. In the past, aggregations of 50-100 animals were frequently recorded. However, population size has been drastically reduced over the last decades, leading to the regular formation of mixed-species pods of common dolphins with other cetacean species, especially striped dolphins (Bearzi et al., 2003). According to Bearzi and many other researchers, big groups would allow *D. delphis* specimens to experience benefits in terms of foraging, predator avoidance and reproduction, even though different species have different ecological needs.

The Mediterranean subpopulation of *D. delphis* is considered “Endangered” (EN) in the IUCN Red List of threatened species, as “*the estimated rate of decline for the subpopulation is likely between 5 and 10% annually, thus substantially exceeding the 0.625% annual decline corresponding to a 20% decline in 2 generations”* (Bearzi et al., 2021).
The major causes of this decline are fishing activities, especially bycatch and prey depletion, contamination through the food web, habitat shifts, and alterations.

Harbor porpoise – Phocoena phocoena
The Harbor Porpoise presents a sturdy body and a poorly demarcated rostrum. The dorsal part is dark grey and fades into a lighter grey towards the belly. Recorded lengths in specimens found in the Black Sea and surrounding areas range from 1.1 m to 1.6 m (Fontaine, 2016; Rosel et al., 2003).
Scientists believe that the population of *P. phocoena* residing in the northern Aegean Sea has originated from the Black Sea population, even though the reason behind this has not been fully understood yet (Rosel et al., 2003). These porpoises distribute at the level of the coastal continental shelf in environments characterized by a high primary production (Fontaine, 2016), where they spend time alone or in small groups of a maximum of 5 individuals.
The IUCN Red List of threatened species lists the *P. phocoena* Black Sea subspecies as “Endangered” (Birkun et al., 2008). Bycatch, contaminants, noise pollution, and prey depletion represent the main threats affecting this species.

2.3.2. Pinnipeds

The only pinniped species inhabiting the Aegean Sea is the Mediterranean monk seal (*Monachus monachus*) (Karamanlidis et al., 2015) (Figure 2.2).

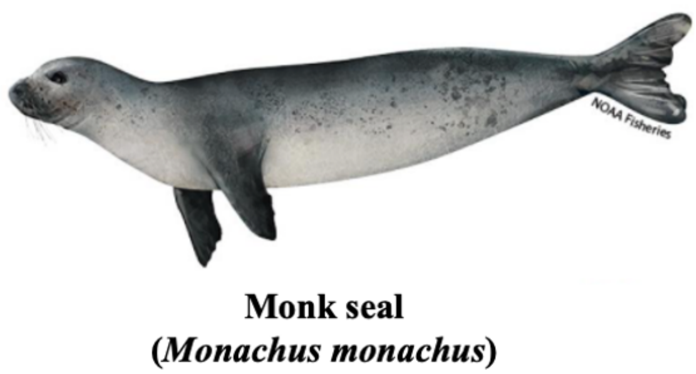


Figure 2.2. Native pinniped species of the Aegean Sea – illustrations by NOAA Fisheries.

Mediterranean monk seal – Monachus monachus

Historically, its distribution range extended throughout the Mediterranean Sea and Black Sea. However, in the last decades, the population size has drastically declined to the point that nowadays, the *M. monachus* population is considered "Endangered" (EN) according to the IUCN Red List, accounting for 400-500 individuals living in the Eastern Mediterranean Sea (Greece, Turkey) (Pietroluongo et al., 2022).

This rapid decline is mainly related to the degradation of its habitat, of which anthropogenic activities, such as fishing and overexploitation, seem to be the primary responsible.

2.3.3. Sea turtles

Three species of sea turtles can be found in the Aegean Sea: the Loggerhead turtle (*Caretta caretta*), which is the most diffused one worldwide; the Green turtle (*Chelonia mydas)*; and the Leatherback turtle (*Dermochelys coriacea*), the most giant in the world (Figure 2.3).

Loggerhead turtle (*Caretta caretta*) | **Green turtle (*Chelonia mydas*)** | **Leatherback turtle (Dermochelys *coriacea*)**

Figure 2.3. Native sea turtle species of the Aegean Sea – illustrations by NOAA Fisheries.

In this study, there is no stranding data regarding the *D. coriacea.*

Loggerhead turtle – Caretta caretta

A reddish-brown carapace, a yellowish plastron and yellow-orange to brown skin characterize loggerhead sea turtles. The head is relatively large with a solid curved beak.

The bony carapace is made up of 5 vertebral scutes running down the midline and 5 pairs of costal scutes on the sides. It connects to the softer plastron by 3 inframarginal scutes on each side. The length of adults varies from 80 cm to 140 cm, with a corresponding weight between 100 kg and 160 kg.

The *C. caretta* is the most common sea turtle species inhabiting the Mediterranean Sea (Casale et al., 2010), moving between coastal and pelagic waters and breeding mainly along the coasts of the eastern Mediterranean basin. Over the past 3 generations, the Mediterranean subpopulation has increased to the point that it is considered “Least Concern” (LC) according to the current IUCN Red List criteria (Casale, 2015). However, it is essential to keep in mind that this increase in population size results from conservation efforts, so the cessation of these actions would be followed by a population decrease (Casale, 2015). Sea turtles are exposed to many threats such as fishery bycatch, nesting habitat degradation due to coastal development, pollution, and boat collisions (Casale et al., 2017; Tomás et al., 2008).

Green turtle – Chelonia mydas

Green turtles are hard-shelled sea turtles with a comparatively small head, a serrated beak and 2 large scales between the eyes.

Their carapace is smooth and varies among black, gray, green, brown, and yellow, while their plastron is yellow-to-white. The carapace has 5 vertebral scutes and 4 scutes on each side. The mean length in adult specimens varies between 90 cm to 120 cm and they weigh from 130 kg to 160 kg.

The *C. mydas* is commonly found in the Mediterranean Sea (Casale et al., 2017), as it inhabits subtropical and temperate regions worldwide. It is highly migratory, presenting both oceanic and neritic phases.

According to the IUCN Red List of Threatened Species, it is considered “Endangered” (EN) as *“extensive subpopulation declines in all major ocean basins over the last three generations as a result of overexploitation of eggs and adult females at nesting beaches, juveniles and adults in foraging areas, and, to a lesser extent, incidental mortality relating to marine fisheries and degradation of marine and nesting habitats”* occurred (Seminoff, 2004).

Sea turtles present slow reproductive dynamics due to their delayed sexual maturity and high natural mortality rate of early juveniles (Belmahi et al., 2020), making their survival harder than other species exposed to the same threats.

2.4. Protection and conservation

During the last decades, several international agreements and conventions have been established to protect marine animals along the Mediterranean basin, of which the main ones are (1) the *Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and contiguous Atlantic area* (*ACCOBAMS*), (2) the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (*CITES*), (3) the *Bern Convention on the Conservation of European Wildlife and Natural Habitats*, (4) the *Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean*, (5) the *Bonn Convention on the Conservation of Migratory Species of Wild Animals*, and (6) the *Marine Strategy Framework Directive 2008/56/EC.*

However, states' commitment to these conventions is variable and the signature is a formal procedure that does not oblige the state at the national level.

In order to protect species and habitats at risk and raise awareness among stakeholders, the European Union has set up the *Life Programme* to make funds available for this purpose.

The Life DELFI project (LIFE18NAT/IT/00942) is part of this program and it aims to lowering the interactions between marine mammals and fishing activities through several actions, such as using advanced technological systems to keep animals away from fisheries and recording sightings, strandings, and animal-human interactions.

It is among many international projects that result in more effective wildlife monitoring, protection, and conservation actions.

2.5. Objectives of the study

The analysis of records of cetaceans, pinnipeds, and sea turtles found stranded or floating in eastern Greece, collected by the Archipelagos Institute of Marine Conservation NGO and integrated with the *Life DELFI* guidelines regarding the *"diagnostic framework for the assessment of fishery interaction in stranded marine mammals"*, is presented with the aim of (I) identifying temporal and spatial trends in marine animals strandings occurring along the coastlines of the islands located in the north-eastern Aegean Sea from 2017 to 2022, highlighting stranding hotspots if present; (II) determining the existence of significant differences in terms of biological characteristics among stranded specimens of the same species; and (III) seeking insights into patterns of anthropogenic impact in the region in order to ascertain the level of anthropogenic mortality on the marine populations.

To a broader extent, this study may represent an example of the redaction of long-term datasets to gain more evidence on the main threats and causes of marine animal strandings and identify potential regions of interest to target further monitoring and conservation actions.

3. Materials and Methods

3.1. Study Area

The study includes the islands of Samos (37°73’N 26°83’E), Ikaria (37°61’N 2°05’E), Fournoi (37°34′N 26°30′E), Patmos (37°18'N 26°29’E), Leros (37°09′N 26°51′E), Agathonisi (37°27’N 26°58′E), and Lipsi (37°17'N 26°44E) (Figure 3.4), located in the Northeastern Aegean and Dodecanese districts of Greece. The coastline extends for approximately 640 km, with a heterogeneous topography dominated by cliffs, and sandy and rocky beaches.

The Aegean Sea is an essential marine ecosystem and habitat for many species, including cetaceans, seals, and sea turtles.

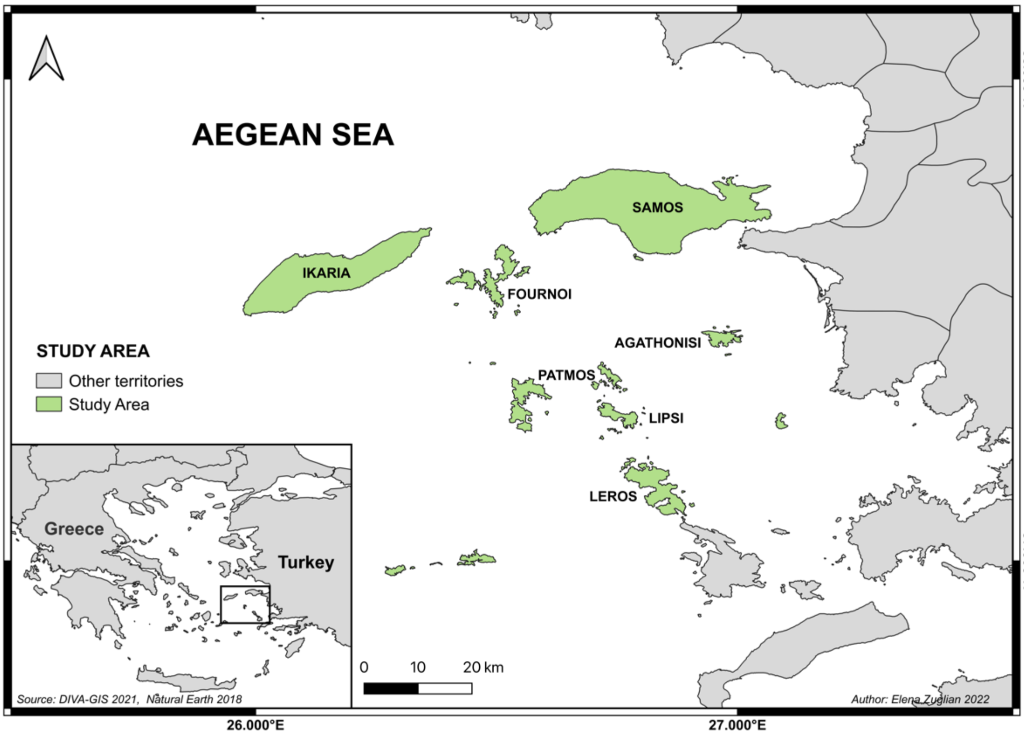


Figure 3.4. The study area includes the Greek islands of Agathonisi, Fournoi, Ikaria, Leros, Lipsi, and Samos. Map generated using QGIS 3.28.0.

3.2. Data Collection

The data analyzed in this study derive from the Archipelagos Institute of Marine Conservation NGO stranding database, recorded from January 2017 to December 2022. Stranding data were acquired through 2 methodologies.

The primary method consisted of receiving information from different sources and going to the stranding location to gather data. Public and private institutions, such as the Coast Guard, and local and port authorities, complied with the information. Additionally, stranding events were directly reported to the Institute by citizens and tourists through phone calls, emails, online reports, regional newspapers, and social media platforms.

The second methodology refers to land-based surveys conducted to record the presence of stranded individuals around the coastline of Samos and Lipsi islands by researchers employed in monitoring activities of the marine ecosystem all year long.

The database does not contain all of the strandings that occurred in the study area during the considered period, but only those reported and with a response. The number of stranding events that have occurred but have not been responded (or seen but not reported) remains unknown.

From January 2017 to December 2022, 113 specimens belonging to 7 species categories were reported as stranded along the shore of Samos, Ikaria, Lipsi, and Agathonisi islands.

A detailed stranding sheet was filled out for each stranded individual to gather information (Annex I, II, III). When the circumstances and the carcass status allowed it, post-mortem examinations were performed *in situ* or *ex-situ* to collect further data, according to the *"Common best practices for a basic post-mortem examination of stranded cetaceans"* (ACCOBAMS-MOP6/Res.6.22/Annex 2 – Mazzariol, 2016), the *"Evidence-based diagnostic assessment frameworks for cetacean necropsies on specific issues/threats"* (ACCOBAMS-MOP7/Res.7.14/Annex 15 – Mazzariol et al., 2019) (Annex IV) and the *"Diagnostic framework for the assessment of fishery interaction in stranded marine mammals"* (LIFE DELFI/Action A3/Tier 3 – Pietroluongo et al., 2021) (Annex V).

All the collected information was then reported in an Excel file, from which the database for this study was obtained (Table 3.1). The database indicates (1) the category, (2) the species, (3) the date when the stranded animal was found (dd-mm-yyyy), (4) the month, (5) the year, (6) the season, (7) the weather condition, (8) the sea state, (9) the island, (10-11) the location coordinates, (12) the state of conservation of the carcass, (13) the sex, (14) the age class, (15-16) the possible evidence of human interaction and (17) the total length of the animal body.

	Category	Species	Date	Month	Year	Season	Weather	Sea State	Island	Latitude (N)	Longitude (E)	DCC	Sex	Age Class	Anthropogenic impact evidence	Description of anthropogenic impact evidence	Total length
1	Sea Turtle	*Caretta caretta*	05-01-2017	Jan	2017	Winter	5	3	Samos	37.708889	26.683056	3	F	Adult	ND	NA	103.0
2	Sea Turtle	*Caretta caretta*	07-01-2017	Jan	2017	Winter	2	5	Samos	37.665828	26.885004	5	ND	Adult	ND	NA	66.0
3	Sea Turtle	*Caretta caretta*	16-04-2017	Apr	2017	Spring	4	4	Samos	37.805556	26.847222	4	M	Juvenile	N	NA	75.0
4	Sea Turtle	*Caretta caretta*	21-04-2017	Apr	2017	Spring	4	4	Samos	37.707414	27.016389	4	F	Juvenile	N	NA	34.0
5	Sea Turtle	*Caretta caretta*	03-05-2017	May	2017	Spring	3	2	Samos	37.698312	26.748337	4	M	Adult	N	NA	90.0
6	Pinniped	*Monachus monachus*	20-05-2017	May	2017	Spring	3	3	Samos	37.709820	26.691860	2	F	Adult	Y	Bullets	229.0
7	Cetacean	*Stenella coeruleoalba*	02-07-2017	Jul	2017	Summer	3	3	Samos	37.804601	26.841991	4	M	Juvenile	N	NA	140.0
8	Sea Turtle	*Caretta caretta*	16-07-2017	Jul	2017	Summer	4	3	Samos	37.806247	26.825230	5	M	Adult	ND	NA	70.0
9	Cetacean	*Stenella coeruleoalba*	11-08-2017	Aug	2017	Summer	4	4	Samos	37.799268	26.700631	1	F	Subadult	N	NA	195.0
10	Cetacean	*Tursiops truncatus*	21-08-2017	Aug	2017	Summer	4	4	Samos	37.698682	26.747563	2	M	Calf	Y	Head trauma	128.0
11	Pinniped	*Monachus monachus*	08-09-2017	Sep	2017	Summer	2	2	Samos	37.779440	26.890290	1	F	Juvenile	N	NA	114.0
12	Sea Turtle	*Chelonia mydas*	20-09-2017	Sep	2017	Summer	2	1	Samos	37.686844	26.921569	1	F	Adult	Y	Fishing line + intestine intussusception	105.0
13	Pinniped	*Monachus monachus*	23-10-2017	Oct	2017	Fall	3	3	Samos	37.638620	26.834370	4	M	Adult	N	NA	257.0
14	Sea Turtle	*Caretta caretta*	09-11-2017	Nov	2017	Fall	2	1	Agathonisi	37.500340	26.915860	3	F	Subadult	Y	Plastic in the stomach and intestine + 2 missed flipper	44.0
15	Cetacean	*Delphinus delphis*	10-11-2017	Nov	2017	Fall	2	2	Samos	37.674910	26.893560	2	F	Juvenile	N	NA	133.0
16	Sea Turtle	*Caretta caretta*	16-11-2017	Nov	2017	Fall	4	4	Samos	37.690966	26.947315	4	F	Adult	ND	NA	110.0
17	Cetacean	*Delphinus delphis*	03-12-2017	Dec	2017	Fall	3	3	Samos	37.674910	26.893560	3	M	Juvenile	ND	NA	135.0
18	Sea Turtle	*Chelonia mydas*	19-12-2017	Dec	2017	Fall	4	4	Samos	37.707414	26.996749	4	F	Adult	ND	NA	130.0
19	Cetacean	*Stenella coeruleoalba*	09-01-2018	Jan	2018	Winter	3	2	Samos	37.788690	26.663205	5	ND	Calf	ND	NA	123.0
20	Sea Turtle	*Caretta caretta*	09-01-2018	Jan	2018	Winter	3	2	Samos	37.700530	26.971246	4	F	Adult	ND	NA	80.0
21	Sea Turtle	*Caretta caretta*	24-01-2018	Jan	2018	Winter	5	4	Samos	37.669944	26.886972	5	M	Subadult	ND	NA	58.0
22	Sea Turtle	*Caretta caretta*	06-02-2018	Feb	2018	Winter	1	0	Samos	37.688434	26.943083	1	ND	Hatchling	N	NA	10.9
23	Sea Turtle	*Chelonia mydas*	09-02-2018	Feb	2018	Winter	0	0	Samos	37.698414	26.968626	1	F	Subadult	N	NA	42.0
24	Sea Turtle	*Chelonia mydas*	12-02-2018	Feb	2018	Winter	2	2	Samos	37.707487	26.986782	4	F	Adult	N	NA	104.0
25	Sea Turtle	*Chelonia mydas*	23-02-2018	Feb	2018	Winter	3	3	Samos	37.686210	26.919326	3	F	Subadult	N	NA	54.0
26	Sea Turtle	*Chelonia mydas*	24-02-2018	Feb	2018	Winter	4	3	Samos	37.689491	26.931523	3	F	Subadult	N	NA	43.0
27	Sea Turtle	*Chelonia mydas*	28-02-2018	Feb	2018	Winter	4	4	Samos	37.686210	26.919326	1	ND	Juvenile	Y	Plastic bag entaglement	30.0
28	Sea Turtle	*Caretta caretta*	01-03-2018	Mar	2018	Winter	1	1	Samos	37.709353	26.689029	3	ND	Adult	ND	NA	ND
29	Sea Turtle	*Caretta caretta*	08-03-2018	Mar	2018	Winter	4	3	Samos	37.677010	26.897276	4	F	Adult	N	NA	110.0
30	Sea Turtle	*Caretta caretta*	08-03-2018	Mar	2018	Winter	4	3	Samos	37.690666	26.947347	5	ND	Adult	ND	NA	ND
31	Sea Turtle	*Caretta caretta*	19-03-2018	Mar	2018	Winter	3	3	Samos	37.707756	26.988190	2	F	Adult	N	NA	84.0
32	Sea Turtle	*Chelonia mydas*	19-03-2018	Mar	2018	Winter	3	3	Samos	37.682671	26.909784	2	F	Adult	N	NA	64.0
33	Sea Turtle	*Caretta caretta*	09-04-2018	Apr	2018	Spring	1	1	Samos	37.755873	26.957904	1	M	Adult	N	NA	108.0
34	Sea Turtle	*Chelonia mydas*	17-04-2018	Apr	2018	Spring	1	1	Samos	37.691150	26.957783	3	F	Adult	N	NA	85.0
35	Sea Turtle	*Caretta caretta*	22-04-2018	Apr	2018	Spring	3	3	Samos	37.795096	26.682887	1	F	Adult	N	NA	98.0
36	Sea Turtle	*Chelonia mydas*	01-05-2018	May	2018	Spring	4	3	Samos	37.689167	26.757056	2	ND	ND	N	NA	ND
37	Cetacean	*Stenella coeruleoalba*	26-05-2018	May	2018	Spring	2	2	Samos	37.806210	26.825410	2	F	Subadult	N	NA	200.0
38	Sea Turtle	*Chelonia mydas*	02-06-2018	Jun	2018	Spring	3	3	Lipsi	37.303333	26.770278	4	ND	ND	ND	NA	ND
39	Cetacean	*Stenella coeruleoalba*	03-06-2018	Jun	2018	Spring	1	1	Samos	37.802620	26.712400	2	F	Subadult	N	NA	201.0
40	Sea Turtle	*Caretta caretta*	07-11-2018	Nov	2018	Fall	1	1	Samos	37.690863	26.953817	3	ND	Juvenile	N	NA	39.0
41	Sea Turtle	*Chelonia mydas*	19-11-2018	Nov	2018	Fall	4	4	Samos	37.684914	26.915813	3	F	Adult	N	NA	84.0
42	Sea Turtle	*Chelonia mydas*	19-11-2018	Nov	2018	Fall	5	5	Samos	37.678771	26.900801	3	F	Adult	N	NA	97.0
43	Sea Turtle	*Caretta caretta*	22-11-2018	Nov	2018	Fall	2	2	Samos	37.690002	26.935330	4	F	Adult	N	NA	93.0
44	Sea Turtle	*Caretta caretta*	27-11-2018	Nov	2018	Fall	4	4	Samos	37.687426	26.923648	3	M	Adult	N	NA	95.0
45	Sea Turtle	*Chelonia mydas*	25-01-2019	Jan	2019	Winter	4	4	Samos	37.688814	26.928020	3	M	Subadult	ND	NA	53.0
46	Sea Turtle	*Caretta caretta*	30-01-2019	Jan	2019	Winter	4	4	Samos	37.672653	26.890082	3	M	Adult	N	NA	96.0
47	Sea Turtle	*Caretta caretta*	30-01-2019	Jan	2019	Winter	5	5	Samos	37.678475	26.900078	3	F	Adult	N	NA	78.0
48	Sea Turtle	*Caretta caretta*	04-02-2019	Feb	2019	Winter	2	1	Samos	37.700773	26.971646	2	F	Adult	N	NA	93.0
49	Cetacean	*Delphinus delphis*	09-02-2019	Feb	2019	Winter	3	3	Samos	37.760500	26.948260	4	F	Subadult	ND	NA	152.0
50	Cetacean	*Stenella coeruleoalba*	25-02-2019	Feb	2019	Winter	4	4	Samos	37.771484	26.916560	4	ND	Juvenile	N	NA	163.0
51	Cetacean	*Stenella coeruleoalba*	01-03-2019	Mar	2019	Winter	3	3	Samos	37.808099	26.808970	1	F	Subadult	Y	Fractured upper jaw	196.0
52	Pinniped	*Monachus monachus*	14-03-2019	Mar	2019	Winter	1	1	Samos	37.727294	27.039582	3	F	Adult	ND	NA	205.0
53	Cetacean	*Stenella coeruleoalba*	16-03-2019	Mar	2019	Winter	3	3	Samos	37.806221	26.825258	3	M	Subadult	ND	NA	180.0
54	Cetacean	*Delphinus delphis*	24-03-2019	Mar	2019	Spring	3	3	Samos	37.729125	27.035610	4	F	Adult	N	NA	208.0
55	Cetacean	*Phocoena phocoena*	31-03-2019	Mar	2019	Spring	4	4	Samos	37.727392	27.040909	5	M	Subadult	ND	NA	125.0
56	Sea Turtle	*Caretta caretta*	05-04-2019	Apr	2019	Spring	2	2	Samos	37.757527	26.956817	4	F	Adult	N	NA	90.0
57	Cetacean	*Delphinus delphis*	12-04-2019	Apr	2019	Spring	2	2	Samos	37.689751	26.933215	4	M	Subadult	N	NA	153.0
58	Pinniped	*Monachus monachus*	17-04-2019	Apr	2019	Spring	4	3	Samos	37.789420	26.663180	3	M	Adult	Y	Bullets	260.0
59	Cetacean	*Tursiops truncatus*	22-04-2019	Apr	2019	Spring	3	4	Samos	37.778782	26.981759	3	F	Adult	Y	Fishing gear + 2 hooks	280.0
60	Cetacean	*Stenella coeruleoalba*	04-05-2019	May	2019	Spring	2	1	Samos	37.796680	26.687130	5	ND	Calf	ND	NA	105.0
61	Cetacean	*Tursiops truncatus*	04-05-2019	May	2019	Spring	2	1	Samos	37.799080	26.857158	1	M	Juvenile	N	NA	180.0
62	Cetacean	*Delphinus delphis*	07-05-2019	May	2019	Spring	3	3	Samos	37.799080	26.857158	1	M	Subadult	N	NA	180.0
63	Sea Turtle	*Caretta caretta*	08-05-2019	May	2019	Spring	1	1	Samos	37.707340	26.984640	4	ND	Hatchling	N	NA	16.0
64	Pinniped	*Monachus monachus*	31-05-2019	May	2019	Spring	1	1	Lipsi	37.282622	26.764343	3	M	Subadult	ND	NA	180.0
65	Sea Turtle	*Chelonia mydas*	07-11-2019	Nov	2019	Fall	2	2	Samos	37.700460	26.971156	3	M	Juvenile	N	NA	36.0
66	Sea Turtle	*Caretta caretta*	28-12-2019	Dec	2019	Winter	1	1	Samos	37.689987	26.937115	3	M	Adult	ND	NA	128.0
67	Sea Turtle	*Caretta caretta*	03-01-2020	Jan	2020	Winter	3	3	Samos	37.795272	26.683118	1	F	Adult	N	NA	89.0
68	Pinniped	*Monachus monachus*	10-01-2020	Jan	2020	Winter	0	2	Samos	37.778330	26.885258	3	F	Juvenile	ND	NA	129.3
69	Sea Turtle	*Caretta caretta*	11-01-2020	Jan	2020	Winter	0	1	Samos	37.805538	26.847317	3	F	Adult	ND	NA	92.0
70	Sea Turtle	*Caretta caretta*	06-02-2020	Feb	2020	Winter	3	2	Samos	37.683822	26.912656	3	F	Adult	N	NA	75.0
71	Sea Turtle	*Chelonia mydas*	04-03-2020	Mar	2020	Winter	6	5	Samos	37.698321	26.968089	2	F	Subadult	N	NA	59.0
72	Sea Turtle	*Chelonia mydas*	03-05-2020	May	2020	Spring	4	3	Samos	37.709303	26.684417	3	F	Subadult	ND	NA	43.0
73	Pinniped	*Monachus monachus*	05-05-2020	May	2020	Spring	4	3	Samos	37.722190	27.001420	3	F	Subadult	ND	NA	198.0
74	Cetacean	*Stenella coeruleoalba*	09-05-2020	May	2020	Spring	2	4	Samos	37.480560	26. 42334	3	ND	Subadult	Y	Gunshot/penetrating wound	177.0
75	Cetacean	*Stenella coeruleoalba*	18-05-2020	May	2020	Spring	3	2	Samos	37.713581	27.051608	3	M	Adult	Y	Gunshot/penetrating wound	225.0
76	Cetacean	*Stenella coeruleoalba*	20-05-2020	May	2020	Spring	0	2	Samos	37.713847	27.056862	4	ND	Juvenile	ND	NA	142.0
77	Cetacean	*Stenella coeruleoalba*	21-05-2020	May	2020	Spring	4	3	Samos	37.713588	27.051608	3	ND	Adult	ND	NA	ND
78	Cetacean	*Stenella coeruleoalba*	28-05-2020	May	2020	Spring	4	2	Samos	37.799137	26.704352	5	ND	Subadult	ND	NA	ND
79	Cetacean	*Stenella coeruleoalba*	31-05-2020	May	2020	Spring	2	1	Samos	37.794660	26.984200	3	F	Subadult	Y	Net marks/Linear signs	183.0
80	Pinniped	*Monachus monachus*	10-06-2020	Jun	2020	Spring	1	1	Lipsi	37.300503	26.735215	2	ND	Pup	ND	NA	ND
81	Sea Turtle	*Caretta caretta*	17-06-2020	Jun	2020	Spring	1	3	Lipsi	37.296130	26.765448	2	F	Adult	N	NA	75.0
82	Sea Turtle	*Caretta caretta*	09-07-2020	Jul	2020	Summer	4	4	Samos	37.662796	26.881472	4	ND	ND	Y	Fishing net	ND
83	Sea Turtle	*Caretta caretta*	10-08-2020	Aug	2020	Summer	1	1	Lipsi	37.285800	26.789230	2	M	Subadult	ND	NA	94.0
84	Cetacean	*Phocoena phocoena*	14-10-2020	Oct	2020	Fall	3	2	Samos	37.707515	26.997027	3	M	Adult	ND	NA	ND
85	Cetacean	*Tursiops truncatus*	12-09-2021	Sep	2021	Summer	2	2	Samos	37.684980	26.954730	4	F	Subadult	ND	NA	197.0
86	Sea Turtle	*Caretta caretta*	05-01-2021	Jan	2021	Winter	3	2	Samos	37.709863	26.673611	4	ND	Adult	N	NA	ND
87	Pinniped	*Monachus monachus*	29-01-2021	Jan	2021	Winter	1	1	Ikaria	37.634550	26.086900	2	ND	Subadult	ND	NA	180.0
88	Sea Turtle	*Chelonia mydas*	02-02-2021	Jan	2021	Winter	2	1	Lipsi	37.282638	26.771600	4	ND	Subadult	N	NA	ND
89	Cetacean	*Delphinus delphis*	16-03-2021	Mar	2021	Winter	4	3	Samos	37.689187	26.942524	3	ND	Subadult	ND	NA	155.0
90	Sea Turtle	*Chelonia mydas*	16-03-2021	Mar	2021	Winter	4	3	Samos	37.689985	26.941372	2	ND	Adult	ND	NA	ND
91	Sea Turtle	*Caretta caretta*	29-03-2021	Mar	2021	Spring	3	3	Samos	37.810380	26.792097	1	ND	ND	ND	NA	ND
92	Pinniped	*Monachus monachus*	08-04-2021	Apr	2021	Spring	3	3	Samos	37.709597	26.690715	ND	ND	ND	ND	NA	ND
93	Sea Turtle	*Chelonia mydas*	08-10-2021	Oct	2021	Fall	0	0	Samos	37.780728	26.992214	4	F	Subadult	ND	NA	47.0
94	Pinniped	*Monachus monachus*	01-11-2021	Nov	2021	Fall	2	2	Samos	37.698600	26.747278	3	ND	Adult	ND	NA	ND
95	Pinniped	*Monachus monachus*	03-11-2021	Nov	2021	Fall	8	7	Samos	37.765530	26.964700	4	M	Pup	ND	NA	87.0
96	Sea Turtle	*Caretta caretta*	29-11-2021	Nov	2021	Fall	4	3	Samos	37.689265	26.930438	2	F	Subadult	Y	Fishing net	85.0
97	Sea Turtle	*Caretta caretta*	01-12-2021	Dec	2021	Fall	5	5	Samos	37.709764	26.699864	1	F	Subadult	N	NA	82.0
98	Sea Turtle	*Chelonia mydas*	08-12-2021	Dec	2021	Fall	1	1	Samos	37.707450	26.997470	4	F	Adult	N	NA	68.0
99	Sea Turtle	*Chelonia mydas*	08-12-2021	Dec	2021	Fall	1	1	Samos	37.688772	26.928043	3	M	Adult	ND	NA	124.0
100	Cetacean	*Stenella coeruleoalba*	22-01-2022	Jan	2022	Winter	8	6	Samos	37.671565	26.888831	2	F	Subadult	Y	Fishing gear	175.0
101	Cetacean	*Stenella coeruleoalba*	22-01-2022	Jan	2022	Winter	8	6	Samos	37.671565	26.888831	1	F	Subadult	Y	Fishing gear	186.0
102	Cetacean	*Stenella coeruleoalba*	22-01-2022	Jan	2022	Winter	8	6	Samos	37.671565	26.888831	2	M	Juvenile	Y	Fishing gear	131.0
103	Cetacean	*Delphinus delphis*	20-02-2022	Feb	2022	Winter	3	1	Samos	37.733810	27.035920	4	M	Adult	ND	NA	215.0
104	Cetacean	*Tursiops truncatus*	21-02-2022	Feb	2022	Winter	3	1	Samos	37.779540	26.995140	4	F	Adult	ND	NA	ND
105	Cetacean	*Tursiops truncatus*	15-03-2022	Mar	2022	Winter	2	1	Samos	37.708800	27.040460	4	F	Subadult	ND	NA	201.0
106	Cetacean	*Delphinus delphis*	02-04-2022	Apr	2022	Spring	2	2	Samos	37.804520	26.841730	3	M	Subadult	Y	Gunshots/Penetrating wounds	198.0
107	Sea Turtle	*Caretta caretta*	23-05-2022	May	2022	Spring	1	1	Samos	37.682800	26.909750	4	F	Adult	ND	NA	99.0
108	Sea Turtle	*Caretta caretta*	27-05-2022	May	2022	Spring	1	1	Samos	37.665190	26.884340	3	F	Adult	Y	Plastic in the mouth	90.0
109	Sea Turtle	*Caretta caretta*	23-08-2022	Aug	2022	Summer	1	1	Lipsi	37.305000	26.787778	4	F	Adult	ND	NA	76.0
110	Sea Turtle	*Chelonia mydas*	22-11-2022	Nov	2022	Fall	0	1	Samos	37.691020	26.957470	2	F	Subadult	Y	Fishing net	43.0
111	Sea Turtle	*Caretta caretta*	12-12-2022	Dec	2022	Fall	2	2	Samos	37.690090	26.935812	4	M	Adult	ND	NA	112.0
112	Sea Turtle	*Chelonia mydas*	12-12-2022	Dec	2022	Fall	2	2	Samos	37.689964	26.934640	4	M	Juvenile	ND	NA	ND
113	Sea Turtle	*Chelonia mydas*	26-12-2022	Dec	2022	Winter	1	1	Samos	37.688410	26.943110	2	F	Subadult	ND	NA	38.0

Table 3.1. Database of the specimens found stranded along the coast of Agathonisi, Ikaria, Lipsi and Samos Islands (Greece) during the period 2017–2022 (Weather: Beaufort Scale; Sea State: Douglas Scale; DCC: Decomposition Condition Code; F: Female; M: Male; ND: Not Determined; NA: Not Applicable; Y: Yes; N: No).

For each stranded animal, one of the following categories was assigned according to its taxonomic group: cetacean, pinniped, or sea turtle. The species was identified by the analysis of the external characteristics.

The year was divided into 4 seasons: winter (December 21st – March 20th), spring (March 21st – June 20th), summer (June 21st – September 22nd), and fall (September 23rd – December 20th).

The weather condition was recorded according to the Beaufort Scale, assigning a number from 0 to 12, which refers to the wind speed (Table 3.2). The sea state was collected according to the Douglas Scale, attributing a number from 0 to 9, indicating the height of the waves (Table 3.3).

BEAUFORT SCALE

Code	Wind speed (km/h)	Description
0	< 2	Calm
1	2 - 5	Light air movement
2	6 - 11	Light breeze
3	12 - 19	Gentle breeze
4	20 - 28	Moderate breeze
5	29 - 38	Fresh breeze
6	39 - 49	Strong breeze
7	50 - 61	Near gale
8	62 - 74	Gale
9	75 - 88	Strong gale
10	89 - 102	Storm
11	103 - 117	Violent storm
12	> 118	Hurricane force

Table 3.2. Beaufort Scale description.

DOUGLAS SCALE

Code	Wave height (m)	Description
0	0.0	Calm (glassy)
1	0.0 - 0.1	Calm (rippled)
2	0.1 - 0.5	Smooth, small wavelets
3	0.5 - 1.25	Slight, large wavelets
4	1.25 - 2.5	Moderate, small waves
5	2.5 - 4.0	Rough, moderate waves
6	4.0 - 6.0	Very rough, large waves
7	6.0 - 9.0	High
8	9.0 - 14.0	Very high
9	> 14.0	Phenomenal

Table 3.3. Douglas Scale description.

All data have been georeferenced using GPS coordinates and the carcass status at the moment of the examination was reported according to the Decomposition Condition Code (DCC) as follows: 1 – when the animal was found alive or died at most 2 hours earlier; 2 – when the carcass was in good condition as death took place within 24 hours of the finding; 3 – when the carcass was in a status of moderate decomposition; 4 – when the carcass was in advanced decomposition; 5 – when there were just mummified or skeletal remains (Mazzariol, 2016).

All animals were assigned to an age class (Calf/Hatchling/Pup, Juvenile, Subadult, Adult) based on their physical characteristics, especially the total body length, which was measured using a soft millimetric tape from snout to notch in cetaceans and pinnipeds, and from the beak to the tip of the carapace in sea turtles (following ACCOBAMS-MOP6/Res.6.22/Annex 2 – Mazzariol, 2016) (Annex VI).

The sex was determined using the tail length of sea turtle adult individuals and looking at the genital area in cetaceans and pinnipeds; furthermore, the gonads were examined during the necropsy when possible.

To assess human-interaction evidence, we looked at the presence of fishing gear, marks on the body surface, lacerations of the skin, scars, amputations, fractures, contusions, marine litter entanglement and ingestion, gunshot/bullet, and penetrating wounds, bleeding from main orifices, nutritional status, and any other evidence of vessel or projectiles interactions according to the *"Handbook for recognizing, evaluating, and documenting human interaction in stranded cetaceans and pinnipeds"* (Moore et al., 2013), the *"Evidence-based diagnostic assessment frameworks for cetacean necropsies on specific issues/threats"* (ACCOBAMS-MOP7 – Mazzariol et al., 2019) and *the "Diagnostic framework for the assessment of fishery interaction in stranded marine mammals"* (LIFE DELFI – Pietroluongo et al., 2021).
In most cases, it was not possible to identify the cause of stranding due to logistical constraints that impeded our ability to perform complete post-mortem examinations.
Additional data not relevant to this study were collected during the carcass examination.

3.3. Data analysis

Maps were drawn using the QGIS Software (v. 3.28.0) to analyze the spatial-temporal tendencies and illustrate the distribution of strandings in the study area for each species and category. The "hotspot" tool in QGIS was used to identify stranding hotspots. This tool calculates the Getis-Ord Gi* statistic for each variable in a dataset, producing z-scores and p-values that explain where variables with high or low values cluster spatially, taking into account the neighboring variables. The area can be defined as a stranding hotspot if the cluster is statistically significant.
The RStudio software (v. 2022.12.0+353) was used to conduct data exploration and statistical analyses. Descriptive statistics of biological and environmental variables were used to gain insight into stranding trends.
A Generalized Linear Mixed-Effects Model (GLMM) was used to estimate the probability of a stranding occurrence in a specific place and time of the year for each species. The results were validated using diagnostic plots.

4. Results and Discussion

4.1. General overview

From January 2017 to December 2022, 113 specimens were found stranded along the shores of Agathonisi, Ikaria, Lipsi and Samos islands: 65 sea turtles, family *Cheloniidae* [*C. caretta* n = 40, *C. mydas* n = 25]; 35 cetaceans, family *Delphinidae* [*S. coeruleoalba* n = 18, *D. delphis* n = 9, *T. truncatus* n = 6] and family *Phocoenidae* [*P. phocoena* n = 2]; and 13 Mediterranean monk seals, family *Phocidae* [*M. monachus*] (Figure 4.5).

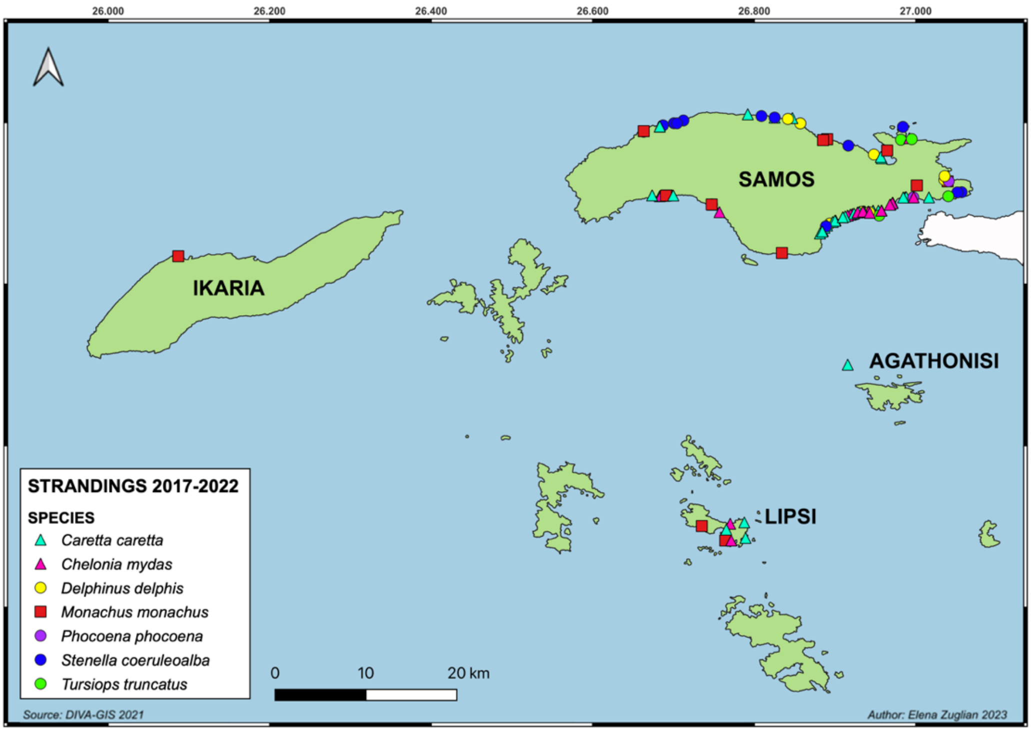


Figure 4.5. Distribution of stranded individuals along Agathonisi, Ikaria, Lipsi and Samos islands shores. Map generated using QGIS 3.28.0.

Among all these stranding episodes, 99% were single strandings (110 individuals), while 1% was a mass stranding (1 event) that occurred in 2022, involving 3 striped dolphins (*S. coeruleoalba*) with the evidence of fishing net trapping the animals (bycatch).
Furthermore, the recorded species were those commonly sighted in the study area; no alien species were identified, and this study did not include unidentified animals.

In particular, sea turtles can be easily found in coastal waters and they come out of the water to lay eggs, exposing themselves to many threats of different nature. The high number of stranding episodes could be predictable for this and many other reasons. The greater number of events involving *C. caretta* specimens compared to *C. mydas* is probably related to the population size; the loggerhead turtle is more abundant than the green turtle in the Mediterranean Sea (Casale et al., 2010).

Among cetaceans, *S. coeruleoalba* is the most abundant. This can justify the higher frequency of strandings compared to the other species of the same category, even though it generally resides in pelagic waters (Frantzis et al., 2003). *D. delphis* and *T. truncatus* spend most of their lives in coastal areas, and, as we can see from the map below (Figure 4.6), several pods reside along the coasts of the islands under investigation.

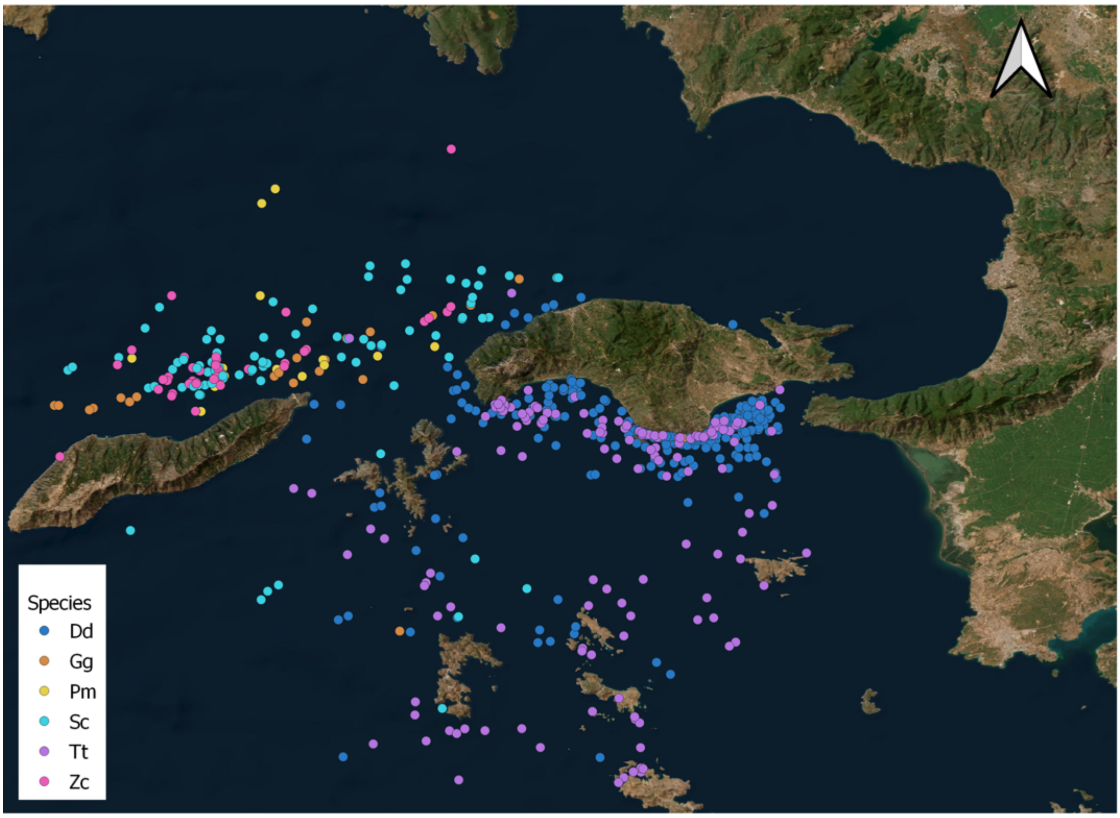


Figure 4.6. Cetacean sightings in the study area from 2017 to 2022. Map generated using QGIS 3.28.0.

The low number of strandings of *P. phocoena* and *M. monachus* is probably associated with the limited number of individuals in the region.

The lack of data for the other resident species is correlated to their being mainly pelagic. However, this does not exclude the possibility that stranding events happened in the region under investigation.

4.2. Spatio-temporal trends

4.2.1. Temporal analysis

Annual stranding frequency fluctuated over the study period. The year presenting more strandings was 2018, with n = 26 (23%), followed by 2019, with n = 22 (20%); 2017 and 2020, with n = 18 (16%); 2021, with n = 15 (14%); and finally, 2022, with n =12 (11%). Although the results highlight a peak in stranding events in 2018, followed by a gradual decline until 2022, it is not appropriate to consider this trend representative of the actual situation due to the different monitoring efforts over the years.

Notably, from 2020 to 2022, external factors impacted data collection, which might have led to distorted results from reality. Furthermore, the annual number of strandings per species did not follow a constant pattern resembling the overall dynamics (Figure 4.7). Therefore, taking into consideration seasonal rather than annual patterns is more appropriate.

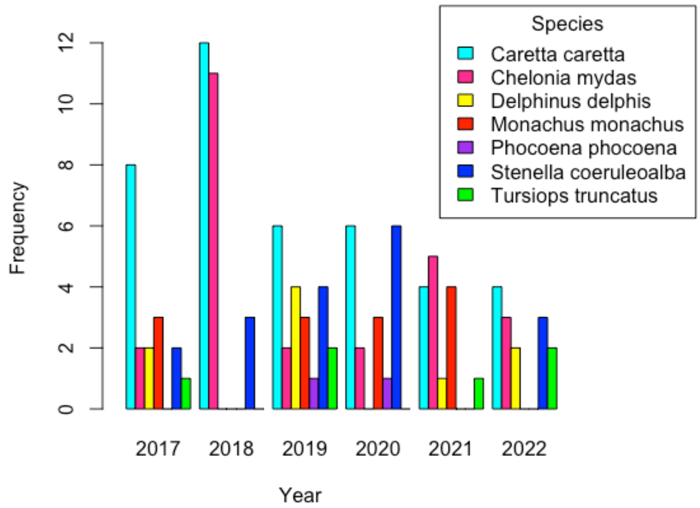


Figure 4.7. Annual frequency of strandings per species

Overall, stranding reports were more frequent during winter (38%; n = 43) and spring (33%; n = 37) compared with fall (20%; n = 23) and summer (9%; n = 10). The seasonal difference varied within the years of study, although not significantly (Table 4.4).

Season/Year	2017	2018	2019	2020	2021	2022	Tot. (season)
Winter	2	14	10	5	5	7	43
Spring	4	7	11	10	2	3	37
Summer	6	0	0	2	1	1	10
Fall	6	5	1	1	7	3	23
Tot. (year)	18	26	22	18	15	14	113

Table 4.4. Number of strandings according to the year and season.

Most strandings occurred during January (n = 17), March (n = 15) and May (n = 19); the lowest number of cases was recorded from June to October (n = 3.6).

Marine mammals and sea turtles showed different peaks in the monthly pattern of strandings.

Among cetaceans, the months with the highest stranding occurrence were May, March, January, and February, with respectively 10 (29%), 6 (17%), and 4 (11%) stranded specimens for the last two, while only 1 to 3 strandings were recorded during the other months.
Stranded seals were found mainly in May (n = 3; 23%) and the other spring months, while only 1 specimen stranded in the summer.
Sea turtle strandings occurred for 17% in January (n = 11), 15% in November (n = 10), and 12% in February, March and December (n = 8). Summer and early fall were the periods during which fewer strandings occurred.
Therefore, stranding episodes were less frequently during summer (Figure 4.8).

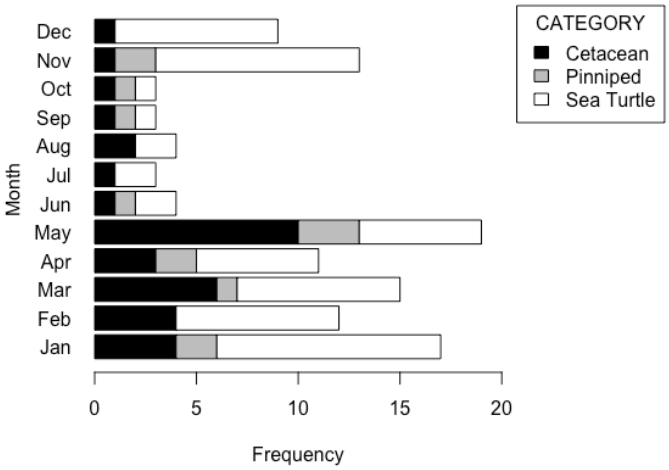


Figure 4.8. Monthly frequency of strandings per category

Variations in marine and meteorological conditions could seem crucial factors in determining seasonality patterns. However, although some stranding episodes happened during severe climatic conditions (sea state > 5 and weather > 5, according to Beaufort and Douglas scales), which never occurred during the summer months, these are not frequent enough to be deemed the primary cause of stranding during fall, winter and spring seasons. Instead, they are limited episodes that could have occurred in combination with other factors.
The elements that could have most influenced the stranding seasonality are season-specific abundance, habitat use, natural mortality, as well as non-biological components such as maritime characteristics and anthropogenic pressures (e.g., spatiotemporal differences in fishing efforts and military training) (IJsseldijk et al., 2020).
In the Northeastern Aegean Sea, marine mammal populations are generally present throughout the year thanks to the temperate climate of the area. Mean sea temperatures are around 16° C during the cold months, from December to March, and reach 24° C during summer.
Sea turtles instead tend to migrate within the Mediterranean Sea, moving towards the east during the coldest months. Hence, the stranding peak reflects the higher abundance of *C. caretta* and *C. mydas* in the Greek area from November to May.

Water temperature could represent a threat in combination with other factors, such as a low body condition score, as a debilitated animal is more exposed to physiological stress, parasites, contraction of diseases, and eventually hypothermia and death. Inadequate energy intake and following poor body condition could result from food depletion. Fishing activities represent the major conflict for marine mammal species in terms of resources and a threat if considering accidental bycatch, especially during spring and fall, when fishing is more intense. Emaciation could also result from excessive energy consumption and inability to maintain stable physical fitness because of particular physiological conditions, such as pregnancy, weaning, other metabolic syndromes or illnesses. Furthermore, military training is widespread in the study area due to the strategic position close to the Turkey border, especially during low-season months when tourism is minimal. The use of sonar and other technologies can interfere with the orientation abilities of marine mammals, increasing stranding risk.

4.2.2. Spatial analysis

The majority of strandings occurred in Samos (n = 104), while only a few cases of sea turtle and pinniped strandings have been sighted along the coastline of Lipsi (n = 7), Agathonisi (n = 1) and Ikaria (n = 1).

The reason behind these results partly is that researchers conduct periodical monitoring surveys only on the islands of Samos and Lipsi while stranding reports regarding the other islands mainly came from the local population and authorities, which are not yet fully engaged in these conservation projects. Secondly, the number of strandings is proportional to the dimension of the island; therefore, a greater number of strandings would be expected for Samos and Ikaria, and a smaller number of strandings would be expected for the other islands.

Lastly, the landscape's topography and the low population index of most of these islands make land-based surveys an inefficient monitoring tool due to the inaccessibility of many areas, and the detection of carcasses through boat-based surveys is challenging to perform.

Analyzing the spatial distribution of the strandings, it was possible to detect some hotspots, which are the areas presenting the highest stranding frequency. As shown in Figure 4.8, these hotspots were the southeastern and northern coasts of Samos, and the southern coast of Lipsi, which coincided with the areas presenting higher anthropogenic disturbance and peculiar coastal geomorphology.

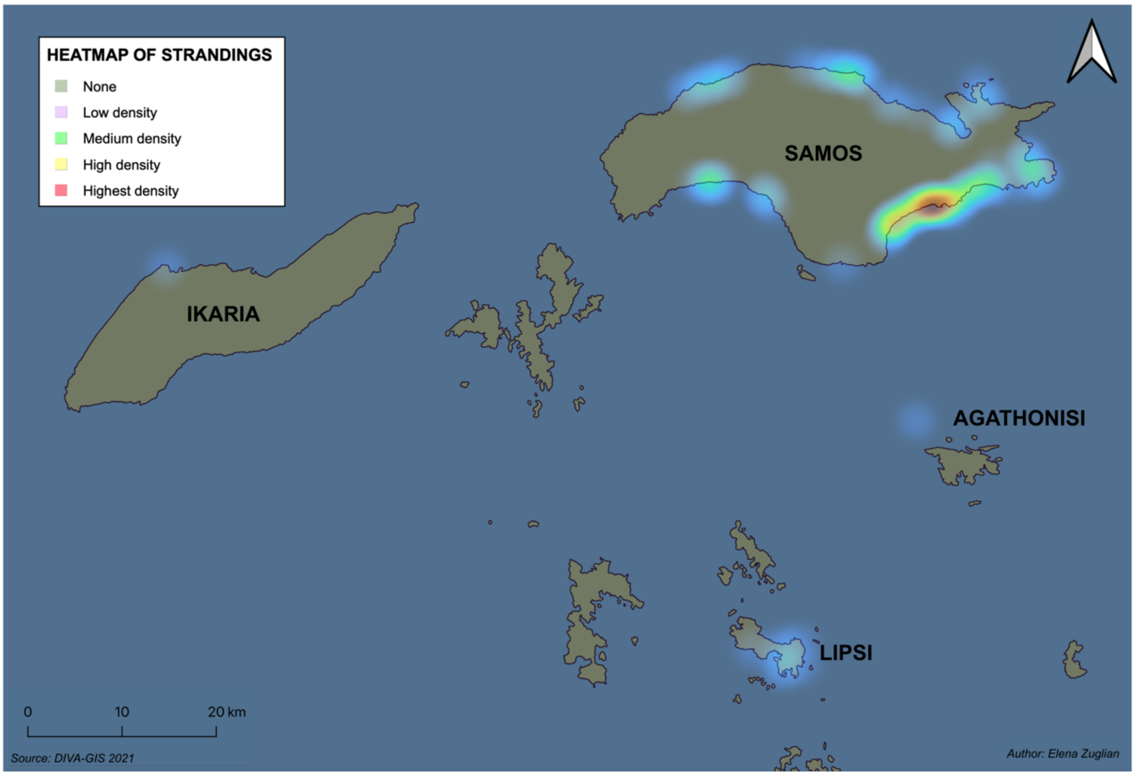
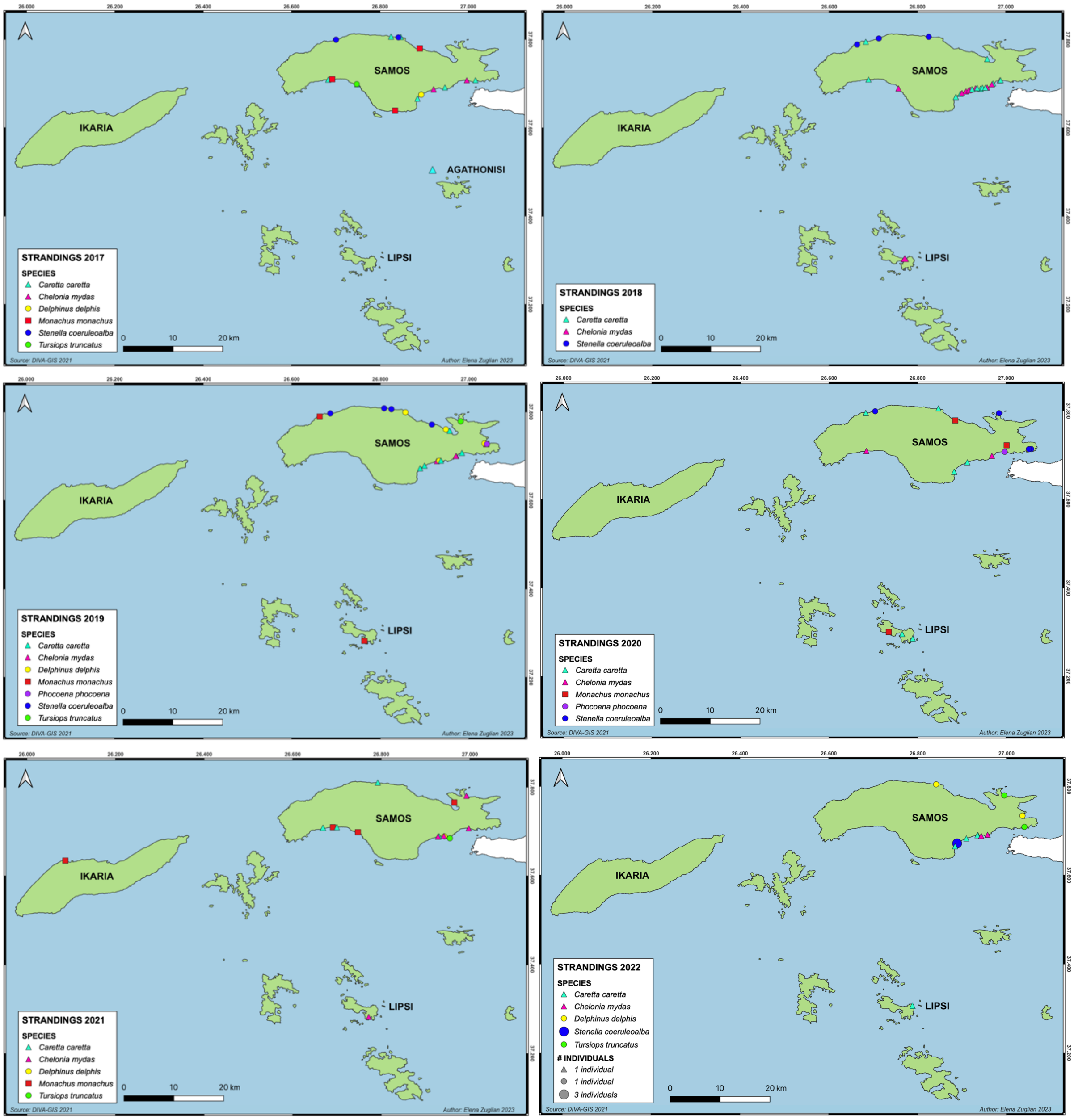


Figure 4.9. Stranding hotspots from 2017 to 2022 in the study area. Map generated using QGIS 3.28.0.

When human-derived stressors concentrate and overlap, the consequence is a higher mortality rate for the marine megafauna in that region, as animals are more exposed to threats. Additionally, the topography of the continental shelf and the bathymetry, considering that the depth of the seabed is less than 40 m in such areas (Soukissian et al., 2017), together with winds, currents, and tides have a considerable impact on the rates of retention on the coast and could drive carcasses distribution (Pikesley et al., 2012). Moreover, the easy access to these locations promotes carcass detection, as opposed to the other areas where the stranding frequency is low and accessibility is challenging. Furthermore, the southeastern coast of Samos is characterized by periodical sightings of these marine populations, justifying the high number of stranded animals.

From a deeper spatiotemporal analysis, we notice how the stranding spatial distribution in 2017 and 2018 was more or less constant, spreading to the south and north of Samos. The same is true for 2020 and 2021, even though the number of events and the frequency per area differ for each year. In 2019, no stranding was recorded in the southwest. In 2022, strandings were distributed on the east side of the island, particularly in the primary hotspot in the southeast (Figure 4.10).



4.10. Spatial distribution of strandings according to the year. Maps generated using QGIS 3.28.0.

Over the years, the seasonal pattern showed a different spatial distribution from the annual trend during fall, as strandings occurred almost entirely along the southeastern coast.

Regarding the species, *C. caretta* specimens were stranded along the northern and southern coasts of Samos and the southern coasts of Lipsi (Figure 4.11 – A), while *C. mydas* individuals were found stranded in the south of both islands (Figure 4.11 – B).

S. coeruleoalba carcasses were distributed mainly north of Samos (Figure 4.11 – C), while *T. truncatus* showed a greater range in its spatial pattern with specimens found south and north (Figure 4.11 – D). *D. delphis* and *P. phocoena* converged on the east coast (Figure 4.11 – E, F). No cetacean carcass was found along the coasts of Ikaria and Lipsi. *M. monachus* specimens were found in different areas, but no carcass was found in the southeastern hotspot of Samos (Figure 4.11 – G).

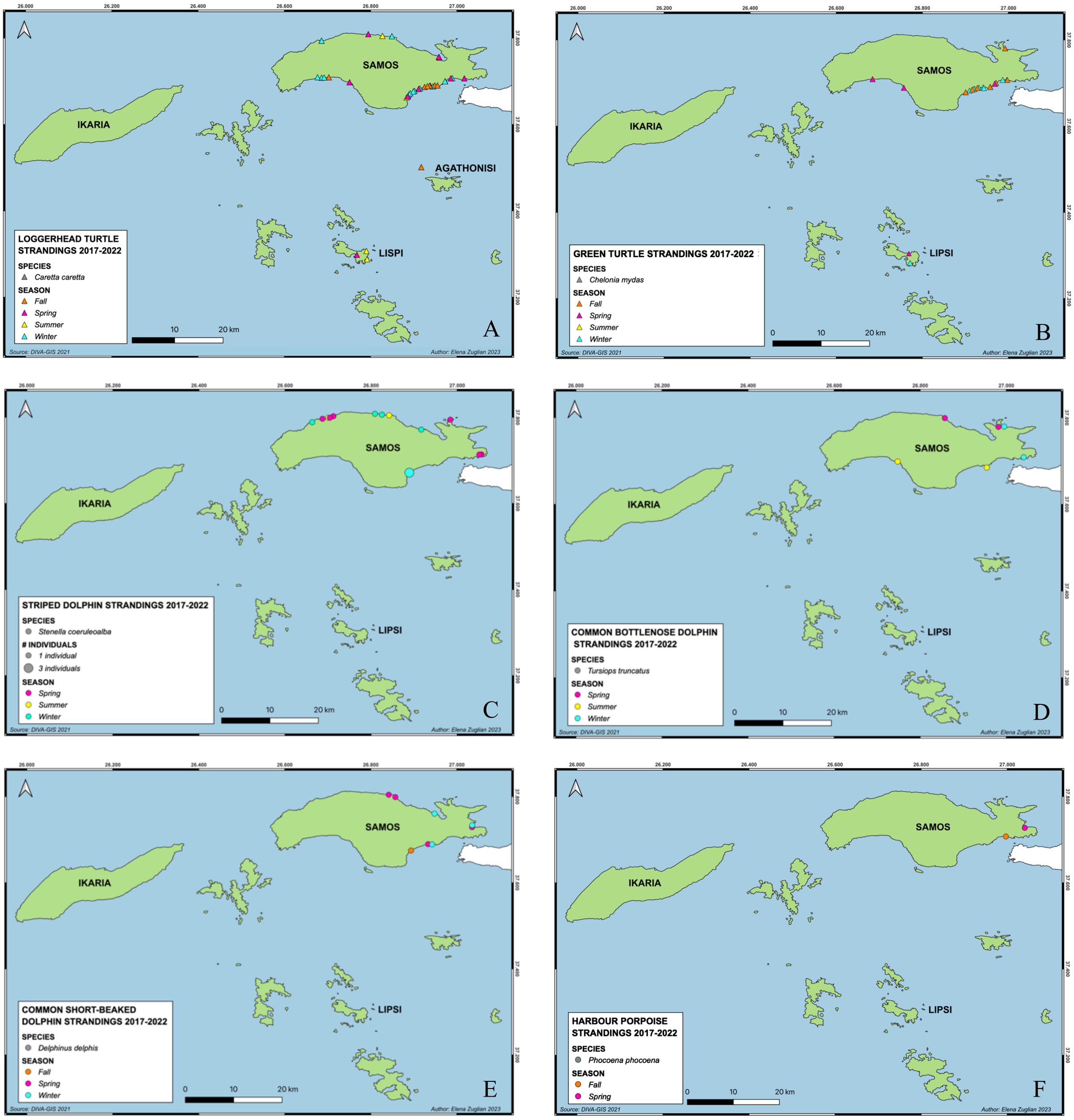
Even though the spatial distribution of all the species has yet to be fully investigated, that of the striped dolphin matches its habitat use, as sightings occurred only in the waters north of Samos. The absence of carcasses of *D. delphis* west of the island could be related to the fact that this species is smaller in dimensions, so it is more exposed to the action of currents. However, there needs to be more proof to confirm this hypothesis.

The lack of Mediterranean monk seal strandings in southeast Samos can be justified by the fact that this area is subject to anthropic disturbance, while seals tend to live in quieter zones (Pietroluongo et al., 2022).

Although some carcasses in the hotspots showed evidence of human interaction, others did not; therefore, anthropogenic impact cannot always be considered the primary factor driving strandings in such areas.

Besides, there is no significant correlation between the spot of stranding and age class, as specimens of different classes for each species were distributed in different areas, except for juveniles, as they were found only on the east side of Samos.

In marine mammals, there appears to be a correlation between spatial distribution and sex, which will be discussed in the next chapters.



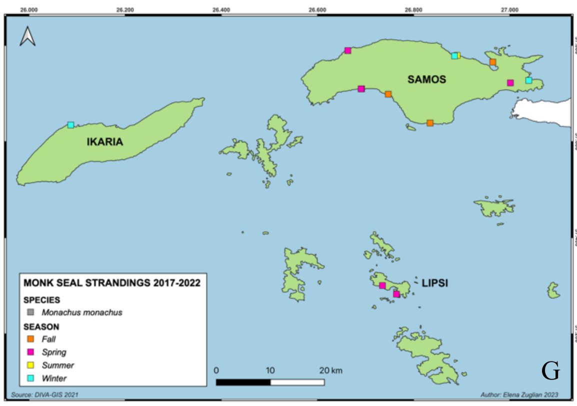


Figure 4.11. Seasonal spatial distribution of stranding events according to the species. Maps generated using QGIS 3.28.0.

4.3. Biological characteristics

4.3.1. Sex

Sex was recorded for 77% of the individuals (n = 87), accounting for 56 females and 31 males among all categories. Without considering the individuals of undefined sex (n = 26; 23%), the sex ratio was skewed towards females due to the significantly higher number of stranded females (n = 37; 57%) with respect to males (n = 14; 21.5%) in sea turtles (F:M = 1:0.4), while the difference was not overall noteworthy for marine mammals: 14 females (40%) versus 13 males (37%) in cetaceans (F:M = 1:0.9), and 5 females (38%) against 4 males (31%) in seals (F:M = 1:0.8). This trend was maintained in all species, apart from *D. delphis* and *P. phocoena*, among which acknowledged males were more than females (Figure 4.12).

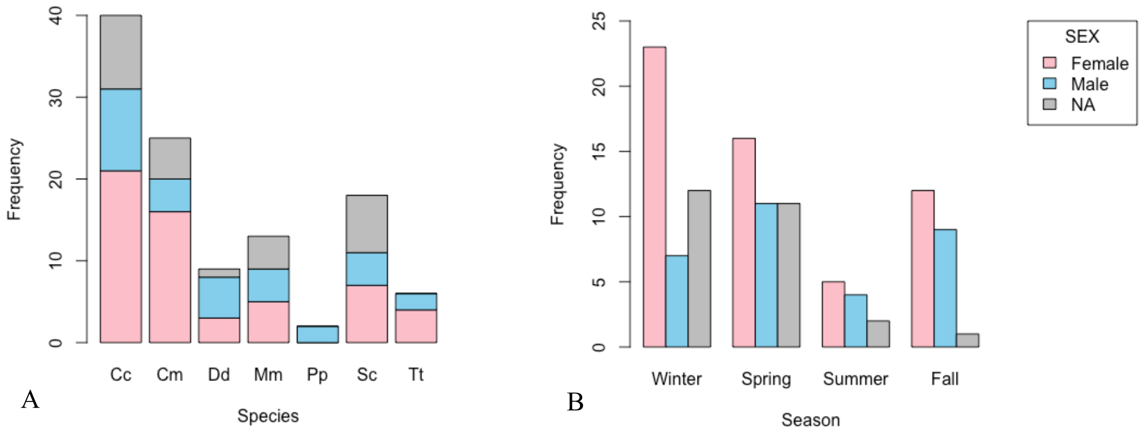


Figure 4.12. Stranding frequency based on the sex, according to the species (A) and the season (B).

Among the 40 specimens of *C. caretta,* 21 females (52.5%) and 10 males (25%) were identified, while the sex of 9 individuals was not determined. Besides summer, all seasons reported an expected higher number of stranded females, especially in winter, when 9 adult females were stranded. Human interaction evidence was reported only in females, particularly the presence of plastic and fishing lines, while it was not assessed in males. This was also true for the green turtle. Regarding age class, most adult specimens were females. Concerning *C. mydas,* 16 specimens were females and 4 were males, while the gender of 5 individuals was not determined. Also, in this case, the number of females per season was greater, with maximal strandings in winter and fall. Differences in age class were observed, with 8 adults and 8 subadults for the females, and 1 adult, 1 subadult, and 2 juveniles for the males. There were no discernible spatial trends related to sex among sea turtles (Figure 4.13 – A). This sex-related disproportion in strandings could be related to seasonal sexual segregation within the populations of sea turtles, which may lead to an over-representation of females (McGovern et al., 2016).

As a consequence, the mortality of adult females is emphasized. Moreover, according to IKsseldijk et al. (2020), higher mortality in juvenile males compared to females is commonly found among marine megafauna.

Among marine mammals, 7 specimens of *S. coeruleoalba* were identified as females, 4 as males, and the sex could not be determined in 7 individuals. All females were subadults, while males belonged to different age classes, from juveniles to adults. There were no differences related to gender at the dimensional and seasonal levels.

The stranded specimens of bottlenose dolphins were 4 females and 2 males. Furthermore, adults and subadults were females, while calves and juveniles were males. At the seasonal level, there was a difference, as the young males were found during spring and summer, while the older females were found also in winter. At the spatial level, males were located north and southwest, while females were concentrated along the east coasts (Figure 4.13 – B). The difference in seasonality can be explained by increased female mortality due to the stress and impaired physiological condition associated with weaning and reproduction (IKsseldijk et al., 2020) and by the precocious death of young individuals.

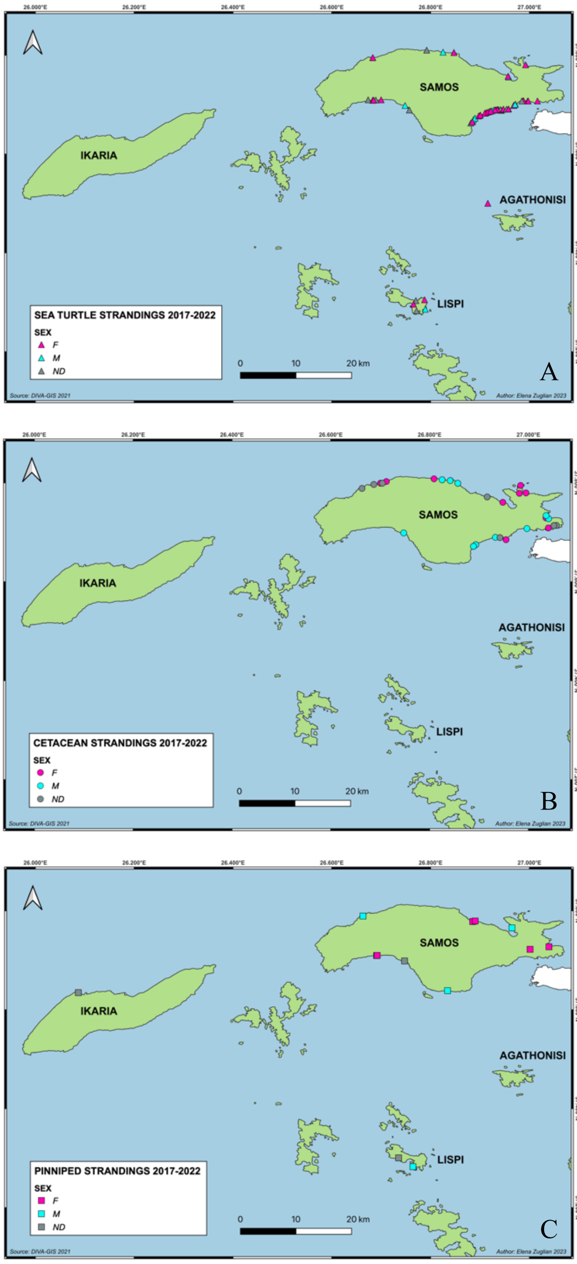


Figure 4.13. Spatial distribution of stranding events according to the sex. Maps generated using QGIS 3.28.0.

The Aegean Sea is an important breeding ground for marine mammals (Frantzis et al., 2003), characterized by resident groups throughout the year. The high population abundance and the high resource exploitation, also due to human activities, increase interspecific competition. Consequently, individuals tend to disperse to limit competition and the adverse effects that could result from it, such as malnutrition.

D. delphis presented a male stranding prevalence, with 5 males against 3 females and 1 individual of unknown gender. There were no particular differences in age class and seasonality among the sexes. The only stranded specimens of *P. phocoena* were 2 males.

Among *M. monachus* specimens, 5 were females and 4 were males, while the sex of 4 was not determined. Both genres included individuals of different ages and there was a sexual dimorphism with males bigger than females ($\bar{l}_M$ = 258.5 cm; $\bar{l}_F$ = 217.0 cm). Strandings occurred in different seasons over the years, with differences in terms of gender, as carcasses of female seals were prevalent in winter and spring, while the contrary was true during fall. The spatial pattern could show sex-related differences, probably linked to the territorial behavior of the species, but this point will be further discussed in the next paragraph (Figure 4.13 – C).

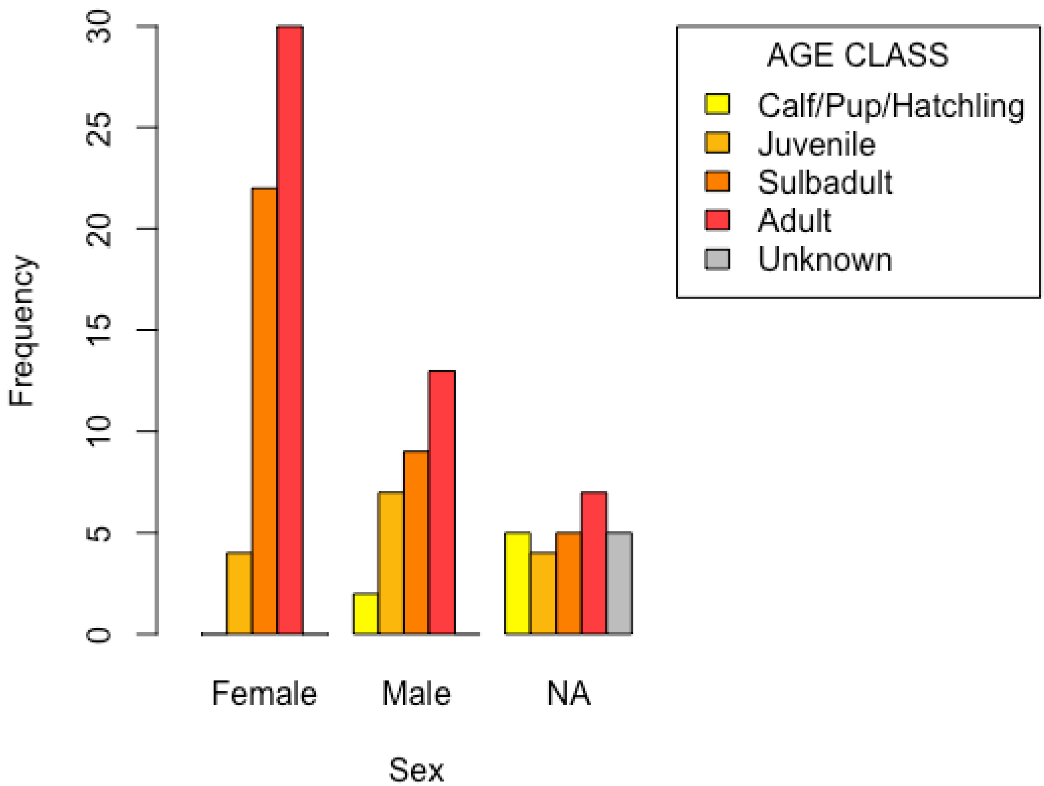


Figure 4.14. Stranding frequency based on the age class, according to the sex.

4.3.2. *Age class and total body length*

The age class was estimated based on the morphometric measurements and the pelage pattern. Body length was measured for 85% of the individuals (n = 96/113) and only the age class of 5 specimens remains unknown.

44% were adults (n = 50), 32% were subadults (n = 36), 13% were juveniles (n = 15), while calves, puppies and hatchlings represented just 6% (n = 7) of the stranded individuals.

Among the different animal categories, stranding events involved prevalently adults in sea turtles (n = 38; 58%) and seals (n = 5; 38%), and subadults in cetaceans (n = 18; 51%). Indeed, mortality rates are generally lower among younger individuals because the older ones are more vulnerable, except for calves and pups (Figure 4.15).

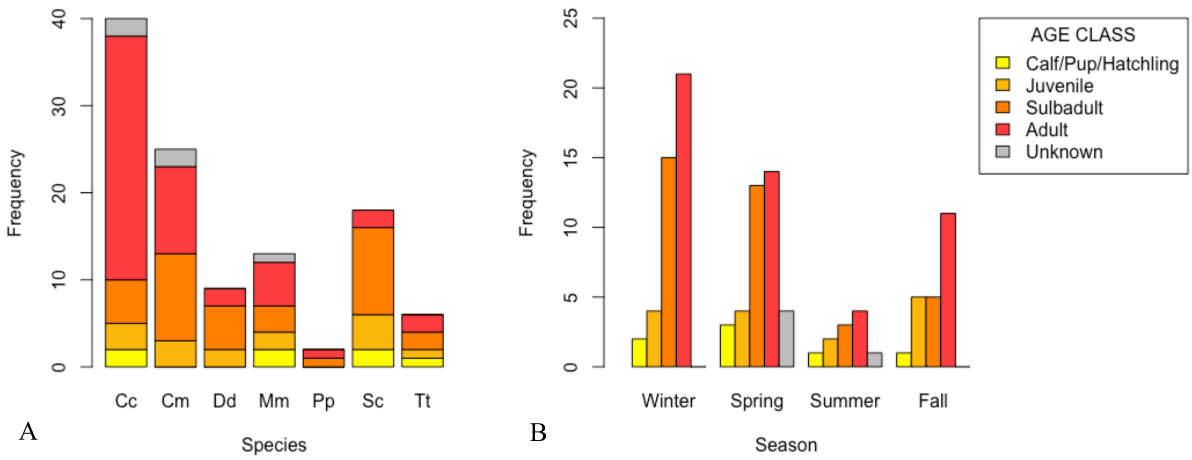


Figure 4.15. Stranding frequency based on the age class, according to the species (A) and the season (B).

In sea turtles, these results were affected by the high number of adult specimens of *C. caretta* found stranded, while stranding episodes of *C. mydas* involved both subadults and adults with the same incidence. On the contrary, the number of young stranded specimens was limited for both species. In summary, 28 adults (70%), 5 subadults (12.5%), 3 juveniles (7.5%), and 2 hatchlings (5%) were identified among stranded *C. caretta* specimens, and 10 adults (40%), 10 subadults (40%), and 3 juveniles (12%) among *C. mydas* individuals. The age class of 4 turtles could not be determined.

For loggerhead turtles, the total body length according to the age class ranged from 11-16 cm in hatchlings ($\bar{l}$ = 13.5 cm), 34-75 cm in juveniles ($\bar{l}$ = 49.3 cm), 44-94 cm in subadults ($\bar{l}$ = 72.6 cm), and 66-128 cm in adults ($\bar{l}$ = 92 cm). For green turtles, the total body length according to the age class ranged from 30-36 cm in juveniles ($\bar{l}$ = 33.0 cm), 38-59 cm in subadults ($\bar{l}$ = 46.9 cm), and 64-130 cm in adults ($\bar{l}$ = 95.7 cm).

Males showed bigger average dimensions than females: $\overline{l_M}$ = 99.6 cm for males and $\overline{l_F}$ = 90.3 cm for females of *C. caretta*, and $\overline{l_M}$ = 124 cm for males and $\overline{l_F}$ = 92.1 cm for females di *C. mydas*; which are generally justified considering that males present longer tails than females in sea turtles, according to sexual dimorphism.

Looking at the spatiotemporal pattern of loggerhead turtles, hatchlings were found in the hotspot southeast of Samos in winter and spring, corresponding to the area where most carcasses of all age classes were found. No subadults were found north of Samos. Additionally, juveniles did not strand in Lipsi, while older individuals did. Green turtle carcasses were found in the hotspot southeast of Samos as well, except for some subadults that were also stranded south of Lipsi and in other regions of Samos (Figure 4.16 – A).

In contrast, there were no significant differences in seasonality among age classes, but both species had a prevalence of adult and subadult females stranded during the cold months.

The presence of hatchlings in southeast Samos suggests that this part of the coast is a nesting area for sea turtles. However, the frequent strandings indicate that it presents many potential threats for sea turtles and marine mammals (Pietroluongo et al., 2021).

Evidence of anthropogenic interaction was found among the different age classes in green turtles, while it was detected only in adult specimens for loggerhead turtles.

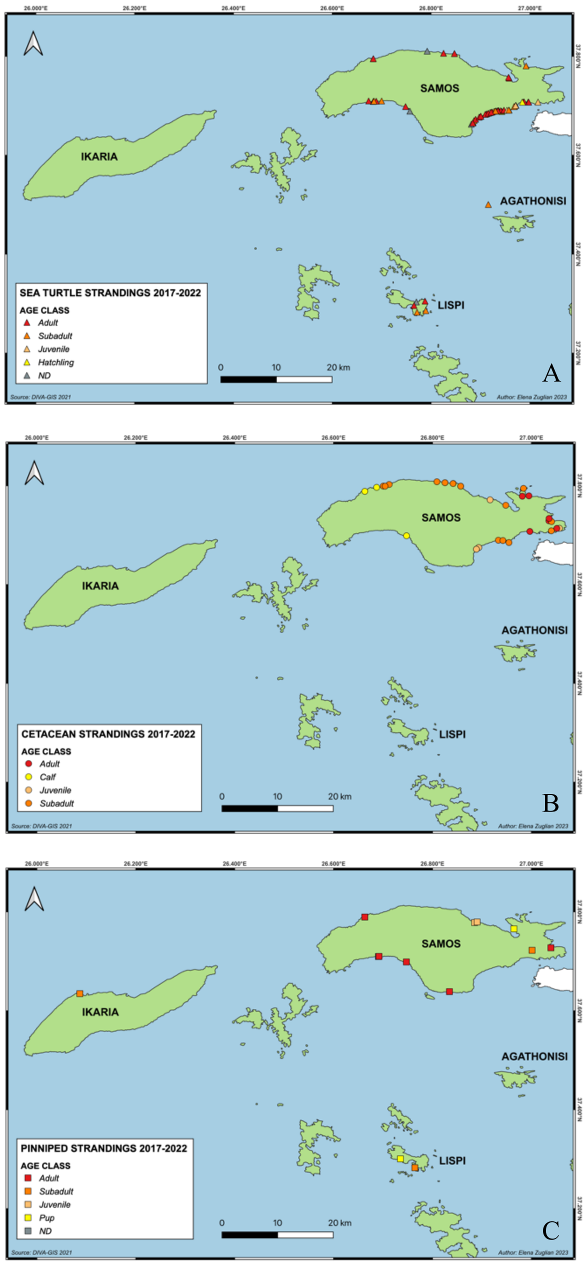


Figure 4.16. Spatial distribution of stranding events according to the age class. Maps generated using QGIS 3.28.0.

In all four species of cetaceans, subadults were the most commonly stranded individuals, although bottlenose dolphins and harbor porpoises presented as many stranded adults. In contrast, the less-represented age classes were different.

Regarding *S. coeruleoalba*, stranded animals belonged to different age classes as follows: 2 calves (11%), 4 juveniles (22%), 10 subadults (56%), and 2 adults (11%). The total body length ranged from 105-123 cm in calves ($\bar{l}$ = 114 cm), 131-163 cm in juveniles ($\bar{l}$ = 144 cm), 175-201 cm in subadults ($\bar{l}$ = 188.1 cm), and 225 cm was the body length of one of the adults. The carcasses of *Delphius delphis* showed the following subdivision based on the age: 2 juveniles (22%), 5 subadults (56%), and 2 adults (22%). Dimensions ranged from 133-135 cm in juveniles ($\bar{l}$ =134 cm), from 152-198 cm in subadults ($\bar{l}$ =167.6 cm), and 208-215 cm in adults ($\bar{l}$ = 211.5 cm). *T. truncatus* strandings involved 1 calf (17%), 1 juvenile (17%), 2 subadults (33%), and 2 adults (33%). The body lengths of the calf and the juvenile were 128 cm and 180 cm, respectively; the body lengths of the subadults were 197 cm and 201 cm ($\bar{l}$ = 199 cm), while the body length of one of the adults was 280 cm. Finally, *P. phocoena* specimens were 1 subadult and 1 adult, and the body length of the subadult was 125 cm. The total length of 4 individuals was not recorded.

Most *S. coeruleoalba* strandings occurred during spring, especially for subadults and adults. Many subadults were females and they were involved in strandings also during winter. Furthermore, many of these strandings presented evidence of anthropogenic interaction, one of which was a mass stranding that also engaged a juvenile and that was probably related to bad climatic conditions as well. The carcasses of calves were located in the northwest of Samos, which represents the region closest to the location where striped dolphin pods are generally sighted. Subadults were generally stranded north too, apart from the individuals involved in the mass stranding that were found southeast. Juveniles and adults were stranded in areas not usually exploited by this species. This spatial distribution suggests that stranding episodes involving calves probably occurred following mother separation, while those involving the other age groups happened while animals were foraging or as a result of disorientation and other causes (Figure 4.16 – B).

Even strandings of subadults and adults of *D. delphis* occurred mainly in winter and spring, while those of juveniles occurred in fall. During spring, the majority of stranded individuals were subadult males, one of which exhibited evidence of interaction with fisheries. The only female subadult stranded in winter. This seasonal pattern supports the hypothesis previously mentioned, and the strandings of juveniles could have occurred following the weaning phase, as post-weaning mortality is very common in marine mammals (IKsseldijk et al., 2020).

According to the spatial distribution, strandings occurred on the east coasts, with juveniles located southeast, subadults southeast and northeast, and adults east (Figure 4.16 – B).

T. truncatus strandings involved female subadults and adults, and younger male individuals. No calf or juvenile was found in winter, as already explained. The calf and the adult specimen that were stranded in spring presented evidence of human interaction. The calf was stranded southwest of Samos, and the subadults were stranded southeast, where bottlenose dolphins are usually sighted and where major threats seem to concentrate, while the juvenile and the adults were north and northeast (Figure 4.16 – B).

P. phocoena male specimens found stranded east of Samos were an adult and a subadult, but the limited data regarding this species make it difficult to identify evidence correlated to the strandings (Figure 4.16 – B).

M. monachus strandings involved 5 adult specimens (38%), 3 subadults (23%), 2 juveniles (15%), and 2 pups (15%). The dimensions according to the age class were 87 cm for one of the pups, 114 cm and 129.3 cm for the juveniles ($\bar{l}$ = 121.7 cm), 180-198 cm in subadults ($\bar{l}$ = 186 cm), and 205-260 cm in adults ($\bar{l}$ = 237.8 cm). The age class of 1 individual was not determined, nor was the total body length of 3 seals.

No adult specimens were found stranded in Lipsi and Ikaria from 2017 to 2022, whereas the strandings in Samos involved individuals of all age groups. Looking at the spatial distribution according to the age class and sex, we notice that the pup, the juveniles, the female subadult and one of the adult females were stranded along the eastern coasts of Samos. In contrast, the carcasses of the adult males were located south and northwest of the island. The other adult female was found southwest during spring (Figure 4.16 – C). Spring was also the season during which the highest number of strandings was recorded for this species, especially for older individuals. Fall was another season when more strandings involving adults occurred, while strandings of younger specimens were spread out across the different seasons.

The spatiotemporal pattern suggests a different seasonal use of Samos coastal waters (Pietroluongo et al., 2022), probably related to the territoriality of the species and reproduction. Evidence of anthropogenic impacts was found in adult specimens during spring, both related to deliberate killing.

4.3.3. DCC

According to the Decomposition Condition Code (DCC), 13% of the stranded individuals were found in extremely fresh conditions (n = 15), 18% of the carcasses were fresh (n = 20), 32% were in a status of moderate decomposition (n = 36), 29% were in advanced decomposition (n = 33), and the 8% were just mummified or skeletal remains (n = 9).

The carcass decomposition represents a limiting factor, as it is one of the major obstacles in post-mortem examinations, both for the collection of data related to biological characteristics and for the detection of evidence related to the cause of death. In fact, in the dataset we can see how the higher the DCC, the more data are missing.

Suppose the carcass is not in good condition. In that case, it is difficult to measure the animal, assess the sex through an external or internal examination, depending on the carcass status, assess the species, especially in cetaceans, and estimate the age and the nutritional status of the animal. Moreover, if normally it is difficult to assess the cause of death of an individual, collecting information needed to detect possible factors that contributed to the stranding event becomes more challenging if the carcass is in moderate-advanced decomposition. One reason is that it is harder to determine if specific evidence is related to an antemortem or post-mortem event. Another cause is that many post-mortem analyses can only be performed if the carcass is in fresh conditions due to physiological and biochemical factors that undergo changes in time, and the reliability of the results.

4.4. Human-interaction evidence

Physical evidence associated with human interaction was recorded in 17% of the strandings (n = 19), and it was mainly related to fishing activities and pollution.

41% of stranded animals (n = 46) did not present evidence of anthropogenic impact. Instead, human interactions remain unknown in the remaining 32% of cases due to the DCC and other reasons related to the inability to recover the carcass, to perform exhaustive post-mortem examinations, or because evidence is indirect, as for malnutrition.

Furthermore, it is important to specify that it was not possible to assess the cause of death, but only the presence of anthropogenic interaction. This is because the analyses performed were insufficient for this purpose; many times, it was not possible to determine if some findings occurred ante-mortem or post-mortem, and the expertise of the interns, as well as the resources of the NGO, were not sufficient to determine the cause of specific evidence, or there was not enough evidence for this purpose. Moreover, stranding events often result from the interaction of multiple factors rather than a single cause.

Based on this premise, it is possible to notice how the DCC of the individuals showing evidence of human interactions was 1 for four carcasses, 2 for six carcasses, 3 for eight carcasses, and 4 for one carcass. No evidence was determined or found when the carcass presented a code 5 or 4, except for one exception.

Collected human-related findings were the presence of marine litter, bullets, fishing nets, lines, and other gear such as hooks; net marks and linear signs on the body, traumas, fractures penetrating wounds due to gunshot, and missing limbs (Table 4.5).

	Category	Species	Date	Month	Year	Season	Weather	Sea State	Island	Latitude (N)	Longitude (E)	DCC	Sex	Age Class	Anthropogenic impact evidence	Description of anthropogenic impact evidence	Total length
6	Pinniped	*Monachus monachus*	20-05-2017	May	2017	Spring	3	3	Samos	37.709820	26.691860	2	F	Adult	Y	Bullets	229.0
10	Cetacean	*Tursiops truncatus*	21-08-2017	Aug	2017	Summer	4	4	Samos	37.698682	26.747563	2	M	Calf	Y	Head trauma	128.0
12	Sea Turtle	*Chelonia mydas*	20-09-2017	Sep	2017	Summer	2	1	Samos	37.686844	26.921569	1	F	Adult	Y	Fishing line + intestine intussusception	105.0
14	Sea Turtle	*Caretta caretta*	09-11-2017	Nov	2017	Fall	2	1	Agathonisi	37.500340	26.915860	3	F	Subadult	Y	Plastic in the stomach and intestine + 2 missed flipper	44.0
27	Sea Turtle	*Chelonia mydas*	28-02-2018	Feb	2018	Winter	4	4	Samos	37.686210	26.919326	1	ND	Juvenile	Y	Plastic bag entaglement	30.0
51	Cetacean	*Stenella coeruleoalba*	01-03-2019	Mar	2019	Winter	3	3	Samos	37.808099	26.808970	1	F	Subadult	Y	Fractured upper jaw	196.0
58	Pinniped	*Monachus monachus*	17-04-2019	Apr	2019	Spring	4	3	Samos	37.789420	26.663180	3	M	Adult	Y	Bullets	260.0
59	Cetacean	*Tursiops truncatus*	22-04-2019	Apr	2019	Spring	3	4	Samos	37.778782	26.981759	3	F	Adult	Y	Fishing gear + 2 hooks	280.0
74	Cetacean	*Stenella coeruleoalba*	09-05-2020	May	2020	Spring	2	4	Samos	37.480560	26. 42334	3	ND	Subadult	Y	Gunshot/penetrating wound	177.0
75	Cetacean	*Stenella coeruleoalba*	18-05-2020	May	2020	Spring	3	2	Samos	37.713581	27.051608	3	M	Adult	Y	Gunshot/penetrating wound	225.0
79	Cetacean	*Stenella coeruleoalba*	31-05-2020	May	2020	Spring	2	1	Samos	37.794660	26.984200	3	F	Subadult	Y	Net marks/Linear signs	183.0
82	Sea Turtle	*Caretta caretta*	09-07-2020	Jul	2020	Summer	4	4	Samos	37.662796	26.881472	4	ND	ND	Y	Fishing net	ND
96	Sea Turtle	*Caretta caretta*	29-11-2021	Nov	2021	Fall	4	3	Samos	37.689265	26.930438	2	F	Subadult	Y	Fishing net	85.0
100	Cetacean	*Stenella coeruleoalba*	22-01-2022	Jan	2022	Winter	8	6	Samos	37.671565	26.888831	2	F	Subadult	Y	Fishing gear	175.0
101	Cetacean	*Stenella coeruleoalba*	22-01-2022	Jan	2022	Winter	8	6	Samos	37.671565	26.888831	1	F	Subadult	Y	Fishing gear	186.0
102	Cetacean	*Stenella coeruleoalba*	22-01-2022	Jan	2022	Winter	8	6	Samos	37.671565	26.888831	2	M	Juvenile	Y	Fishing gear	131.0
106	Cetacean	*Delphinus delphis*	02-04-2022	Apr	2022	Spring	2	2	Samos	37.804520	26.841730	3	M	Subadult	Y	Gunshots/Penetrating wounds	198.0
108	Sea Turtle	*Caretta caretta*	27-05-2022	May	2022	Spring	1	1	Samos	37.665190	26.884340	3	F	Adult	Y	Plastic in the mouth	90.0
110	Sea Turtle	*Chelonia mydas*	22-11-2022	Nov	2022	Fall	0	1	Samos	37.691020	26.957470	2	F	Subadult	Y	Fishing net	43.0

Table 4.5. Database reporting the specimens presenting anthropogenic impact evidence.

All these were related to fishing activities because of accidental bycatch or deliberate killing, boat collision episodes, and plastic pollution. The evidence of bullets and gunshots could also be due to episodes unrelated to fishing activities, and traumas, fractures, and missing limbs could result from several factors.

Plastic pollution is a ubiquitous issue for marine animals, including herbivorous species like *C. mydas*, as they often consume contaminated prey, or accidentally ingest plastic in its different forms due to it being traded for food. This trophic transfer of plastic can have harmful effects on the health and survival of these animals because it can lead to problems in resource acquisition, malnutrition, reproduction, intoxication, and the development of pathologies (Pietroluongo et al., 2022). The same is true for plastic entanglement. Furthermore, interaction with fisheries is a major issue for sea turtles and marine mammals in the Mediterranean Sea (McGovern et al., 2016; Tomás et al., 2008), especially with longline fishery.

In this study, anthropic activity interaction affected sea turtles, cetaceans, and pinnipeds to different extents. Evidence was detected in carcasses stranded along the coasts of Samos and in a carcass of *C. caretta* floating in the proximity of Agathonisi. The seasons when most evidence was found were spring and winter, while the region where it focused the most was southeast of Samos, although signs of interactions with human activities were found all around the island during the primary months (Figure 4.17).

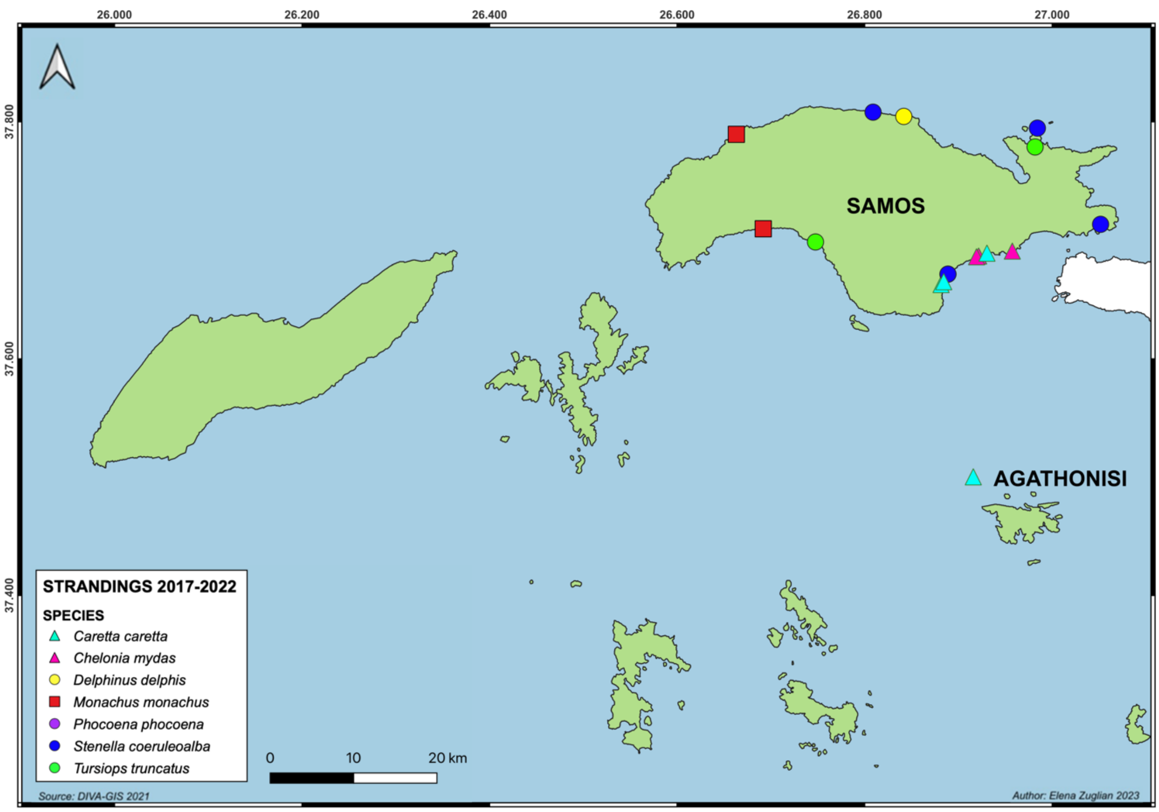


Figure 4.17. Distribution of stranded individuals presenting human-interaction evidence. Map generated using QGIS 3.28.0.

Among sea turtles, 4 *C. caretta* and 3 *C. mydas*, the majority of which were female adults and subadults, showed evidence of anthropogenic impact related to bycatch (cases 12; 82; 96; 110), plastic ingestion (cases 14; 108), and entanglement (case 14; 27). These results agree with Tomás et al. (2008), according to whom longline bycatch is more frequent in adult-sized turtles. Moreover, when the turtles ingest the fishing lines, these can lead to intussusception and other gut pathologies that, in most cases, result in death (Tomás et al., 2008). The dispersion of stranding episodes related to bycatch is explained by the fact that older turtles feed mainly in neritic areas, where small-scale fisheries operate (Belmahi et al., 2020). Bycatch and marine litter can also result in entanglement and subsequent chronic physical injuries, such as amputation of limbs.

The only 2 seals presenting signs of human interaction were adults; 1 female presenting bullets in the ventral region of the body (case 6) and 1 male presenting bullets in the head (case 58). The carcasses were located along the northwest and southwest coasts of Samos during spring. Even though the motive behind these episodes of deliberate killing remains unknown, there is reason to believe that it was related to the exploitation of resources, as a growing hostility towards Mediterranean monk seals is publicly recognized in Samos.

This antagonism is also well documented between fishermen and dolphins in Greece (Miliou et al., 2018) due to the depredation of nets and the overall decrease of common prey stocks. Therefore, it is not surprising that cetaceans were the taxonomic group presenting most evidence. The stranding of 7 specimens of *S. coeruleoalba,* 2 of *T. truncatus* and 1 of *D. delphis* seemed to be correlated to human activities, and the carcasses were found all around Samos in spring, winter, and summer. The specimen that was stranded in summer was a male bottlenose dolphin calf, presenting head trauma probably caused by a ship strike (case 10). The other specimen of *T. truncatus* was an adult female that stranded in spring, showing evidence of bycatch (case 59). A male subadult of *D. delphis* was found north of Samos during spring, presenting several penetrating wounds, supposedly caused by gunshots (case 106). The same findings were noticed in 1 adult male and 1 subadult of *S. coeruleoalba* (cases 74; 75)*,* always during spring. These events could be explained by the more aggressive feeding behavior that usually characterizes male specimens (Miliou et al., 2018). Furthermore, bottlenose and short-beaked common dolphins are regularly sighted while plunding fishing gear and trawlers in the Aegean Sea, especially solitary ones that are probably males.

Female striped dolphin subadults that stranded during winter and spring showed evidence of interaction with fisheries, presumably due to bycatch (cases 79; 100; 101), and 1 had a fractured upper jaw (case 51). Specifically, 2 of these were part of a mass stranding that also included a male juvenile (cases 100; 101; 102).

4.5. Conclusions

The number of stranded animals recorded in this study represents a minimum measure of real at-sea mortality. Furthermore, stranding data are inherently biased due to the physical, social, and biological processes from which stranding events result. A change in strandings, indeed, could reflect a variation in abundance and mortality, or it could be a function of environmental sea and climate conditions, observer effort, or a combination of these.

At the same time, tracking stranding episodes helps gain knowledge about species ecology and anthropogenic interactions, which are fundamental for deciding species management and conservation.

For this purpose, the redaction of an efficient stranding network based on standardized guidelines should be implemented in Greece, which could also be compared to stranding data from other parts of the Mediterranean Sea to enable a larger-scale analysis of the health of marine megafauna populations. To do so, a constant research effort over designated areas is required. A Generalized Linear Mixed-Effects Model (GLMM), or any other analysis that allows to estimate the probability of stranding occurrence in a specific place and time of the year starting from already identified stranding hotspots, is a valuable tool that could help in the enforcement of stranding response teams and monitoring surveys. Furthermore, identifying the major anthropogenic threats for each species in the area would contribute to applying preventive mitigation measures to protect and conserve the marine ecosystem.

5. Bibliography

Bearzi, G.; Bonizzoni, S. & Santostasi, N.L. *Stenella coeruleoalba* (Gulf of Corinth subpopulation). *The IUCN Red List of Threatened Species* **2022**, e.T210188066A210188619.

Bearzi, G.; Fortuna, C.M. & Reeves, R.R. Ecology and conservation of common bottlenose dolphins *Tursiops truncatus* in the Mediterranean Sea. *Mammal Review* **2008**, *39*, 92-123.

Bearzi, G.; Genov, T.; Natoli, A.; Gonzalvo, J. & Pierce, G.J. *Delphinus delphis* (Inner Mediterranean subpopulation). *The IUCN Red List of Threatened Species* **2021**, e.T189865869A189865884.

Bearzi, G.; Reeves, R.R.; Notarbartolo-Di-Sciara, G.; Politi, E.; Cañadas, A.; Frantzis, A. & Mussi, B. Ecology, status and conservation of short-beaked common dolphins *Delphinus delphis* in the Mediterranean Sea. *Mammal Review* **2003**, *33,* 224–252.

Belmahi, A.E.; Belmahi, Y.; Benabdi, M.; Bouziani, A.L.; Darna, S.A.; Bouslah, Y.; Bendoula, M.; Bouderbala, M. First study of sea turtle strandings in Algeria (western Mediterranean) and associated threats: 2016-2017. *Herpetozoa* **2020**, *33*, 113-120.

Birkun Jr, A. & Frantzis, A. *Phocoena phocoena ssp. relicta*. *The IUCN Red List of Threatened Species* **2008,** e.T17030A6737111.

Braulik, G.; Minton, G.; Amano, M. & Bjørge, A. *Phocoena phocoena*. *The IUCN Red List of Threatened Species* **2020**, e.T17027A50369903.

Casale, P. *Caretta caretta* (Mediterranean subpopulation). *The IUCN Red List of Threatened Species* **2015**, e.T83644804A83646294.

Casale, P.; Affronte, M.; Insacco, G.; Freggi, D.; Vallini, C.; Pino D'Astore, P.; Basso, R.; Paolillo, G.; Abbate, G. & Argano, R. Sea turtle strandings reveal high anthropogenic mortality in Italian waters. *Aquatic Conserv: Mar. Freshw. Ecosyst.* **2010**, *20*, 611-620.

Casale, P.; Freggi, D.; Basso, R. & Argano, R. Size at Male Maturity, Sexing Methods and Adult Sex Ratio in Loggerhead Turtles (*Caretta caretta*) from Italian Waters Investigated Through Tail Measurements. Herpetological Journal **2005**, *15*, 145-148.

Casale, P.; Freggi, D. & Rocco, M. Mortality induced by drifting long-line hooks and branchlines in loggerhead sea turtles, estimated through observation in capitivity. *Aquatic Conserv: Mar. Freshw. Ecosyst*. **2008**, *18*, 945–54.

Casale, P. & Tucker, A.D. *Caretta caretta*. *The IUCN Red List of Threatened Species* **2017**, e. T3897A119333622.

Dendrinos, P.; Adamantopoulou, S.; Tounta, E.; Koemtzopoulos,K. ; Karamanlidis, A.A.; The Mediterranean Monk Seal (*Monachus monachus*) in the Aegean Sea. “The Living Myth of the Sirens”. **2022**

Fontaine, M.C. Harbour Porpoises, *Phocoena phocoena*, in the Mediterranean Sea and Adjacent Regions: Biogeographic Relicts of the Last Glacial Period. *Advances in Marine Biology* **2016**, *75*

Frantzis, A.; Alexiadou, P.; Paximadis, G.; Politi, E.; Gannier, A.; Corsini-Foka, M. Current knowledge of the cetacean fauna of the Greek Seas. *J. Cetacean res. Manage.* **2003***, 5(3)*: 219-232.

Geldiay, R.; Koray, T. & Balik, S. Status of the sea turtle population (*Caretta caretta* and *Chelonia mydas*) in the northern Mediterranean Sea, Turkey. *Biology and Conservation of Sea turtles* **1982**, *583*, 425-434.

Geraci, J.R., Lounsbury, V.J. & Yates, N. Marine Mammals Ashore: A Field Guide for Strandings. *National aquarium Baltimore* **2005.**

Hammond, P.S.; Bearzi, G.; Bjørge, A.; Forney, K.A.; Karkzmarski, L.; Kasuya, T.; Perrin, W.F.; Scott, M.D.; Wang, J.Y.; Wells, R.S. & Wilson, B. *Tursiops truncatus*. *The IUCN Red List of Threatened Species* **2012**, e.T22563A17347397.

IJsseldijk, L.L.; Brownlow, A.C. & Mazzariol, S. Best practice on cetacean post mortem investigation and tissue sampling. *ACCOBAMS-MOP 7* **2019**, 33.

IJsseldijk, L.L.; Doeschate, M.T.I.t.; Brownlow, A.; Davison, N.J.; Deaville, R.; Galatius, A.; Gilles, A.; Haelters, J.; Jepson, P.D.; Keijl, G.O.; Kinze, C.C.; Olsen, M.T.; Siebert, U.; Thøstesen, C.B.; Broek, J.v.d.; Gröne, A.; Heesterbeek, H. Spatiotemporal mortality and demographic trends in a small cetacean: Strandings to inform conservation management. *Biological Conservation* **2020***, vol. 249,* 108733.

Karamanlidis, A.A. & Dendrinos, P. *Monachus monachus. The IUCN Red List of Threatened Species* **2015**, e.T13653A117647375.

Karamanlidis, A.A.; Dendrinos, P.; De Larrinoa, P.F.; Gücü, A.C.; Johnson, W.M.; Kiraç, C.O.; Pires, R. The Mediterranean monk seal *Monachus monachus*: Status, biology, threats, and conservation priorities. *Mamm. Rev.* **2016**, *46*, 92–105.

Kasuya, T. Review of the biology and exploitation of striped dolphins in Japan. *J. Cetacean Res. Manage* **1999**, *1(1),* 81-100.

Margaritoulis, D. & Rees, A.F. Loggerhead Nesting Effort and Conservation Initiatives at the Monitored Beaches of Greece during 2002. *Marine Turtle Newsletter* **2003**, *102, 1-13.*

Mazzariol, S. Common best practices for a basic post-mortem examination of stranded cetaceans. *ACCOBAMS-MOP 6* **2016**, *Resolution 6.22*, Annex 2.

Mazzariol, S. & Centelleghe, C. Evidence-based diagnostic assessment frameworks for cetacean necropsies on specific issues/threats. *ACCOBAMS-MOP 7* **2019**, *Resolution 7.14,* Annex 15.

McGovern, B.; Culloch, R.M.; O'Connell, M.; Berrow, S. Temporal and spatial trends in stranding records of cetaceans on the Irish coast, 2002-2014. *Journal of the Marine Biological Association of the United Kingdom* **2016***,* 1-13.

Milani, C.B.; Vella, A.; Vidoris, P.; Christidis, A.; Koutrakis, E.; Frantzis, A.; Miliou, A. & Kallianiotis, A. Cetacean stranding and diet analyses in the North Aegean Sea (Greece). *Journal of the Marine Biological Association of the United Kingdom* **2017**, 1-18.

Miliou, A.; Akkaya Baş, A.; Pietroluongo, G. Interactions between marine mammals and fisheries: Case studies from the Eastern Aegean and the Levantine Sea. *CIESM Workshop Monograph "Engaging Marine Scientists and Fishers to Share Knowledge and Perceptions—Early Lessons",* **2018***, 50,* 119– 124.

Moore, K.T. & Barco, S.G. Handbook for recognizing, evaluating, and documenting human interaction in stranded cetaceans and pinnipeds. *NOAA Technical Memorandum NMFS-SWFSC* **2013**, 510.

Moore, M.J.; Van der Hoop, J.; Barco, S.G.; Costidis, A.M.; Gulland, F.M.; Jepson, P.D.; Moore, K.T.; Raverty, S. & McLellan, W.A. Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma. *Dis. Aquat. Organ.* **2013**, *103*, 229–264.

Norman, S.A.; Bowlby, C.E.; Brancato, M.S.; Calambokidis, J.; Duffield, D.; Gearin, P.J.; Gornall, T.A.; Gosho, M.E.; Hanson, B.; Hodder, J.; Jeffries, S.J.; Lagerquist, B.; Lambourn, D.M.; Mate, B.; Norberg, B.; Osborne, R.W.; Rash, J.A.; Riemer, S. & Scordino, J. Cetacean strandings in Oregon and Washington between 1930 and 2002. *J. Cetacean Res. Manage* **2004**, *6(1)*, 87–99.

Pardalou, A. & Tsikliras, AC. Factors influencing dolphin depredation in coastal fisheries of the northern Aegean Sea: Implications on defining mitigation measures. *Mar Mam Sci.* **2020**, *36,* 1126–1149.

Pietroluongo, G.; Cipriano, G.; Ashok, K.; Antichi, S.; Carlier, H.; Miliou, A.; Maglietta, R.; Fanizza, C. & Carlucci, R. Density and Abundance of *Delphinus delphis* in Waters South of Samos Island, Greece (Eastern Mediterranean Sea). *J. Mar. Sci. Eng.* **2020**, *8*, 218.

Pietroluongo, G.; Corazzola, G.; Centelleghe, C.; Mazzariol, S. Diagnostic framework for the assessment of fishery interaction in stranded marine mammals. *LIFE DELFI – Dolphin Experience: Lowering Fishing Interactions LIFE18 NAT/IT/000942* **2021**, Action A3.

Pietroluongo, G.; Miliou, A.; Antichi, S.; Ashok, K.; Falquina, F. J. & Montalvo, B. Q. M. The role of citizen reports in turtle nests discovery: the case of Samos Island, Greece. *International Workshop on Metrology for the Sea; Learning to Measure Sea Health Parameters (MetroSea)* **2021**, 153-157.

Pietroluongo, G.; Quintana Martín-Montalvo, B.; Antichi, S.; Miliou, A.; Costa, V. First Assessment of Micro-Litter Ingested by Dolphins, Sea Turtles and Monk Seals Found Stranded along the Coasts of Samos Island, Greece. *Animals* **2022**, *12*, 3499.

Pietroluongo, G.; Quintana Martin-Montalvo, B.; Ashok, K.; Miliou, A.; Fosberry, J.; Antichi, S.; Moscatelli, S.; Tsimpidis, T.; Carlucci, R.; Azzolin, M. Combining Monitoring Approaches as a Tool to Assess the Occurrence of the Mediterranean Monk Seal in Samos Island, Greece. *Hydrobiology* **2022**, *1*, 440-450.

Pikesley, S.K.; Witt, M.J.; Hardy, T.; Loveridge, J.; Loveridge, J.; Williams, R.; Godley, B. J. Cetacean sightings and strandings: evidence for spatial and temporal trends? *Journal of the Marine Biological Association of the United Kingdom* **2012***, 92(8)*, 1809-1820.

Rosel, P.E.; Frantzis, A.; Lockyer, C. & Komnenou, A. Source of Aegean Sea harbour porpoises. *Mar. Ecol. Prog. Ser.* **2003**, *247*, 257-261.

Saintignan, S.; Costantino, M.; Milou, A.; Moscatelli, S.; Pietroluongo, G.; Azzolin, M. Habitat use of *Delphinus delphis* (Linnaeus, 1758) in the southern waters of Samos island (Aegean Sea, Greece). *2020 IMEKO TC-19 International Workshopon Metrology for the Sea* **2020**, Naples, Italy

Seminoff, J.A. *Chelonia mydas*. *The IUCN Red List of Threatened Species* **2004**, e.T4615A11037468.

Soukissian, T.; Papadopoulos, A.; Skrimizeas, P.; Karathanasi, F.; Axaopoulos, P.; Avgoustoglou, E.; Kyriakidou, H.; Tsalis, C.; Voudouri, A.; Gofa, F. & Katsafados, P. Assessment of offshore wind power potential in the Aegean and Ionian Seas based on high-resolution hindcast model results. *AIMS Energy* **2017**, *5(2),* 268-289.

Tomás, J.; Gozalbes, P.; Raga, J.A. & Godley, B.J. Bycatch of loggerhead turtles: insights from 14 years stranding data. *Endang Species Res* **2008**, *5*, 161-169.

Truchon M-H.; Measures, L.; L'Hérault, V.; Brêthes, J-C.; Galbraith, P.S.; Harvey, M; Lessard, S.; Starr, M.; Lecomte, N. Marine Mammal Strandings and Environmental Changes: A 15-Year Study in the St. Lawrence Ecosystem. *PLoS ONE* **2013***, 8(3):* e59311.

6. Annexes

Annex I. Sea Turtle Stranding Report. *Archipelagos Institute of Marine Conservation.*

Annex II. Cetacean Stranding Report. *Archipelagos Institute of Marine Conservation.*

Annex III. Pinniped Stranding Report. *Archipelagos Institute of Marine Conservation.*

Annex IV. Evidence-based diagnostic assessment frameworks for cetacean necropsies on specific issues/threats. *ACCOBAMS-MOP7/2019/Doc38/Annex15/Res.7.14.*

Annex V. Diagnostic framework for the assessment of fishery interaction in stranded marine mammals. *LIFE DELFI/2021/ActionA3/Tier3.*

Annex VI. Common best practices for a basic post-mortem examination of stranded cetaceans. *ACCOBAMS-MOP6/2016/Res.6.22/Annex 2/Appendix II.2-II.3.*

SEA TURTLE STRANDING REPORT

Stranding number (AIMCYY_##Sp): ____

Examiners names: ____

Report type: ☐ Dead Stranding ☐ Alive Stranding

Confidence code: ☐ Unconfirmed public report ☐ Confirmed Public Report

Species: ___________________________

INITIAL OBSERVATION

Date: Year: __________Month: ______Day: ______

LOCATION:

Name of Beach: ___________________________

Beach topography: ___________________________

Lat: ___________________________________

Long: __________________________________

Nearest village & Distance: ________________

Sea state: ______

Weather: _____________________________

Stranding time/ death time: _________________

Cause of death:

Deliberate kill

Natural

Bycatch

Unknown

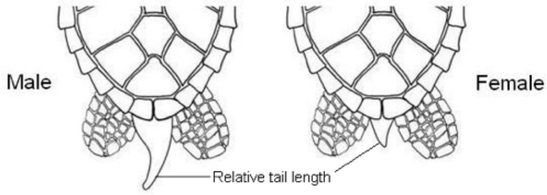
Other: ___________________________

Photographs taken (tick if yes):

☐ Location ☐ Parasites ☐ Position relative to shore ☐ Any fishing gear present Damage, scars, lesions. ☐ Throughout first aid

Photos taken by:_________________________

GENERAL EXAMINATION



Sex of animal:

☐ Female ☐ Male

Condition at Examination:

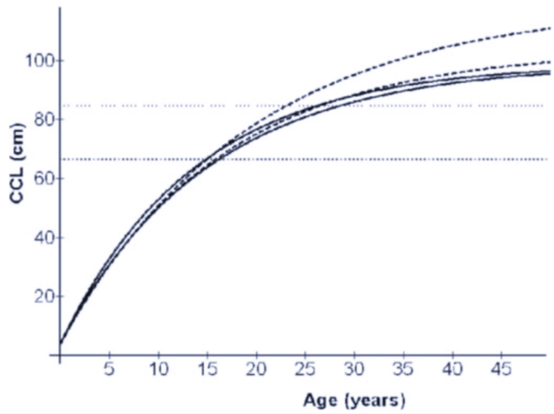
☐ 1. Alive ☐ 4. Advanced decomposition

☐ 2. Fresh dead ☐ 5. Severed decomposition

☐ 3. Moderate decomposition

Age class:

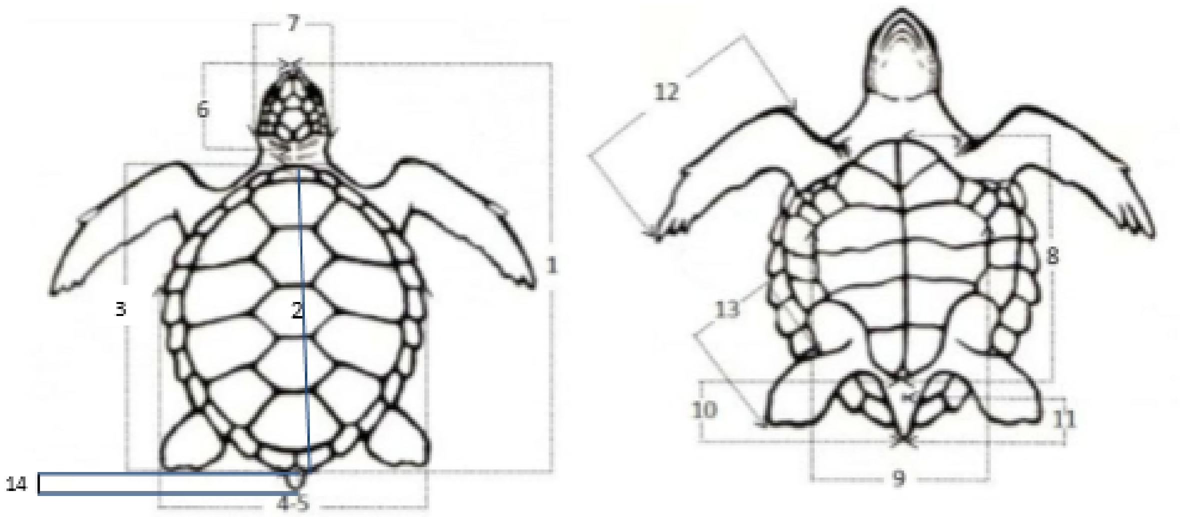
☐ Adult ☐ Subadult ☐ Calf ☐ Unknown



IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

MORPHOMETRICS (cm):

If live, only take measurements once the animal is calm and first aid has been administered. Take as many measurements as possible without disturbing the animal. Hold the tape at a distance and do not touch.



- ☐ 1.**TL** Total length ____
- ☐ 2. **CCL** curved carapace length notch to tip__
- ☐ 3. **SCL** straight-line carapace length ___
- ☐ 4. **CCW** curved carapace width ___
- ☐ 5. **SCW** straight-line carapace width ___
- ☐ 6. **HL** head length ___
- ☐ 7. **HW** Head width__
- ☐ 8. **PL** plastron length ___
- ☐ 9. **PW** plastron width ___
- ☐ 10. **TTL** total tail length ___
- ☐ 11. **VTL** vent-tip length__
- ☐ 12. **FoL** foreleg length___
- ☐ 13. **HiL** (hindleg length) ___
- ☐ 14. **CaTT**(tip of carapace-tip of tail)__

LIVE STRANDING	**DEAD STRANDING:**
Number of Animals: ☐ Single ☐ a nest _______ **Animal Condition:** ☐ Healthy ☐ Malnourished ☐ Sick ☐ Wounds/Lesions ☐ Injured ☐ Fishing gear present **FIRST AID:** **Breathing Rate:** ☐Normal ☐Accelerated ☐ Reduced/minimal **Reflexes:** The blowhole should close when touched ☐ Yes ☐ No **Heart Rate:** ☐Normal ☐ Accelerated ☐ Reduced/Minimal	**CARCASS STATUS:** ☐ 1. Alive or just dead. ☐ 2. Fresh carcass (<24h) ☐ 3. Moderate Decompositions ☐ 4. Advanced Decomposition ☐ 5. Mummified or partial carcass **NUTRITIONAL CONDITION:** ☐ 1. Emaciated ☐ 3. Normal ☐ 2. Thin ☐ 4. Robust (fat) **EXTERNAL EXAM (Tick if Yes):** ☐ Signs of fishing net or lines: ________________________________ ☐ Penetrating wounds (gunshots/puncture wounds): ________________________________ ☐ Mutilations (cuts/cracks in the body wall/ missing appendages): ______________________ ☐ Post-mortem damage from scavengers and opportunists: ______________________ ☐ Parasites and foreign bodies _____________

TICK IF ADMINISTRATED:

☐ Animal supported in an upright position

☐ Covered with wet sheets/towels/seaweed

☐ Sea turtle and sheets continually covered with water-sea turtle remained wet at all times

☐ Path to sea cleared of debris

☐ Eyes flushed with water

☐ Cold/Windy weather: windbreak constructed around the animal

☐ Sunny weather: shade provided by constructing a tarpaulin over the cetacean

☐ Fishing line or nets cut away and disentangled

☐ Back end of turtle raised by 30° to drain the lungs (to be done only if inactive). This can be done by making a small pile of sand beneath it.

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

NEXT STEPS:

- ☐ Left on beach, no further action required
- ☐ Left on beach, will monitor for next 24-48 hrs
- ☐ Taken back to base, will send to rescue center

If the animal is in a stable and healthy condition, attempt to refloat it with the rising tide. Only release the animal after acclimatisation: the animal can lift its head and breath on its own. If the animal beaches again, continue rocking and try again an hour later. If the animal beaches again it is probably diseased and a vet needs to be called.

NOTES:

Name of Reporter: ____

Contact number: ____

Time reported: ____

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

CETACEAN STRANDING REPORT

Stranding number (AIMCYY_##Sp): ______________________________________

Examiners names: ____

Report type: ☐ Dead Stranding ☐ Alive Stranding

Confidence code: ☐ Unconfirmed public report ☐ Confirmed Public Report

Species: _________________________

INITIAL OBSERVATION

Date: Year: __________Month: ______Day: ______

LOCATION:

Name of Beach: ____________________________

Beach topography: __________________________

Lat: ____

Long: ____

Nearest village & Distance: ________________

Sea state: ______

Weather: ____________________________________

Stranding time/ death time: _________________

Cause of death:

Deliberate kill

Natural

Bycatch

Unknown

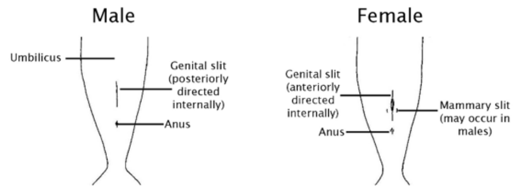
Other: ______________________________

Photographs taken (tick if yes):

☐ Location ☐ Parasites ☐ Position relative to shore ☐ Any fishing gear present Damage, scars, lesions. ☐ Throughout first aid

Photos taken by:____________________________

GENERAL EXAMINATION



Sex of animal:

☐ Female ☐ Male

Condition at Examination:

☐ 1. Alive ☐ 4. Advanced decomposition

☐ 2. Fresh dead ☐ 5. Severed decomposition

☐ 3. Moderate decomposition

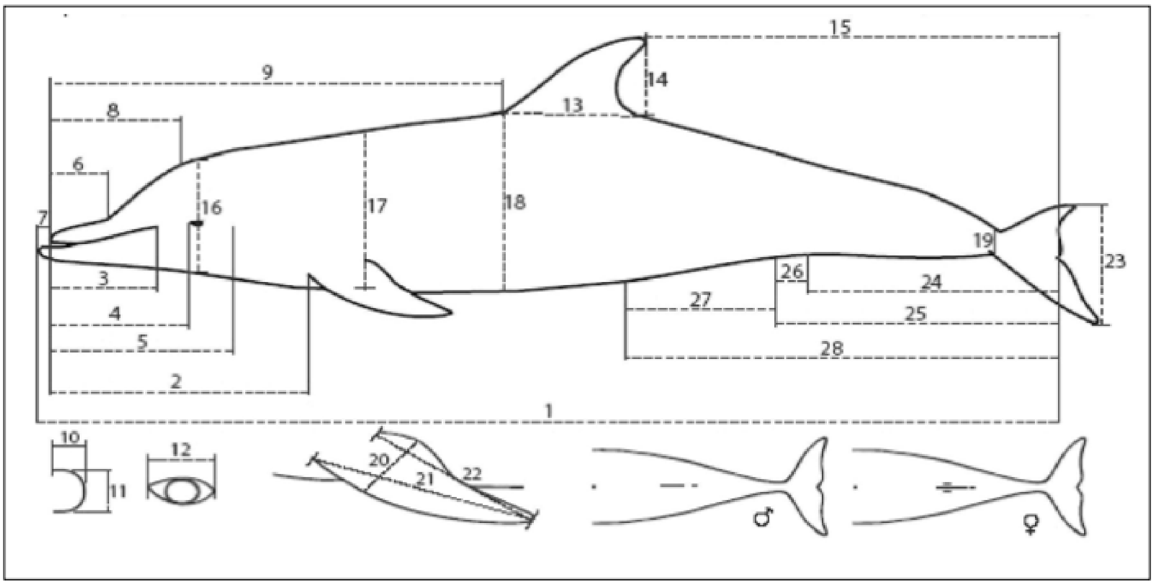
Age class:

☐ Adult ☐ Subadult ☐ Calf ☐ Unknown

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

MORPHOMETRICS (cm):

If live, only take measurements once the animal is calm and first aid has been administered. Take as many measurements as possible without disturbing the animal. Hold the tape at a distance and do not touch.



- ☐ 1.Total length, snout to notch____
- ☐ 2. Snout to flipper (left and right) __
- ☐ 3. Length of gape (mouth) (left and right) ___
- ☐ 4. Snout to centre of eye (left and right) ___
- ☐ 5. Snout to ear (left and right) ___
- ☐ 6. Length of rostrum ___
- ☐ 7. Front end of mandibula__
- ☐ 8. Snout to centre of blowhole___
- ☐ 9. Snout to tip of dorsal fin___
- ☐ 10. Blowhole width ___
- ☐ 11. Blowhole length___
- ☐ 12. Eyes width___
- ☐ 13. Length of dorsal fin base__
- ☐ 14. Height of dorsal fin__
- ☐ 15. Dorsal fin to end of fluke__
- ☐ 16. Girth at eye__
- ☐ 17. Girth at axilla__
- ☐ 18. Girth at leading edge of dorsal fin___
- ☐ 19. Circumference of caudal peduncal___
- ☐ 20. Maximum width of flipper (left and right) __
- ☐ 21. Flipper length (ant) (left and right) ___
- ☐ 22. Flipper length (post) (left and right) ___
- ☐ 23. Width of fluke___
- ☐ 24. End of genital slit to the end of fluke___
- ☐ 25. Beginning of genital slit to end of fluke__
- ☐ 26. Length of genital slit____

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

LIVE STRANDING	DEAD STRANDING:
Number of Animals: ☐ Single ☐ Mass ________ **Animal Condition:** ☐ Healthy ☐ Malnourished ☐ Sick ☐ Wounds/Lesions ☐ Injured ☐ Fishing gear present **FIRST AID:** **Breathing Rate:** *Normal:* *Small cetaceans (e.g. common dolphin) = 2-5 breaths/min* *Medium cetaceans (e.g. pilot whale) = 1 breath/min* *Large cetaceans (e.g. sperm whale) = up to 1 breath/20mins* ☐ Normal ☐ Accelerated ☐ Reduced/minimal **Reflexes:** The blowhole should close when touched ☐ Yes ☐ No **Heart Rate:** *Normal: 30-100 bpm dependent on size. A firm hand placed under a front flipper should be sufficient.* ☐ Normal ☐ Accelerated ☐ Reduced/Minimal	**CARCASS STATUS:** ☐ 1. Alive or just dead. ☐ 2. Fresh carcass (<24h) ☐ 3. Moderate Decompositions ☐ 4. Advanced Decomposition ☐ 5. Mummified or partial carcass **NUTRITIONAL CONDITION:** ☐ 1. Emaciated ☐ 3. Normal ☐ 2. Thin ☐ 4. Robust (fat) **EXTERNAL EXAM (Tick if Yes):** ☐ Signs of fishing net or lines (head, pectoral fin, caudal peduncle, dorsal fin, pectoral fin right: ____ ☐ Penetrating wounds (gunshots/puncture wounds): ____ ☐ Mutilations (cuts/cracks in the body wall/ missing appendages): ____________________________ ☐ Post-mortem damage from scavengers and opportunists: ____________________________ ☐ Parasites and foreign bodies ________________

TICK IF ADMINISTRATED:

☐ Animal supported in an upright position

☐ Trenches dug under the pectoral fins (flippers)

☐ Covered with wet sheets or towels

☐ Rock the animal gently from side to side to maintain the blood flow and reduce muscle stiffness

☐ Blowhole is kept free of water and debris

☐ Eyes flushed with water

☐ Cold/Windy weather: Dorsal fin and tail fluke covered with cloths soaked in vegetable or mineral oil to reduce heat loss.

☐ Cold/Windy weather: windbreak constructed around the animal

☐ Sunny weather: shade provided by constructing a tarpaulin over the cetacean

☐ Fishing line or nets cut away and disentangled

If the animal is in a stable and healthy condition, attempt to refloat it with the rising tide. Only release the animal after acclimatisation: the animal can lift its head and breath on its own. If the animal beaches again, continue rocking and try again an hour later. If the animal beaches again it is probably diseased and a vet needs to be called. <u>Keep the blowhole clear of water at all times</u>.

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

NOTES:

Name of Reporter: ____

Contact number: ____

Time reported: ____

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

PINNIPED STRANDING REPORT

Stranding number (AIMCYY_##Sp): ____

Examiners names: ____

Report type: ☐ Dead Stranding ☐ Alive Stranding

Confidence code: ☐ Unconfirmed public report ☐ Confirmed Public Report

Species: _____________________________

INITIAL OBSERVATION

Date: Year: ________Month: _____Day: _____

LOCATION:

Name of Beach: _________________________

Beach topography: _______________________

Lat: _________________________________

Long: ________________________________

Nearest village & Distance: _____________

Sea state: _____

Weather: _____________________________

Stranding time/ death time: ____________

Cause of death:

Deliberate kill

Natural

Bycatch

Unknown

Other:

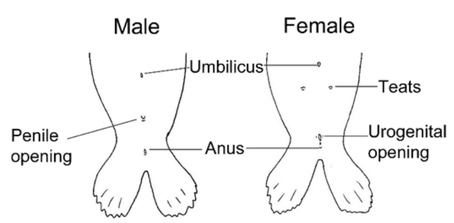
____

Photographs taken (tick if yes):

☐ Location ☐ Parasites ☐ Position relative to shore ☐ Any fishing gear present Damage, scars, lesions. ☐ Throughout first aid

Photos taken by:________________________

GENERAL EXAMINATION



Sex of animal:

☐ Female ☐ Male

Condition at Examination:

☐ 1. Alive ☐ 4. Advanced decomposition

☐ 2. Fresh dead ☐ 5. Severed decomposition

☐ 3. Moderate decomposition

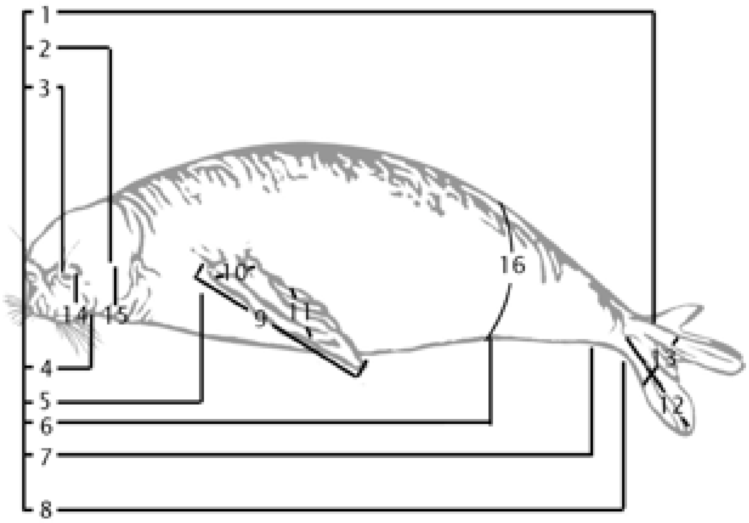
Age class:

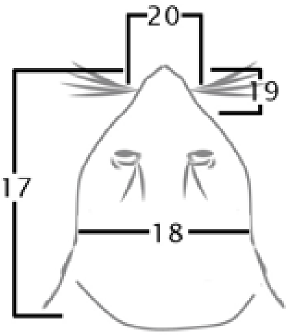
☐ Adult ☐ Subadult ☐ Calf ☐ Unknown

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

MORPHOMETRICS (cm):

If live, only take measurements once the animal is calm and first aid has been administered. Take as many measurements as possible without disturbing the animal. Hold the tape at a distance and do not touch.





- ☐ 1.Total length, snout to notch____
- ☐ 2. Snout to centre of eye (left) __
- ☐ 3. Snout to centre of eye (left) ___
- ☐ 4. Length of gape (mouth) (left)___
- ☐ 5. Snout to foreflipper (left)___
- ☐ 6. Snout to umbilical scar ___
- ☐ 7. Snout to genital opening__
- ☐ 8. Snout to anus___
- ☐ 9. Foreflipper length (left)___
- ☐ 10. Foreflipper width at the base (left) ___
- ☐ 11. Maximum width of foreflipper (left)___
- ☐ 12. Length of hindflipper (left)___
- ☐ 13. Maximum width of hindflipper (left)__
- ☐ 14. Eye length__
- ☐ 15. Ear length__
- ☐ 16. Girth at umbilicus__
- ☐ 17. Length of head__
- ☐ 18. Width of head___
- ☐ 19. Length of snout___
- ☐ 20. Width of snout__

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

LIVE STRANDING

Individual ID number:

- ☐ Single
- ☐ with pup ____________________

Animal Condition:

- ☐ Healthy
- ☐ Malnourished
- ☐ Sick
- ☐ Wounds/Lesions
- ☐ Injured
- ☐ Fishing gear present

FIRST AID:

Breathing Rate:

☐ Normal ☐ Accelerated ☐ Reduced/minimal

Reflexes:

☐ Yes ☐ No

Heart Rate:

☐ Normal ☐ Accelerated ☐ Reduced/Minimal

TICK IF ADMINISTRATED:

- ☐ Fishing line or nets cut away and disentangled
- ☐ Cold/Windy weather: windbreak constructed around the animal

DEAD STRANDING:

CARCASS STATUS:

- ☐ 1. Alive or just dead.
- ☐ 2. Fresh carcass (<24h)
- ☐ 3. Moderate Decompositions
- ☐ 4. Advanced Decomposition
- ☐ 5. Mummified or partial carcass

NUTRITIONAL CONDITION:

- ☐ 1. Emaciated
- ☐ 3. Normal
- ☐ 2. Thin
- ☐ 4. Robust (fat)

EXTERNAL EXAM (Tick if Yes):

- ☐ Signs of fishing net or lines (head, pectoral fin, caudal peduncle, dorsal fin, pectoral fin right:
  ____
- ☐ Penetrating wounds (gunshots/puncture wounds):
  ____
- ☐ Mutilations (cuts/cracks in the body wall/ missing appendages): ______________________________
- ☐ Post-mortem damage from scavengers and opportunists: ______________________________
- ☐ Parasites and foreign bodies _________________

LOOK FOR:

- ☐ Skin damage (viral infections & parasites, seal pox lesions on stomach, flippers and/or chest)
- ☐ Continuous monitoring of breathing pattern
- ☐ Eye condition (scratches, trauma or infections)
- ☐ Umbilical infections in pups
- ☐ Oral lesions, dental fractures or missing teeth
- ☐ Presence of oil indicative of local spill
- ☐ Malnutrition & dehydration

NEXT STEPS:

- ☐ Left on beach, no further action required
- ☐ Left on beach, will monitor for next 24-48 hrs
- ☐ Taken back to base, will send to rescue center

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

NOTES:

Name of Reporter: ____

Contact number: ____

Time reported: ____

IF IN DOUBT, CALL THE MARINE MAMMAL TEAM SUPERVISOR FOR HELP

ANNEX

EVIDENCE-BASED DIAGNOSTIC ASSESSMENT FRAMEWORKS FOR CETACEAN NECROPSIES ON SPECIFIC ISSUES/THREATS

Sandro Mazzariol & Cinzia Centelleghe
Department of Comparative Biomedicine and Food Science - University of Padova

Interpreting post-mortem findings and evidences collected during a thorough necropsy, not limited to gross examination, needs specific skills and expertise. More in detail, these data should be elaborated by skilled professionals to properly hypothesize the possible cause, mechanism and manner of death.

A necropsy is a specialized medical procedure comprising of a thorough examination of a carcass by dissection. Sampling and testing should be complete and not be driven by any previous hypothesis or speculation; interpretation of evidences should be based on the best existing literature and protocols already published and/or used, ruling out any possible causes of death without bias. Even if it depends on the specific country's legal framework, post-mortem investigations with diagnostic aims should be performed by a veterinarian trained in animal pathology with an experience in marine mammal diseases.

In the present document, best practices and criteria associated with diagnoses of the most relevant threats for marine mammals (i.e. bycatch, marine litter ingestion, underwater noise) found during cetacean post-mortem examinations are resumed along with the most recent pertinent literature. These set of findings constitute an evidence-based diagnostic assessment framework and could support the interpretation of data and observations collected during a thorough and complete necropsy by a veterinary pathologist and/or a governmental veterinarian.

It should be stressed that the following frameworks are not shortcuts that justifies rapid diagnoses from inexperienced personnel; rather they are a support tool for trained and authorized professionals to harmonize interpretation and evaluation. Total or partial presence or absence of the reported evidences obtained through the reported best practices should be considered along with the other results from the entire necropsy in order to gain the final diagnosis, and evidences should be interpreted by the experienced veterinarian or biologist involved after a complete necropsy. Without a complete post-mortem investigation, carried out according to a standardized procedure by expert and trained personnel, final diagnoses are not supported and have no value.

The following issues are herein resumed:

a. bycatch
b. entanglement
c. marine litter ingestion
d. underwater noise
e. ship strikes
f. infectious diseases

All the most relevant findings and diagnostic criteria for each single issue and reported in the most relevant literature will be summarized in tables including the type of examination, the tiers at which it could be detected according the European Cetaceans post-mortem investigations best-practices and some notes. It is not necessary that all the listed evidences are contemporary present, but they should be interpreted with the results of the complete necropsy and all the other possible causes of death should be ruled out. Since this information is included in the most recent literature, it is highly recommended a periodic update.

a. Bycatch

The challenge of identifying the cause of death in bycaught cetaceans arises from the nonspecific nature of the lesions of drowning/asphyxiation, lack of previous history of the dead animal and the varied nature of fishing gear, with no pathognomonic changes recognized for acute underwater entrapment. Several publications recognize signs of acute external entanglement, bulging or reddened eyes, recently ingested gastric contents, pulmonary changes, and decompression-associated gas bubbles as most commonly reported changes, but these findings cannot be surely related to acute bycatch and many others could support the interpretation and final diagnosis.
All these findings should be collected during a standardized, thorough necropsy performed by skilled personnel. The necropsy could allow to interpret all the reported findings, to exclude any other cause of death and to advance a final diagnosis.

Investigation	Evidences	Tier	Notes	Literature
External examination	Nutritional condition: very good to sub-optimal	1	difficult to detect in case of interaction with gillnets and trawling	1. Bernaldo de Quiros et al., 2018 2. Moore et al., 2013 3. Kuiken et al., 1994
	In vivo evidence of entanglement: . contact with fishing gear (superficial: impressions, depigmentation etc.) . presence of fishing gear . physical injuries (amputation, laceration, fracture etc.) . haemorrhagic findings			
	Bulging/red eyes			
Pathological examination (gross and/or microscopic)	Evidence of undigested gastro-oesophageal contents	1	nonspecific and/or pathognomonic	1. Bernaldo de Quiros et al., 2018 2. Moore et al., 2013 3. Kuiken et al., 1994 4. Bernaldo de Quiros et al., 2016
	Multi-organ congestion	2		
	Multi-organ gas bubbles with high score in coronary, renal, iliac, subcutaneous vessels and perirenal tissues	2	requires training	
	Pulmonary oedema	2	nonspecific finding associated to many other pathological conditions	
Chemical analyses of gas bubbles	Gas bubbles are not consistent with post-mortem gases.	3	sampling requires training and very few laboratories are skilled in this type of analyses	5. Bernaldo de Quiros et al., 2013 6. Bernaldo de Quiros et al., 2011
Microscopic and immunohistochemical examination	Muscle changes consistent with stress	3	sampling requires training and very few laboratories are skilled in this type of analyses	7. Sierra et al., 2017.
Pathological and microbiological examinations	Absence of infectious agents impairing animal health	3	results from microbiology should be compared to microscopic examination	2. Moore et al., 2013 3. Kuiken et al., 1994
Diatoms research technique	Diatoms in the long bones	3	not pathognomonic; may support diagnosis	8. Rubini et al., 2018

1. *Bernaldo de Quirós Y, Hartwick M, Rotstein DS, Garner MM, Bogomolni A, Greer W, Niemeyer ME, Early G, Wenzel F, Moore M.* ***Discrimination between bycatch and other causes of cetacean and pinniped stranding.*** *Dis Aquat Organ. 2018 Jan 31;127(2):83-95.*
2. *Moore MJ, der Hoop Jv, Barco SG, Costidis AM, Gulland FM, Jepson PD, Moore KT, Raverty S, McLellan WA.* ***Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma.*** *Dis Aquat Organ. 2013 Apr 11;103(3):229-64. Kuiken T, Simpson VR, Allchin CR, Bennett PM, Codd GA, Harris EA, Howes GJ, Kennedy S, Kirkwood JK, Law RJ, et al.* ***Mass mortality of common dolphins (Delphinus delphis) in south west England due to incidental capture in fishing gear****. Vet Rec. 1994 Jan 22;134(4):81-9*
3. *Bernaldo de Quirós Y, Saavedra P, Møllerløkken A, Brubakk AO, Jørgensen A, González-Díaz O, Martín-Barrasa JL, Fernández A.* ***Differentiation at necropsy between in vivo gas embolism and putrefaction using a gas score.*** *Res Vet Sci. 2016 Jun;106:48-55.*
4. *Bernaldo de Quirós Y, Seewald JS, Sylva SP, Greer B, Niemeyer M, Bogomolni AL, Moore MJ.* ***Compositional discrimination of decompression and decomposition gas bubbles in bycaught seals and dolphins****. PLoS One. 2013 Dec 19;8(12):e83994.*
5. *Bernaldo de Quirós Y, González-Díaz O, Saavedra P, Arbelo M, Sierra E, Sacchini S, Jepson PD, Mazzariol S, Di Guardo G, Fernández A.* ***Methodology for in situ gas sampling, transport and laboratory analysis of gases from stranded cetaceans.*** *Sci Rep. 2011;1:193*
6. *Sierra E, Espinosa de Los Monteros A, Fernández A, Díaz-Delgado J, Suárez-Santana C, Arbelo M, Sierra MA, Herráez P.* ***Muscle Pathology in Free-Ranging Stranded Cetaceans.*** *Vet Pathol. 2017 Mar;54(2):298-311.*
7. *Rubini S, Frisoni P, Russotto C, Pedriali N, Mignone W, Grattarola C, Giorda F, Pautasso A, Barbieri S, Cozzi B, Mazzariol S, Gaudio RM.* ***The diatoms test in veterinary medicine: A pilot study on cetaceans and sea turtles****. Forensic Sci Int. 2018 Sep;290:e19-e23*

b. Entanglement

Entanglement refers to the wrapping of materials of anthropogenic origin like lines, ropes or nets around the body of an animal and differs from bycatch, which refers to the unintentional capture of species such as small cetaceans in fishing nets.

Entangled animals do not die immediately after wrapping but the materials around the cetacean's body could injure it and impair its swimming, diving and feeding, inducing a chronic condition. In these conditions, death could be due to progressive starvation due to a reduction in food intake and an increase of energetic cost. Possible secondary infections could infect wounds associated with entanglement or affect the animal due to an impairment of the immune system. The following table resumes the main finding that could be reported during post-mortem examinations on entangled cetaceans.

Investigation	Evidences	Tier	Notes	Literature
External examination	Nutritional condition: poor to cachectic	1		1. Moore et al., 2006 2. Moore et al., 2013
	In vivo entanglement evidence: . contact with anthropogenic materials around the body of the animal (superficial changes) . presence of anthropogenic materials around the body of the animal . chronic physical injuries (laceration, scars, etc.)			
Gross examination	Muscular atrophy	2		1. Moore et al., 2006 2. Moore et al., 2013
	Absence of food remains in the stomach	2		
	Pale discoloration of muscle and tissues	2	possible findings that may be detected singularly or associated with muscular atrophy	
	Severe parasitic infestation	2		
	Gelatinous atrophy of the subcutaneous tissues	3		
	Haemorrhagic changes to subcutaneous and serosal surfaces (petechiae, bruises, etc.)	3		
	Opportunistic infections	3		
Microscopic examination	Muscular atrophy with scattered fiber necrosis	3		3. Sierra et al., 2017.
	Liver steatosis and/or hemosiderotic pigment in Kupffer cells	3	described in terrestrial mammals; only in single case reports in cetaceans	4. Gerdin et al., 2016
	Splenic hemosiderophages	3		
	Opportunistic infections	3		2. Moore et al., 2013
Microbiological investigations	Possible infectious diseases	3		2. Moore et al., 2013

1. *Moore MJ, der Hoop Jv, Barco SG, Costidis AM, Gulland FM, Jepson PD, Moore KT, Raverty S, McLellan WA.* ***Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma.*** *Dis Aquat Organ. 2013 Apr 11;103(3):229-64.*

2. *Kuiken T, Simpson VR, Allchin CR, Bennett PM, Codd GA, Harris EA, Howes GJ, Kennedy S, Kirkwood JK, Law RJ, et al.* ***Mass mortality of common dolphins (Delphinus delphis) in south west England due to incidental capture in fishing gear****. Vet Rec. 1994 Jan 22;134(4):81-9*
3. *Moore MJ, Bogomolni AL, Bowman R, Hamilton PK.* ***Fatally entangled whales can die extremely slowly.*** *Ocean'06 MTS/IEEE, Boston, MA: 2006.*
4. *Sierra E, Espinosa de Los Monteros A, Fernández A, Díaz-Delgado J, Suárez-Santana C, Arbelo M, Sierra MA, Herráez P.* ***Muscle Pathology in Free-Ranging Stranded Cetaceans.*** *Vet Pathol. 2017 Mar;54(2):298-311.*
5. *Gerdin JA, McDonough SP, Reisman R, Scarlett J.* ***Circumstances, Descriptive Characteristics, and Pathologic Findings in Dogs Suspected of Starving****. Vet Pathol. 2016 Sep;53(5):1087-94.*

c. Marine litter

The ingestion of marine litter can occur in many cetacean species and the number of reports of foreign bodies found in the stomachs of stranded marine mammals is increasing. Despite these numbers, it should be noted that findings of plastic debris are not often deemed to be the main cause of stranding and are poorly reported in pathology literature. Recent papers published in the Canary Islands (Diaz Delgado et al., 2018; Puig-Lozano et al., 2018) underline that only a few species seem to be lethally affected by plastic ingestion, with deep divers such as sperm whales and beaked whales more affected than others; young age and poor nutritional condition seems to be another relevant factor. With regards to the nutritional condition, it is not yet clear if it is a predisposing factor for the ingestion of marine litter, or a consequence thereof.

While, during necropsy, it is easy to state the possible ingestion of marine debris, it is more difficult to assess the impact it has on the animal's health. The findings summarized in the above table could be observed, alone or associated, and they can support the interpretation of the pathologist in the assessment of the cause of death during the complete necropsy.

Investigation	Evidences	Tier	Notes	Literature
External examination	Nutritional condition: normal to poor	1		1. Puig-Lozano et al., 2018 2. Diaz-Delgado et al., 2018
Gross examination	Plastic debris	1		
	Gastric perforation	2	may lead to acute death	
	Ulcerative gastritis	2		
	Gastric impaction/obstruction	2		
	Muscular atrophy	2	only when a poor nutritional condition has been determined	
	Severe parasitic infestation	2		
	Opportunistic infections	3		
Microscopic examination	Muscular atrophy	3		3. Sierra et al., 2017.
	Opportunistic infections	3		
Microbiological investigations	Possible infectious diseases	3		

1. *Puig-Lozano R, Bernaldo de Quirós Y, Díaz-Delgado J, García-Álvarez N, Sierra E, De la Fuente J, Sacchini S, 1. Suárez-Santana CM, Zucca D, Câmara N, Saavedra P, Almunia J, Rivero MA, Fernández A, Arbelo M.* ***Retrospective study of foreign body-associated pathology in stranded cetaceans, Canary Islands (2000-2015)****. Environ Pollut. 2018 Dec;243(Pt A):519-527.*
2. *Díaz-Delgado J, Fernández A, Sierra E, Sacchini S, Andrada M, Vela AI, Quesada-Canales Ó, Paz Y, Zucca D, Groch K, Arbelo M.* ***Pathologic findings and causes of death of stranded cetaceans in the Canary Islands (2006-2012)****. PLoS One. 2018 Oct 5;13(10):e0204444.*
3. *Sierra E, Espinosa de Los Monteros A, Fernández A, Díaz-Delgado J, Suárez-Santana C, Arbelo M, Sierra MA, Herráez P.* ***Muscle Pathology in Free-Ranging Stranded Cetaceans.*** *Vet Pathol. 2017 Mar;54(2):298-311.*

d. Underwater impulsive noise-related strandings

The diagnostic assessment framework for the investigation of underwater impulsive noise as a possible cause of strandings is not as complete as for other causes due to lack of knowledge. In fact, only a spatial and temporal association of middle and low frequency military sonar to a gas and fat embolic syndrome developed in beaked whales has been reported, while for any other species and/or sound sources there is not yet enough literature to draw possible diagnostic criteria. Investigations performed on the inner ear conducted according to a specific protocol could support the diagnosis of cochlear damage.
Due to these limitations, to date, it is only possible to exclude any other possible cause through a complete and detailed necropsy. The stranding pattern (active vs. passive, location of strandings, marine currents etc.), the number of animals involved (individual or multiple animals in good nutritional condition stranded within hours or a few days of a military exercise), the spatial and temporal association with a functioning impulsive noise source are fundamental to support the diagnostic hypothesis. From a pathological point of view, the post-mortem findings included in the following table may be observed.

Investigation	Evidences	Tier	Notes	Literature
External examination	Bleeding from main orifices	1		1. Fernandez et al., 2005 2. Bernaldo de Quiros et al., 2019
	Good nutritional status	1		
Gross examination	food remnants in the first gastric compartment ranging from undigested food to squid beaks	2		
	abundant gas bubbles widely distributed in veins (subcutaneous, mesenteric, portal, coronary, subarachnoid veins, etc.)	2	requires training	
	gross subarachnoid and/or acoustic fat hemorrhages;	2		
	absence of other relevant diseases	2		
Microscopic examination	microscopic multi-organ gas and fat emboli associated with bronchopulmonary shock	3		
	diffuse, mild to moderate, acute, monophasic myonecrosis (hyaline degeneration) in fresh and well-preserved carcasses	3		
	multi-organ microscopic hemorrhage of varying severity in lipid-rich tissues such as the central nervous system, spinal cord, and the coronary and kidney fat (when present)	3		
	Hemorrhage in the inner ear visible with HE-stain after decalcifing tympano-periotic complex	3	decalcification process may alter microscopic findings	3. Jepson et al., 2013
	absence of other relevant diseases	3		
Chemical analyses of gas bubbles	mainly N2	3	requires training to collect bubbles from veins and perform chemical analyses	4. Bernaldo de Quiros et al., 2011
Electron microscopy	scars and damage to the cochlear hair cells of the inner ear	3	requires training to collect and preserve inner ear; possible until 30 hours after death	5. Morell et al., 2017

1. *Fernández A, Edwards JF, Rodríguez F, Espinosa de los Monteros A, Herráez P, Castro P, Jaber JR, Martín V, Arbelo M.* ***"Gas and fat embolic syndrome" involving a mass stranding of beaked whales (family Ziphiidae) exposed to anthropogenic sonar signals.*** *Vet Pathol. 2005 Jul;42(4):446-57*
2. *Bernaldo de Quirós Y, Fernandez A, Baird RW, Brownell RL Jr, Aguilar de Soto N, Allen D, Arbelo M, Arregui M, Costidis A, Fahlman A, Frantzis A, Gulland FMD, Iñíguez M, Johnson M, Komnenou A, Koopman H, Pabst DA, Roe WD, Sierra E, Tejedor M, Schorr G.* ***Advances in research on the impacts of anti-submarine sonar on beaked whales.*** *Proc Biol Sci. 2019 Jan 30;286(1895):20182533*
3. *Jepson PD, Deaville R, Acevedo-Whitehouse K, Barnett J, Brownlow A, Brownell RL Jr, Clare FC, Davison N, Law RJ, Loveridge J, Macgregor SK, Morris S, Murphy S, Penrose R, Perkins MW, Pinn E, Seibel H, Siebert U, Sierra E, Simpson V, Tasker ML, Tregenza N, Cunningham AA, Fernández A.* ***What caused the UK's largest common dolphin (Delphinus delphis) mass stranding event?*** *PLoS One. 2013 Apr 30;8(4):e60953. doi: 10.1371/journal.pone.0060953.*
4. *Bernaldo de Quirós Y, González-Díaz O, Saavedra P, Arbelo M, Sierra E, Sacchini S, Jepson PD, Mazzariol S, Di Guardo G, Fernández A.* ***Methodology for in situ gas sampling, transport and laboratory analysis of gases from stranded cetaceans.*** *Sci Rep. 2011;1:193*
5. *Morell M, Brownlow A, McGovern B, Raverty SA, Shadwick RE, André M.* ***Implementation of a method to visualize noise-induced hearing loss in mass stranded cetaceans****. Sci Rep. 2017 Feb 6;7:41848. doi: 10.1038/srep41848*

e. Ship strikes

In the last decades, collisions between vessels and cetaceans have significantly increased worldwide and they are deemed to be a major threat for large cetaceans living in the ACCOBAMS area. In case of collisions, external features may be pathognomonic with extensive subcutaneous, muscular and visceral hemorrhage and hematomas, indicating unequivocal *ante-mortem* trauma. However, when carcasses are highly autolyzed, it is challenging to distinguish whether the trauma occurred *ante-* or *post-mortem*. The presence of fat emboli within the lung microvasculature is used to determine a severe "*in vivo*" trauma in other species, and they can be used also in these cases. These aspects are summarized in the following tables.

Investigation	Evidences	Tier	Notes	Literature
External examination	Sharp traumas with one or more linear to curvilinear laminar incising wounds that cause damage to axial muscles, skull and vertebral column	1	mainly on the back and sides	1. Moore et al., 2013 2. Campbell-Malone et a., 2008
Gross examination	Blunt traumas with hemorrhage and edema in the blubber, subcutaneous tissue, and skeletal muscle	2		
	fractures and luxations	2		
Microscopic examination	Muscular hemorrhages and edema	3		3. Sierra et al., 2014.
	flocculent, granular or/and hyalinised segmentary degeneration; contraction band necrosis; discoid degeneration or fragmentation of myofibres	3		
	Fat emboli in the lung tissue	3	not relevant if death is immediate after trauma	4. Arregui et al., 2019

1. *Moore MJ, der Hoop J, Barco SG, Costidis AM, Gulland FM, Jepson PD, et al.* ***Criteria and case definitions for serious injury and death of pinnipeds and cetaceans caused by anthropogenic trauma.*** *Dis Aquat Organ. 2013; 103 (3): 229–264*
2. *Campbell-Malone R, Barco SG, Daoust PY, Knowlton AR, McLellan WA, Rotstein DS, et al.* ***Gross and histologic evidence of sharp and blunt trauma in North Atlantic right whales (Eubalaena glacialis) killed by vessels.*** *J Zoo Wildl Med. 2008; 39 (1): 37–55.*
3. *Sierra E, Fernández A, Espinosa de los Monteros A, Arbelo M, Díaz-Delgado J, Andrada M, et al.* ***Histopathological muscle findings may be essential for a definitive diagnosis of suspected sharp trauma associated with ship strikes in stranded cetaceans.*** *PLoS One. 2014*
4. *Arregui M, Bernaldo de Quirós Y, Saavedra P, Sierra E, Suárez-Santana CM, Arbelo M, Díaz-Delgado J, Puig-Lozano R, Andrada M and Fernández A (2019)* ***Fat Embolism and Sperm Whale Ship Strikes.*** *Front. Mar. Sci. 6:379*

f. Infectious diseases

Cetaceans can be affected by many infectious agents that can cause diseases and death. Among these pathogens, Cetacean Morbillivirus (CeMV), Brucella spp. and Toxoplasma gondii are the most relevant ones.

As in terrestrial mammals, the diagnosis of a disease is supported by the contemporary evidence of pathological changes, immunohistochemical and microbiological findings. If all three are not present at the same time, the diagnosis is weak, and it should be interpreted in accordance with other findings. In the following table, main findings for CeMV diseases are reported in order to aid pathologists in their diagnosis for this virus considered as the more dangerous for the cetaceans' conservation in the ACCOBAMS waters. Other pathogens are often reported as single case reports.

Investigation	Evidences	Tier	Notes	Literature
Gross examination	Meningeal congestion	2	not always present at the same time	1. Van Bressem et al., 2014
	Lymph node enlargement	2		
	Bronchopneumonia	2		
	Secondary infections and parasitic infestation	2		
Microscopic examination	Chronic meningoencephalitis with astrogliosis and possible demyelinization	3		
	Interstitial bronchopneumonia	3		
	Lymphoid depletion with multinucleated giant cells	3		
	Secondary infections and parasitic infestation	3		
Immunohistochemistry	Positive using anti-CDV antibodies	3		
Molecular analyses	Positive target organs (brain, lymph nodes, spleen, thymus, lungs)	3	highly specific but limited by conservation code	2. Verna et al., 2017
		3	time-consuming but highly sensitive for large cetaceans and conservation codes 3-4	3. Centelleghe et al., 2016
		3	all CeMV strains	4. Rubio-Guerri et al., 2013

1. *Van Bressem MF, Duignan PJ, Banyard A, Barbieri M, Colegrove KM, De Guise S, Di Guardo G, Dobson A, Domingo M, Fauquier D, Fernandez A, Goldstein T, Grenfell B, Groch KR, Gulland F, Jensen BA, Jepson PD, Hall A, Kuiken T, Mazzariol S, Morris SE, Nielsen O, Raga JA, Rowles TK, Saliki J, Sierra E, Stephens N, Stone B, Tomo I, Wang J, Waltzek T, Wellehan JF. Cetacean morbillivirus: current knowledge and future directions. Viruses. 2014 Dec 22;6(12):5145-81.*
2. *Verna F, Giorda F, Miceli I, Rizzo G, Pautasso A, Romano A, Iulini B, Pintore MD, Mignone W, Grattarola C, Bozzetta E, Varello K, Dondo A, Casalone C, Goria M. Detection of morbillivirus infection by RT-PCR RFLP analysis in cetaceans and carnivores. J Virol Methods. 2017 Sep;247:22-27.*
3. *Centelleghe C, Beffagna G, Zanetti R, Zappulli V, Di Guardo G, Mazzariol S. Molecular analysis of dolphin morbillivirus: A new sensitive detection method based on nested RT-PCR. J Virol Methods. 2016 Sep;235:85-91.*
4. *Rubio-Guerri, C. et al. Simultaneous diagnosis of Cetacean morbillivirus infection in dolphins stranded in the Spanish Mediterranean Sea in 2011 using a novel Universal Probe Library (UPL) RT-PCR assay. Vet Microbiol 165, 109–114 (2013).*

LIFE 18/NAT/IT/000942

www.lifedelfi.eu

LIFE DELFI

Dolphin Experience: Lowering Fishing Interactions

LIFE18 NAT/IT/000942

Action A3
Framework for fishery interaction

9th April 2021

Beneficiario coordinatore

CNR IRBIM

Beneficiari associati

Tavolara Punta Coda Cavallo
Torre del Cerrano Area Marina Protetta
Blue World Institute
Filicudi WildLife Conservation
Legambiente
Università degli Studi di Padova
Università di Siena 1240

TIER 3

Evaluation at Tier 3 requires appropriate skills and expertise as well as logistical and laboratory equipment. In addition to a complete necropsy, the following must be confirmed/stated:

- the carcass decomposition condition code (DCC)
- Confirmation of fishery interaction
- Presence or absence of other ongoing diseases
- Assessment of mechanism of death

The first table below summarizes the list of the main categories and associated findings related to fishery interaction that should be assessed during a post-mortem investigation. Evidence is categorised as "certain/pathognomonic" (labeled as C/P), "consistent" (labeled as C) and "suggestive" (S) with respect to the type of interaction with the fishery (i. e. by-catch with active fishing gear, by-catch with passive fishing gear, chronic entanglement, laryngeal entanglement, ingestion).

CATEGORIES	FINDINGS	B(A)	B(P)	LE	CE	I	II	DCC
Direct evidences of fishing interaction	fishing interaction in the animal history (specific for each category) (12, 18, 20)	C/P	C/P	C/P	C/P	C/P	C/P	1-5
	presence of fishing gears (active v/s passive) (12, 18, 20)	C/P	C/P		C/P			1-4
	net marks/linear signs (acute) (12, 18, 20)	C/P	C/P					1-3
	net marks/linear signs (chronic, i.e. constriction lesions) (12, 18, 20)				C/P			1-3
	presence of fishing gear around larynx (11)			C/P				1-4
	penetrating wounds (12,18)	C	C				C/P	1-3
	mutilation with acute inflammatory reaction	C	C				C/P	1-3
	mutilation with chronic inflammatory reaction	S	S		S			1-3
	gunshot/bullet wounds (12,18)						C/P	1-3
	contusions (12,18)	C						1-3
	fractures (12,18)	C						1-4
Other fishery interaction - associated lesions	capture myopathy (to be confirmed with histology and IHC) (20)	C/P	C/P					1-3
	separation of the rectus abdominis muscles (6)	C	C					1-2
	gas bubbles in main vessels (2)	C	C					1-2
	linea alba erniation (6)	C	C					1
Nutritional findings	presence of fresh oesophagic/gastric content (12,18)	C	C					1-4
	absence of fresh gastric content (12,18)			S	C			1-4
	good NCC (12,18)	C	C					1-3
	poor NCC (12,18)			S	C			1-3
Aspecific findings	bulging eyes/red eyes (2)	C						1-2
	microscopic muscular haemorragies (histology) (20)	S	S					1-3
	pulmonary and vascular changes (epicardial petechiae, edema, froth/ blood-tinged watery fluid in the airways, congestion, bullae in the lung parenchyma, incomplete collapse of the lungs, chyle in the ductus thoracicus and) (2)	S	S					1-3

	multiorgan congestion (2)	S	S					1-3
Other pathologies	absence of other ongoing diseases (2, 12, 20)	C	C					1-3

TABLE LEGEND

B(a) = By-catch with active fishing gear

B (p) = By-catch with passive fishing gear

CE = Chronic entanglement

LE = Larynx entanglement

I = Ingestion

II = Intentionally injured

DCC = interval of decomposition code of the carcass where the finding can be assumed as true

NCC = nutritional code of the carcass

(no.) = references describing findings, sampling and analytical approaches

While a single C/P evidence will deem a specific fishing interaction confirmed, the experience and skills of the examiner will consider the type and number of C/P and S/P evidences in diagnosing the fishing interaction. As a suggestion, a minimum of 3 between C/P and S/P can deem the occurrence of a specific fishing interaction probable or suspected. The following table will help to hypothesise the cause of death with some degree of certainty by coupling previous fishery interaction findings with other post mortem findings.

For statistical purposes, the first 2 scores (Certain and Probable) should be considered among human-induced mortality, while the uncertainty of the 3rd score (Suspected) does not allow to be included. For the 4th score, it should only be included when interaction with fishing is considered the ultimate cause of death.

Certain/Pathognomonic (only in carcasses with code of decomposition 1 and 2)	The fishery interaction is confirmed + absence of other severe pathologies + the mechanism of death is assessed
Probable (only in carcasses with code of decomposition 1 and 2)	The fishery interaction is confirmed or suspected + absence of other pathologies
Suspected/Possible (if the carcass present a decomposition code higher than 2)	The fishery interaction is confirmed + absence of other pathologies
Fishery interaction as a consequence of underlying pathologies	The fishery interaction is confirmed + neurological, systemic and other severe pathologies that could have predisposed the animal to the fishery interaction

ANNEX 2
COMMON BEST PRACTICES FOR A BASIC POST-MORTEM EXAMINATION OF STRANDED CETACEANS

Sandro Mazzariol
DVM, PhD

Appendix II
BASIC NECROPSY PROTOCOL

2 State of Conservation of the Carcass

It is possible to classify the state of conservation of a carcass found along the coastline using the criteria outlined by the most important manuals on the management of cetacean strandings. The following table delineates the criteria, which is based on physical parameters easily identified even by persons without any veterinarian experience, used to classify the state of conservation of a carcass and the code number assigned to each category; it also lists other investigations, depending on its status, that should be carried out.

Code	State of conservation	Description	Possible investigations
1	Alive/just died	Animal found alive or died at most 2 hrs earlier	Clinical examination, blood and urine exams, Microbiology/histology swabs, cytology, virology (from the tissue/PCR), serology, microbiology (cultures from tissues or PCR), parasitology, contaminants, biotoxins, genetics, biology (life history)
2	Carcass in good condition	Death took place within 24 hrs of the finding; minimal scavenger damage; normal smell; minimal drying or wrinkling of skin or eyes; eyes clear; no bloating; tongue and penis not protruded	Histology, cytology, virology, (from the tissue/PCR), serology, microbiology (cultures from tissues or PCR), parasitology, contaminants, biotoxins, genetics, biology (life history)
3	Moderate decomposition	Integral carcass with evident bloating (tongue and penis protruding) skin not integral with some sloughing, some damage by scavengers possible, mild odor, mucous membranes dry, eyes shrunken or missing	Histology (limited) virology (PCR) parasitology, contaminants, biotoxins, genetics, biology (life history)
4	Advanced decomposition	The carcass may be integral but collapsed; ample areas of sloughing skin, serious scavenger damage, strong odor, muscles and blubber easily detached from the bone, liquefaction of internal organs	Histology, (limited) virology (PCR), parasitology(PCR), contaminants (limited) biology, paleopathology (on the skeleton) (life history), genetics
5	Mummified or skeletal remains	Dehydrated, dry skin draped over desiccated bones	Biology (life history), genetics, paleopathology (on the skeleton)

3 Life history and physiological parameters estimation

3.1 Age estimation

It is useful to estimate the age of beached cetaceans as this can modify the prognosis and all of the operations that need to be carried out.

Age estimation of cetaceans can be based on microscopic evaluation of the exemplar's teeth, but the procedure cannot be carried out on live animals. Age estimates can also be based on the dimensions and on other properties of the layer of dentin (calf, juvenile, young adult, old). The specimen's total length is the physical parameter that help to define both physiologic parameters that is age class and estimated weight. The mean lengths ascertained in particular make it possible to differentiate between neonates (dimensions similar to the mean ones outlined in the literature for the species) and adults. Neonates a few days old can be identified by the presence of lingual papillae and a patent umbilical cord. Other factors of importance are obviously length and in some species the season.

Animals which are suspected to be dependent maternally should not be liberated unless there is clear evidence of members of that same species in the vicinity.

Intermediate length conditions falling between adult and neonates make it possible to classify the subject as young. That estimate can be confirmed by the color of the livery in some species of odontocetes (Risso's Dolphin, Beaked whale, etc.) and the limited number of signs attributable to intra-specific interaction.

Older specimens are characterized by dimensions comparable to those of an adult cetacean with perhaps some signs of muscular atrophy along the trunk or absent or worn out teeth. The table below outlines typical correlations between approximate lengths and age classes in species that are frequently beached on Mediterranean coastlines.

Species	Total length at birth (cm)	Total length calf (cm)	Total length 1 year (cm)	Total length 2 years (cm)	Approx age weaning (years)	Total length Weaning (cm)	Total length Adult (m)
Striped Dolphin Stenella coeruleoalba	93-100	100	166	180		170	2.2-2.6
Bottlenose Dolphin Tursiops Truncatus	117	100-130	170-200	170-225	1.5-2	225	2.2-3 cost. 2.5-6 pel.
Risso's Dolphin Grampus Griseous	110-150	120-160					3-4
Common Dolphin Delphinus Delphis	80-90	80-100				110-120	2.3-2.5
Rough Toothed Dolphin Steno Bredanensis	100						2.4-2.7
Long-finned pilot whale Globicephalea melas	177	160-200			2-3	240	4.5-5 F 4.5-6 M
Beaked Whale Ziphius cavirostris	270	200-300					6.7-7
Sperm Whale Physeter macrocephalus	300	350-500		670	>2	670	11-13 F 15-18 M