

Title: Pathological findings and causes of death of Angelshark (*Squatina squatina*) in Canary Islands

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#### ABSTRACT

The Canary Islands are a very important cradle of marine biodiversity. The Angelshark (*Squatina squatina*) is a species of elasmobranch that uses the coasts of the Canary Islands as its habitat, since its population in other places has decreased significantly.

The main objective is to know the epidemiology and pathologies related to the causes of death of Angelsharks stranded in the Canary Islands between 2021 and 2024. In order to carry out this final project, we used a database from "Red Vigía" of the Gobierno de Canarias" and Institute of Animal Heath and Food Safety of the University de Las Palmas de Gran Canaria, that collected the parameters and pathological results obtained from the necropsies performed on 47 individuals of Angelsharks between February 2021 and April 2024.

Between February 2021 and April 2024, we received 47 carcasses of Angelsharks that appeared off the coast of the Canary Islands. A complete standardized necropsy was performed on each specimen to identify injuries and to make a final diagnosis of the cause of death. Of these, 5/47 (10.64%) Angelsharks had injuries related to anthropogenic activities, such as fishing interactions or boat trauma. Non-anthropogenic "natural as death" are referred to and were diagnosed causes in 8/47 (17.02%) Angelsharks and were associated with infectious processes, noninfectious pathologies and interspecific interactions. The causes of death could not be diagnosed in 34/47 (72.3%) of the animals subjected to necropsies. This work compiles for the first time all available information on Angelshark strandings in the Canary Islands from 2021 to April 2024 and try to provide future guidelines to understand the causes of the stranding of these elasmobranch species.

#### **INTRODUCTION**

The Angelshark (*Squatina squatina*) is one of the most significant species of elasmobranchs found in the Canary Islands, given that our coasts serve as a breeding area and habitat for these animals (Gordon *et al.*, 2022). Nevertheless, the population of these animals has declined significantly over time, largely due to a multitude of factors such as fishing, habitat destruction or pollution. Currently, stranded individuals continue to be discovered on our beaches, yet the underlying causes of stranding remain largely unknown. This is due to the fact that these animals are less frequently studied than, for instance, some marine mammals that also strand on our coasts.

#### **MOTIVATIONS**

The Marine Mammal Protection Act, enacted in the United States in 1972, defines a stranding as "the finding of an animal, while dead, stranded anywhere on the coast or floating within the jurisdictional waters of the State." A stranding is defined as an animal that is alive and stranded on the shore, unable to return to the water or requiring apparent veterinary attention. In addition, the term can be defined as an animal that is within state jurisdictional waters and unable to return to its natural habitat by its own means or without assistance. The term can also be applied to fish species, which is why it is also referred to as a stranding when a shark appears on our beaches.

It is of the utmost importance to comprehend the ongoing threat and decline of these shark populations in order to develop effective conservation strategies. By expanding our understanding of these intriguing creatures, we can contribute to their conservation and prevent their extinction.



#### BLACKGROUND FROM CHONDRICHTHYES TO ANGELSHARKS

A shark is any animal belonging to the class Chondrichthyes, which groups all cartilaginous fish. This class of fish has an internal skeleton formed by cartilage, which allows for greater flexibility and strength than the skeleton formed by bones. This gives sharks a significant evolutionary advantage (Compagno *et al.*, 2005). Furthermore, they possess a lower and upper jaw, in contrast to primitive lampreys, and nostrils in the ventral region of the head (Compagno *et al.*, 2005).

#### History

Vertebrates are believed to have originated from primitive fish-like creatures that inhabited the oceans approximately 500 million years ago. These primitive fish-like creatures were elongated and flat, with musculature attached to a central axis that ran lengthwise along their structure. These creatures were devoid of characteristics such as fins, eyes, or bones, and their capacity for swimming was constrained (Compagno *et al.*, 1990).

Over a period of approximately ten million years, these ancestors underwent a process of evolution that resulted in the emergence of more fish-like forms. The original shaft underwent a transformation, developing a cartilaginous backbone and single eyes and odd fins. The head and upper body were covered with bony plates. Initially, these organisms lacked jaws, presumably feeding on small particles or detritus. Subsequently, they developed biting jaws, potentially derived from their original cartilaginous structures (Compagno *et al.*, 1990). These improvements, in conjunction with the evolution of swimming ability, permitted diversification and adaptation to a broad range of prey, including forms that would eventually breathe air and move on land (Compagno *et al.*, 2005).

Approximately 400 million years ago, the evolution of ancient fishes gave rise to two major groups that have survived to the present day, as well as other groups found only in the fossil record (Figure 1). One of these groups, the class Chondrichthyes, retains a cartilaginous skeleton and lacks both internal bones and flat external bony scales.

The other group, the class Osteichthyes (Greek "*osteon*": bone, "*ichthus*": fish), replaced cartilage with bone, giving rise to the bony fishes, which are the most abundant vertebrates, with some 25,000 extant species. However, sharks and their relatives were much more abundant and varied in the fossil record, with more than 300 species. The number of living species is currently estimated to be in excess of 1,100, with this figure continuing to increase over time (Compagno *et al.*, 2005).



Figure 1: Fossil record of Xenacanthus (Source Wikimedia Commons).



#### **Class** Chondrichthyes

The class *Chondrichthyes*, or chondrichthyans, is divided into two major groups: the subclass Holocephali (*Holocephalos*, from the Greek "*hólos*": all, and "*kephalé*": head) and the subclass Elasmobranchii (*Elasmobranchii*, from the Greek "*elasmós*": plate, and "*brachys*": gills).

The subclass Holocephali is a small group of living animals with extensive fossil records, including the chimaeras. The most pertinent finding for their differentiation is the presence of a soft gill cover with a slit, which protects the four gill slits on both sides of the head (Compagno *et al.*, 2005).

The subclass Elasmobranchii encompasses rays and sharks. They are readily identifiable due to the presence of numerous pairs of brachial slits on both sides of the head. The group comprises approximately 800 living species of sharks and rays (Compagno *et al.*, 2005). The latter category includes a wide range of sizes, from species such as those of the genus *Urolophus*, which are no larger than the size of a hand, up to 6 meters in length. There are more than 600 species of rays or batoid fishes (flat sharks), which are characterized by the presence of two flat pectoral fins fused to the head above the brachial fissures (Compagno *et al.*, 2005).

With respect to sharks, the size can vary considerably, from a few centimeters to over 12 meters, as exemplified by the whale shark (*Rhincodon typus*; Figure 2). A total of more than 500 species of sharks have been identified, with the majority exhibiting a cylindrical shape (although flat species do exist). They are characterized by the presence of five to seven pairs of brachial slits on either side of the head and the development of pectoral fins that are not fused to the head of the individual. Furthermore, in these species, the caudal fin is observed, which allows the animal to orientate and propel itself in the water. Additionally, one or two dorsal fins may be equipped with a spine, and pelvic fins are present.



Figure 2: Whale shark (*Rhincodon typus*) swimming close to the surface (Source Goodfon.com).



#### Anatomy

The reputation of sharks as marine predators, their ecological importance, and their unique body structure have led to their designation as icons of marine ecosystems (Compagno *et al.*, 2005).

A) External Anatomy (Figure 3): Sharks exhibit a distinctive external anatomy, with streamlined bodies adapted for efficient swimming in the aquatic environment. The body structure of sharks is composed of the head, which extends from the snout to the gills. The trunk, or body, is the space between the pelvic girdle and the anus. The tail is divided



into a precaudal tail and a caudal fin. With regard to the fins, there is considerable diversity in their shape and size (Compagno *et al.*, 2005).

Figure 3: External characteristics and body structure of sharks. 1. Ampullae of Lorenzini: These are tiny blackish dots, externally observable on the lower region of the muzzle. They act as sensitive detectors of electric fields. 2. The nostrils may exhibit a triangular anterior flap (simple shape) or may be more complex, as is the case in the majority of demersal sharks. 3. The eves of some species are protected by a lower nictitating eyelid. The majority of species lack movable eyelids. 4. Spiracles. In sharks, this structure is observed caudal to the eyes and is typically larger in demersal animals. 5. Brachial slits may be present in five, six, or seven instances. The size of these structures is directly proportional to the size of the shark in question. 6. Pectoral fins serve to elevate the animal in conjunction with pelvic fins. 7. Dorsal fins are typically composed of two fins, with the more anterior one being larger than the more posterior one. Some species exhibit a spine on the first dorsal fin, while others display a spine on both dorsal fins. 8. In males, pelvic fins are equipped with pterygopodia, which are male reproductive organs. 9. The presence of an anal fin is not universal among species. 10. Lateral keels are dorsoventral flattening that continue with a widening of the region anterior to the caudal fin. It is typically observed in fastswimming sharks and is believed to enhance stability during aquatic locomotion. 11. The caudal fin is typically asymmetrical, with the upper lobe being larger than the lower lobe in most species. In the fastest species, such as the shortfin mako shark (Isurus oxyrhinchus), the tail is known to be almost entirely symmetrical.





B) <u>Skin and scales</u>: Sharks are protected by a thick, firm skin that is normally covered by dermal denticles, which are acute, dentiform placoid scales (Figure 4). These denticles exhibit a structure analogous to teeth, as they are covered with enamel and are attached to the skin by dentine bases (Compagno *et al.*, 2005).



Figure 4: shark dermal denticles under the microscope (Source CSIC).

C) <u>Internal Anatomy</u>: The skeleton of sharks is more simplified than that of bony fish. This is evidenced by the fact that their skeleton is composed of cartilage, which renders it more flexible and lighter in weight. Furthermore, it is notable that in older sharks, the skeleton can become partially calcified, making it harder and more similar to the skeleton of bony fish. The skeleton is composed of a skull with associated bones that support the gills, the jaws (with several longitudinal series of teeth), a vertebral column formed by hourglass-shaped vertebrae that protect the spinal cord, and cartilaginous regions that support the fins. Furthermore, in males, these structures also serve to support the pterygopodia (Compagno *et al.*, 2005).

#### **Taxonomic groups**

The animal that serves as the subject of this final degree project belongs to one of the eight orders of sharks that are included in the class of animals known as chondrichthyans (Table 1). More specifically, it belongs to the order of the squatiniformes. It is therefore of interest to ascertain the differences between this species and the sharks included in the other orders.

Shark orders (Euselachii)	Key features
Hexanchiformes (2 families and 4 genders)	The species in question has six or seven brachial slits, a single dorsal fin, and an anal fin. Furthermore, they are distributed globally, occurring in temperate, cold, and deep waters. These fish are typically caught unintentionally.
Chumyuoseuchus unguineus	
	(Compagno & Fowler, 2005)
Squaliformes (7 families y 25 genders)	They have 5 brachial slits, 2 dorsal fins (with spine), short snout and no anal fin. They can be found in deep waters and are highly commercialized for their liver oil and meat, being vulnerable to overfishing.
Somniosus pacificus	(Compagno & Fowler, 2005)

Table 1. Presentation of the differential characteristics of the 8 orders of Euselachii.



1	Shark orders (Euselachii)	Key features
	Pristiophoriformes (1 family y 2 genders)	They have 5 or 6 brachial slits, 2 dorsal fins and a long snout. They can be found in a variety of oceans, although some species are very restricted. They are highly vulnerable to accidental fishing due to the shape of the snout, getting caught in nets. In addition, "saws" are commercialized in some places.
	<b>Pristiophorus japonicus</b>	
	Squatiniformes (1 family y 1 gender)	The species in question is characterized by the presence of five brachial slits, two dorsal fins, a terminal mouth, and a flattened body. Outside of Europe and the Northwest Pacific, its distribution is poorly known. A significant number of species are included in the IUCN Red List, with a high degree of vulnerability, due to the intense fishing to which they are subjected for their meat, leather, and oil.
	Squatina squatina	
		(Compagno & Fowler, 2005)
	Heterodontiformes (1 family y 1 gender)	These sharks are characterized by five brachial slits, two dorsal fins (with spines), and an anal fin. Their distribution is highly restricted, and the primary threat to their survival is posed by accidental and sport fishing. Additionally, they are readily amenable to breeding in aquariums.
	Heterodontus portujacksoni	(Compagno & Fowler, 2005)
	Orectolobiformes (7 families y 14 genders)	The shark in question has five brachial slits, two dorsal fins, a mouth situated in front of the eyes, and an anal fin. It is found throughout the world, and a significant number of species are threatened by accidental capture.
	Stegostoma fasciatum	(Compagno & Fowler, 2005)
	Lamniformes (7 families y 10 genders)	The subject sharks exhibit the following characteristics: five brachial slits, two dorsal fins, a mouth situated behind the eyes, an anal fin, and the absence of a nictitating eyelid. They are distributed worldwide and are threatened by overfishing for their meat and sport fishing.
	Alopias pelagicus	(Compagno & Fowler 2005)





Shark orders (Euselachii)	Key features
Carcharhiniformes (8 families y 50 genders)	The shark in question has five brachial slits, two dorsal fins, a mouth situated behind the eyes, an anal fin, and a nictitating eyelid. These sharks are distributed globally, from tropical to cold seas and from medium to deep depths. Some species are adept swimmers, enabling them to traverse considerable distances and undertake extensive migrations. The status of these sharks varies depending on the species.
	$(C_{\text{composing}} \in \text{Fewler} \ 2005)$

#### **Family Squatinidae**

The order Squatiniformes is comprised of a single family, Squatiniformes, which contains a single genus, Squatina. The animals of this genus exhibit demersal behavior and are characterized by a wide and flat body, with large pectoral and pelvic fins that are lateralized from the body, giving the animal an "angel" appearance (Compagno *et al.*, 2005). With regard to the eyes, they are situated in the dorsal region of the head, and just caudal to them, we can find the spiracles, which are openings that facilitate the animal's breathing while it is buried in the marine sediment (Gordon *et al.*, 2022). The snout is relatively short and bears a large mouth surrounded by barbels. The jaw is capable of protrusion, enabling the animal to catch prey. In the lower region of the animal, the gills are located (Compagno *et al.*, 2005).

A considerable number of species exhibit variations in layers and colors between adults and juveniles, as well as sexual dimorphism. Furthermore, within the same species, there are sometimes distinctive characteristics that depend on the region in which they are found (Gordon *et al.*, 2022). The animals of this genus are often misidentified due to their morphological and behavioral similarities to other elasmobranchs (Gordon *et al.*, 2022), such as rays or even the bony fish known as anglerfish (*Lophius piscatorius*). The diet of these animals is based on the ingestion of bony fish, mollusks, and small crustaceans (Gordon *et al.*, 2022).

The species are ovoviviparous or viviparous aplacental, forming litters of between one and 25 offspring. These offspring are able to feed from the yolk sac before birth (Compagno *et al.*, 2005).

With regard to the relationship between these animals and humans, it is generally observed that they are harmless unless they are disturbed. It is also noteworthy that many of the species are subject to intensive fishing for their meat, meal, oil, and leather. Furthermore, they are susceptible to bycatch in trawl and bottom-set nets (Compagno *et al.*, 2005).

A total of at least 23 accepted species of Angelsharks have been identified globally (Gordon *et al.*, 2022). Among these, the following are of particular interest (Table 2).



Table 2. Relationship between 4 Angelsharks from different parts of the world and their state of vulnerability.



**Common name**: Eastern Angelshark **Scientific name**: *Squatina albipunctata* 

Due to bycatch and maintenance in trawl fisheries, its population has declined by around 98% in three generations. It is quite likely that the species is more threatened than its assessment suggests (Murch, 2021).



**Common name**: Pacific Angelshark **Scientific name**: *Squatina californica* 

Due to fisheries, it suffered a widespread decline in the 1970s, although conservation measures assessed it as near-threatened (Murch, 2021).



**Common name:** Japanese Angelshark **Scientific name**: *Squatina japonica* 

They are often caught by land-based fisheries or as bycatch, and are conserved for their meat, fins and fishmeal. In 69 years, the Japanese Angelshark population has declined by more than 80% (Murch, 2021).



**Common name:** Angular Angelshark **Scientific name**: *Squatina guggenheim* 

It showed a high decline in individuals shortly before 2000 due to fishing. Currently protected in Brazil, and still very important commercially in Argentina (Murch, 2021).

Due to the precipitous decline in the numbers of a multitude of squatiniform species, the squatinidae has been designated as the second most endangered chondrichthyan family. Consequently, there is an immediate necessity to implement improvements in fisheries and trade management in order to forestall extinctions and facilitate population recovery (Dulvy *et al.*, 2014). It is noteworthy that of the 23 species of Angelsharks described, 13 are classified as threatened according to the IUCN Red List of Threatened Species (Gordon *et al.*, 2022).

Awareness of the species of Angelsharks that exist in other regions provides insight into the factors contributing to the decline in their populations and the critical and imminent danger they currently face. However, the situation of the Angelshark found on the Canary coasts remains unclear.



#### Angelshark in the Canary Islands

The Canary Islands constitute an archipelago in the North Atlantic Ocean, which is part of Spain. The archipelago is composed of eight volcanic islands. The islands of Tenerife, Gran Canaria, La Palma, Fuerteventura, Lanzarote, La Gomera, El Hierro, and La Graciosa, in addition to three islets, comprise the archipelago. The islands of Alegranza, Lobos, and Montaña Clara. Each island exhibits distinct biogeographical conditions, shaped by altitude and the northeast trade winds (Barker *et al.*, 2016).

The Canary Islands encompass a coastline of over 1,500 km<sup>2</sup> and an underwater region of 2,256 km<sup>2</sup> (Pascual, 2004). The waters of the Canary Islands archipelago, situated in the Atlantic Ocean, serve as a cradle for the formation and maintenance of a vast array of marine biodiversity. It is estimated that around 5,232 different species of marine animals inhabit the waters of the Canary Islands (Hernández *et al.*, 2012). Of these, 81 species of elasmobranchs have been reported (Moro *et al.*, 2003), including the common Angelshark (*Squatina squatina*), the manta ray (*Gymnura altavela*) and the horned dogfish (*Sphyrna spp.*).

From a biogeographic perspective, the Canary Islands are part of the Macaronesia region, which comprises four archipelagos: the Azores, Madeira, the Canary Islands, and Cape Verde (Barker *et al.*, 2016). The area is home to a high diversity of fish, with the highest concentration of fish in threatened states in European waters (Nieto *et al.*, 2015).

The Angelshark has exhibited a decline in population density across a significant portion of its range in recent years (Barker *et al.*, 2016), resulting in its categorization as Critically Endangered according to the Red List of Threatened Species of the International Union for Conservation of Nature (Morey *et al.*, 2019). Nevertheless, the species persists in the Canary Islands, underscoring the imperative for the conservation of this last known stronghold to prevent its extinction (Barker *et al.*, 2016).

#### ANGELSHARK (Squatina squatina)

The common Angelshark exhibits a broad, dorsoventrally compressed body, a characteristic shared by all other squaliformes. The head is broad, with small eyes

lacking a nictitating membrane positioned in the dorsal region of the head. Additionally, the nasal barbels are cone-shaped or simple, with a straight tip (Narváez, 2012). The teeth are small and arranged in ten rows per hemimandible, with more than one row being functional (Moreno, 2004).



Figure 5: Drawing of an adult common Angelshark (Gervais, 1877. *Les poissons*)



The dermal denticles observed on the body of the animal vary in type depending on the area being examined (Reif, 1985). The pectoral fins are broad and rounded at the posterior end, while their base extends up to half their length (Narváez, 2012).

The coloration of the back is typically grey to reddish or greenish brown, with scattered blackish and white patches (Compagno *et al.*, 2005). In young individuals, the original coloration is typically obscured by whitish reticules and large, dark patches (Roux 1989; Compagno *et al.*, 2005).

#### Distribution

With regard to its distribution, the species has been recorded in the northeastern Atlantic Ocean, from Norway through Mauritania, the Canary Islands, the Mediterranean Sea, and the Black Sea (Figure 6). However, it is now extinct in many of these coastal areas (Compagno *et al.*, 2005). The Canary Islands represent a singular stronghold for this species, serving as the last known location where they can still be frequently encountered (Gordon *et al.*, 2017).

They inhabit temperate waters at depths ranging from 5 to 150 meters on the continental shelf (Narvaez, 2012). They are typically found in habitats characterized by the presence of sand and mud (Compagno *et al.*, 2005).



Figure 6: Historical distribution of *Squatina squatina* (Source Wikimedia Commons).

### Biology

In terms of reproduction, the number of offspring typically ranges from seven to 25 per litter, with the number of offspring being positively correlated with the size of the mother (Figure 7). This correlation indicates that the larger the mother, the greater the number of offspring she usually has (Roux, 1989; Compagno *et al.*, 2005). Furthermore, it is essential to highlight that the reproductive period of the broodstock extends from eight to 10 months. In the Mediterranean Sea, the majority of pups are born between December and February (Compagno *et al.*, 2005). In the Canary Islands, the peak breeding season occurs between April and July (Meyer *et al.*, 2017). Furthermore, the precise age and periodicity of reproduction, as well as the natural mortality rate, remain uncertain. Indeed, the estimated life expectancy for this species is approximately 15 years, based on data from another species, the Pacific Angelshark (*Squatina californica*) (Cailliett *et al.*, 1992). The majority of their diet consists of rays, flatfish, crustaceans, and mollusks (Compagno *et al.*, 2005). They are known to ambush their prey from the seabed.





Figure 7: Angelshark maturity scale in the Canary Islands (Osaer *et al.*, 2015).

- 1. <u>Juvenile</u>: after birth there is growth without development of the reproductive system.
- 2. <u>Sub-adult</u>: there is growth with development of the reproductive organs until adulthood.
- 3. <u>Adult</u>: there is growth, but it is ready to reproduce.

Angelsharks, like most elasmobranchs, exhibit sexual dimorphism between males and females. In many cases, the pterygopodia are located near the cloacal region in males, whereas in females, they are absent (Figure 8).



Figure 8: Sexual differences in female and male Angelsharks (Source Angelshark (Squatina squatina) necropsy and sample collection protocol).

#### Behavior

The creature is typically observed in a state of repose on the seabed, as illustrated in Figure 9. During the day, it is buried with its eyes protruding. During the night, the species becomes more active, with individuals observed swimming along the seabed. Furthermore, the species exhibits



Figure 9: Adult common Angelshark (*Squatina squatina*) (Source elasmocan.org).

seasonal migration towards cooler waters, with observations indicating that it may migrate northwards during the summer months (Compagno *et al.*, 2005).





#### Threats to their survival

Although the Canary Islands archipelago represents the last remaining habitat for the Angelshark to reproduce, it is not immune to activities that can exacerbate the vulnerability status of this species, as illustrated in Figure 10.

	Categorías de ame	enazas prioritarias		Categorías de amenazas adicionales							
Utilización de los recursos biológicos	Intrusión e interferencia humana	Modificación del sistema natural	Contaminación	Agricultura y acuicultura	Cambio climático y climatología severa	Especies invasoras y otras especies problemáticas, genética y enfermedades	Construcciones residenciales y comerciales	Transportes y rutas de servicio			
Pesca ilegal, no declarada y no reglamentada (IUU, en inglés)	Aumento del número de turistas	Construcciones en el agua que modifican la dinámica costera (p.ej. rompeolas)	Contaminación del agua, incluyendo escorrentía y restos de protector solar	Impacto indirecto de las jaulas de acuicultura	Temperatura del agua en aumento	Patógenos que afectan a la biología	Construcción de edificios e infraestructuras en la costa	Conductos y cableado eléctrico			
Pesca comercial	Interferencia física o daños infligidos por los bañistas		Micro y macro plásticos		Tormentas que destruyen el hábitat	Especies invasoras que conducen a una mayor competición	Construcción de puertos	Resonancia procedente del cableado eléctrico			
Barcos de excursiones de pesca recreativa	Interferencia por parte de los buceadores		Contaminación de plantas desalinizadoras que resulta en la producción de agua hipersalina		Escorrentías de agua desde la isla que destruyen el hábitat						
Pesca recreativa (pesca submarina/ pesca con caña)	Degradación del hábitat de praderas submarinas que resulta en menor sedimentación		Aguas residuales (efluentes sin tratar que llegan al agua)								
Sobrepesca de las presas predilectas de estas especies	Impacto de los bañistas/ actividades en áreas de cría costeras		Derrames de petróleo (durante la perforación o el transporte)								
	Daño a hábitats importantes por las anclas		Antibióticos en el cauce de agua								
	Persecución debido a los ataques de tiburones										

Figure 10: Potential threats identified for the Angelshark in the Canary Islands. Angelshark Action Plan for the Canary Islands (Barker et al., 2016).



#### Legislation and protection figures

The protection of the Angelshark in the Canary Islands is governed by several regional, national and international legislations. These are some of the regulations that protect this species:

- <u>Regional Legislation (Canary Islands)</u>:
  - **Decree 139/2011, of 10 June**: this decree establishes protection measures for endangered species of flora and fauna in the Canary Islands. It includes the common Angelshark in the Canary Islands Catalogue of Protected Species.
- <u>National Legislation (Spain)</u>:
  - **Royal Decree 139/2011 of 4 February**: developing the List of Wildlife Species under Special Protection Regime and the Spanish Catalogue of Threatened Species. The common Angelshark is included in the catalogue as a species "in danger of extinction".
  - Law 42/2007, of 13 December, on Natural Heritage and Biodiversity: this law establishes the bases for the protection of endangered species in Spain, including the protection of their habitat.
- <u>International legislation</u>:
  - **Barcelona Convention (Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean)**: Spain is a signatory to this convention and is committed to protecting endangered marine species.
  - Convention on the Conservation of Migratory Species of Wild Animals (CMS or Bonn Convention):
  - **Bern Convention**: This convention on the conservation of Europe's wildlife and natural habitats includes the Angelshark among the species requiring special protection measures.
- European legislation:
  - Regulation (EU) 2018/120 of 23 January 2018 fixing the fishing opportunities for 2018 in Union waters and, for Union fishing vessels, in certain non-Union waters: this regulation prohibits fishing for Angelshark in all European Union waters, including the Canary Islands. Such regulations are updated annually, but the Angelsharkspecific ban has been a constant.

#### **OBJECTIVE**

The principal objective of this Final Degree Project is to conduct a comprehensive review of strandings, epidemiology and pathologies related to the cause(s) of death of Angelshark (*Squatina squatina*) specimens stranded in the Canary Islands between 2021 and 2024.



#### MATERIALS AND METHODS

The material used for the development of this work corresponds to individuals of the common Angelshark stranded in the Canary Islands between February 2021 and April 2024. The animals once detected are identified and collected but trained personnel of the network "Red Vigía del Gobierno de Canarias". Usually, the animals are preserved frozen and later sent to the Unit of Histology and Veterinary Pathology at the Institute of Animal Health and Food Safety (IUSA), Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, Arucas, Spain, where systematic necropsies are conducted. This work includes the results of 47 Angelsharks stranded in the Canary Islands during this period.

Necropsies were conducted on all animals as long as the necessary conservation and logistical conditions were met. A protocol developed by the team of the Unit of Fish Pathology of the IUSA was employed, which delineates the specific steps to be taken during necropsy of these specimens, with adaptations made to accommodate their unique anatomical characteristics. This approach ensures that the procedure is conducted in a precise and regulated manner.

During the necropsies, samples of each of the organs present in the animal were obtained for histology, if the state of putrefaction allowed it. These samples were fixed in 10% buffered formalin. After that, the samples were processed in accordance with standard procedures.

It is essential to record the date on which the animal carcass was discovered and the date on which it was received at the ULPGC Veterinary School facilities in order to monitor the time taken to perform the necropsy of the animal.

With regard to the weights, the animal's weight in kilograms was determined, and subsequently, the weight of its liver, once removed from the abdominal region. Subsequently, this permitted the calculation of the proportion of the liver weight relative to the total weight of the animal. The length of the animal was measured from the most cranial region to the most caudal region of its body.

The age of each animal was estimated by means of a biomorphometric analysis, which involved the examination of each individual animal in comparison to species data. This approach permitted the delineation of two age categories: baby and adult (Figure 11 and 12).



Figure 11: Baby of Angelsharks (Source Angelshark (Squatina squatina) necropsy and sample collection protocol).



Figure 12: Adult of Angelsharks (Source Angelshark (Squatina squatina) necropsy and sample collection protocol).



The body condition of each animal was established morphologically with reference to anatomical parameters such as the presence of certain prominent bones, the dorso-axial muscular mass, taking account of the species and the age of the animal. Thereby the body condition status was classified as good (grade 1), moderate (grade 2), poor (grade 3) or emaciated (grade 4).

The state of decomposition was classified as very fresh (grade 1), fresh (grade 2), moderate autolysis (grade 3) (Figure 13), advanced autolysis (grade 4) and very advanced autolysis (grade 5) (Figure 14) according to the protocol established by IJsseldijk *et al.*, (2019).





Figure 13: individual of Angelshark in ventral decubitus with a lower degree of decomposition (Source Angelshark (Squatina squatina) necropsy and sample collection protocol).

Figure 14: individual of Angelshark in ventral decubitus with a higher degree of decomposition (Source Angelshark (Squatina squatina) necropsy and sample collection protocol).

With regard to the morphological diagnosis, we describe the findings observed in the individuals included in the study, with the objective of subsequently leading them to a definitive diagnosis and even to evaluate the etiological causes that may have produced these findings in the form of lesions. The aforementioned descriptions can be found in Appendix A (Appendix A shows the full dataset used in this Final Degree Project).





### **RESULTS AND DISCUSSION**

This section will present and interpret the data obtained from the necropsies of 47 Angelsharks that stranded in the Canary Islands between February 2021 and April 2024.

#### **Results per location**

As illustrated in Figure 15, 39/47 (**82.98%**) of the individuals who completed this study were found on the island of Lanzarote, 4/47 (**8.51%**) on Fuerteventura, 3/47 (**6.38%**) on Tenerife, and 1/47 (**2.12%**) on Gran Canaria.

These data reflect that these animals strand mainly in Lanzarote. However, our hypothesis is that the lack or scarcity of Angelshark strandings in the rest of the islands is due to the lack of a stable and standardized elasmobranch stranding service in islands such as Fuerteventura, Gran Canaria and Tenerife.



Figure 15: Distribution of Angelshark strandings by island between February 2021 and April 2024.

#### **Results according to the sex**

With regard to the sex of the animals depicted in Figure 16, it can be observed that 25 of the 47 specimens (53%) were male, 22 (47%) were female. No significant trends were identified regarding the observed sex differences.



Figure 16: Sex determination of the 47 individuals of Angelshark belonging to the study, stranded between February 2021 and April 2024.



#### Results associated to the animal's age

With regard to the age of the animals, 43/47 (91.49%) were adults, 4/47 (8.51%) were shark babies (Figure 17).

Notably, there is a considerable proportion of adults versus babies. In addition, it should be noted that all four shark babies included in the study were identified as female. However, this observation is not sufficiently conclusive to confirm a higher prevalence of strandings in female babies compared to male babies.



Figure 17: Age determination of the 47 individuals of Angelshark belonging to the study, stranded between February 2021 and April 2024.

#### **Results of animal's body condition**

The body condition is reported in the Figure 18. In grade 1 and grade 4 we found 0/47 (0%), in grade 2 there were 6/47 (12.8%), in grade 3 and undetermined 21/47 (44.68%) and 20/47 (42.55%) respectively. We can observe that the most commonly repeated value is grade 3, although we cannot ignore that we have the same number of carcasses with an undetermined body condition.



Figure 18: Determination of the body condition of the 47 individuals of Angelshark belonging to the study, stranded between February 2021 and April 2024.



It is noteworthy that, of the 27 individuals correctly evaluated, none exhibited a body condition that could be classified as "good" (grade 1). This observation may indicate that the animals had a low body condition at the time of death. However, it should also be considered that the body condition of Angelsharks during necropsy may be underestimated. The body condition of an organism may be influenced by a number of factors, including reproductive timing and age (Lloret *et al.*, 2014).

In addition, food availability exerts a direct influence on the body condition of fish, as in mammals, a balanced diet is essential for maintaining a correct body condition. Another factor that should be considered is the environmental condition, as water quality is essential for the proper condition of these animals. A reduction in body condition may be observed in fish as a consequence of alterations in temperature, pH, oxygen levels, or the presence of contaminants, which can result in the onset of stress and disease (Wedemeyer, 1996).

It is important to note that stress is not solely a consequence of environmental changes; it can also be caused by the presence of predators or human handling. In the case of diseases, parasites, bacteria, or viruses, they are capable of reducing the animal's body index, affecting its capacity to metabolize nutrients and feed (Barton, 2002; Stevenson & Woods, 2006).

#### Results related to the degree of decomposition

The degree of decomposition was evaluated using a numerical rating scale of 1-5 (grades 1-5). In the study, 4/47 (8.51%) of the animals were observed to be in a state of very fresh condition, 0/47 (0%) were classified as fresh, 10/47 (21.27%) exhibited moderate autolysis, 16/47 (34.04%) displayed advanced autolysis, 9/47 (19.15%) demonstrated very advanced autolysis, and 8/47 (17.02%) were not evaluable (Figure 19).



Figure 19: Determination of the degree of decomposition of the 47 individuals of Angelshark belonging to the study stranded between February 2021 and April 2024.

It is noteworthy that the four necropsies conducted in situ in Lanzarote the same day (Table A2) when they were discovered showed a minimum degree of decomposition (grade 1). This study confirms that when the discovery and collection of the animal carcass on the beach is delayed, the carcass shows a higher degree of decomposition. If animals are subjected to adverse environmental conditions for a prolonged period of





time, post-mortem phenomena progress rapidly in marine environments (IJsseldijk et al., 2019).

For these reasons, the anatomopathological diagnosis is highly improve when the necropsies are performed at the time and place of discovery with very fresh or fresh animals not previously frozen.

#### **Results of the strands yearly distribution**

Figure 20 illustrates the number of Angelshark specimens that have been stranded each year, providing a quantitative measure of the annual incidence of strandings. A closer examination of the data reveals that the year with the highest number of strandings at our facilities was 2023, with 24 out of 47 (51%) of the specimens included in this study. Moreover, it can be postulated that a comparable pattern of individuals will be observed in 2024, given that in only four months of the year, we have reached the half of the animals found in 2023 (12 animals).

It can be reasonably assumed that the number of deceased animals in 2021 and 2022 was higher than reflected in the data. This evidence reflects that the collection service through the "Red Vigía - Gobierno de Canarias" is currently being more efficient.



Figure 20: Findings by year of the 47 individuals belonging to the study, stranded between February 2021 and April 2024 (2021: 8.33%; 2022: 14.89%; 2023: 50%; 2024: 25%).

Figure 21 presents the number of strandings per month. This figure includes 43 of the 47 animals studied in this study. The four animals that were exempted from this figure did not have a specific date of finding, and thus would not be significant to include in this graph. An exponential trend is observable (41/43, or **95.35%**), commencing in November and declining at the end of April. The maximum peak of strandings is estimated to occur around February or March.





Figure 21: Findings by month for 43 of the 47 individuals belonging to the study, stranded between February 2021 and April 2024 (January: 11.63%; February: 23.25%; March: 23.25%; April: 16.27%; May: 0%; June: 4.65%; July: 0%; August: 0%; September: 0%; October: 0%; November: 9.3%; December: 11.63%).

It is noteworthy that the reproductive phase of these animals typically concludes between January and March in the Canary Islands, coinciding with the period between April and June when offspring are born (Meyer *et al.*, 2017). This heightened incidence during the months of November to April may be attributed to the gestational period of these animals or their overall reproductive stage, potentially resulting in increased energy expenditure that could precipitate these strandings.

- Females: they may appear in these months due to high energy expenditure during gestation or dystocia. In addition, at this time, pregnant females approach the coast to deposit their young, so they may be more exposed to anthropogenic causes of death.
- Males: may appear in these months due to the large energy expenditure involved in courtship and copulation with the female, or due to interspecific interactions with other males seeking the same female.

#### Results related to liver weight and the ratio total body weight/liver weight

The distribution of liver weight in adult Angelsharks (35/47) is presented in Figure 22. It can be seen that most of the liver weights of the adults evaluated in our study (91.4%) is within the range of 0.1 to 0.4 kg (Figure 24). Therefore, it can be concluded that this is a significant range of weights. However, there are three outliers, which are values that deviate from the norm. In this case, the outliers are higher weights (Figure 23). They are observed in the classes with lower frequency. The mean weight of the livers evaluated in this study was 0.23 kg.



Figure 22: Representation of the weights of the livers of 35 adult Angelsharks belonging to the study, stranded between February 2021 and April 2024.







Figure 23: Liver of Angelshark (216/24) weighing 0.6 kg.



Figure 24: Liver of Angelshark (494/24) weighing 0.18 kg.

Figure 25 shows a histogram correlating the liver weights of the Angelshark with respect to the total body weight of each individual (hepatosomatic index). This evaluation shows that the data that deviate the most from the mean are not significantly exaggerated. Therefore, it can be assumed that the ratio of liver weight to total body weight of the animal is usually in the range of approximately **1.96%**.



Figure 25: Representation of the percentages resulting from the relationship between liver weight and total weight of the 35 adults Angelsharks belonging to the study, stranded between February 2021 and April 2024.

Our results suggest that the liver of sharks plays a pivotal role in their survival and its adaptation to the marine environment. In certain species, it can occupy a significant portion of the body cavity (Compagno *et al.*, 2005). In sharks, the liver fulfills a number of functions.

• <u>Buoyancy</u>: The liver of sharks is typically large and contains a high concentration of lipids, particularly a fat called squalene (Compagno *et al.*, 2005). This content is less dense than water, which allows sharks to maintain their buoyancy in water. Furthermore, sharks lack a swim bladder, an organ used



by other fish to control their buoyancy. Consequently, the liver assumes a more crucial role in this function.

- <u>Metabolism and Energy Storage</u>: The liver is capable of storing significant quantities of fats and oils, which are essential for the metabolic processes of sharks. Furthermore, the stored nutrients serve as an energy reserve that sharks can draw upon during periods of food scarcity (Kiessling *et al.*, 2003). This is particularly important in species that undertake long migrations or have erratic feeding habits.
- <u>Detoxification and Immune System</u>: The liver, as in other vertebrates, is capable of filtering toxins and waste products from the blood. Furthermore, the liver plays a role in the synthesis of vital compounds that form part of the immune system, which protects sharks from disease and infection.

#### Results according to the causes of death

The cause of death could be established in 13/47 (**28%**) of the Angelsharks submitted to a standardized necropsy, classified as natural (**17%**) or anthropogenic (**11%**). In 34/47 (72%) animals, the cause of death could not be established (Figure 26.a), probably due to the low state of preservation of the carcass, which was mainly above grade 3 (see Table 3 and Figure 26.b for more detailed information).



Figure 26.a: Pathological entities of the causes of death in 47 individuals belonging to the study, stranded between February 2021 and April 2024. ND: Not Determined.

The Table 3 illustrates the relationship between the 34/47 specimens (72%) whose morphological diagnosis could not be determined, and the degree of decomposition observed in each specimen. It is important to notice that those animals classified in grade 3 and above do not provide feasible tissues to perform morphological and histological evaluation and, therefore, is still highly difficult to provide an accurate diagnosis. As showed in Figure 26.b, a notable **28%** of the studied animals were classified in the maximum grade (5) and other **31%** in grade 4. In grade 3 we found **19%** of the stranded animals. Those animals not rated (21%) could be associated to the initial establishment of the protocol and the standardized procedures for this species.





TABLE 3. Relationship between undetermined morphological diagnoses and the degree of decomposition of 34/47 undiagnosed individuals.

Nº SA	MORPHOLOGICAL DIAGNOSIS	DEGREE OF DECOMPOSITION	Nº SA	MORPHOLOGICAL DIAGNOSIS	DEGREE OF DECOMPOSITION
157/21	ND	4	568/23	ND	3
158/21	ND	4	2//24	ND	ND
1445/21	ND	5	3//24	ND	ND
1446/21	ND	3	4//24	ND	ND
223/22	ND	5	402/24	ND	5
387/22	ND	5	462/24	ND	5
459/22	ND	3	403/24	ND	4
15/23	ND	5	428/24	ND	ND
16/23	ND	5	463/24	ND	4
38/23	ND	4	429/24	ND	3
68/23	ND	4	526/24	ND	5
569/23	ND	3	571/24	ND	3
567/23	ND	4	495/24	ND	4
571/23	ND	5	570/24	ND	4
-	ND	ND	595/24	ND	4
-	ND	ND	-	ND	ND



Figure 26.b. Distribution of the 72% animals without a morphological diagnosis according to the decomposition degree.

In this category of natural deaths, we observe the following:



- Pathologies associated with a significant loss of nutritional status, characterized by a moderate to poor nutritional status and the presence of subacute or chronic organic lesions.
- Pathologies where the Angelshark displays a favorable body and nutritional status, accompanied by acute organic lesions.
- Mortality resulting from interspecific interactions involving trauma with other sharks.

In this study, the etiological diagnoses that correspond to this type of death are as follows (Figure 27): 1/8 infectious processes (12.5%); 5/8 not determined (62.5%); 1/8 interspecific interaction (12.5%); and 1/8 caused by dystocia (12.5%). The limited number of animals evaluated and diagnosed in their totality renders the data insignificant. Further investigation is necessary to better determine the causes of death in these animals.



Figure 27: Etiological diagnoses of 8/47 individuals belonging to the study, diagnosed with natural death, stranded between February 2021 and April 2024. ND: Not Determined.

**Cases of Natural Deaths.** The following section will present a series of cases in which natural pathologies has been observed in Angelsharks.

1. <u>127/22</u>: Infectious process (finding of oophoritis).



Figure 28 and 29: Multifocal fibrinohemorrhagic oophoritis in both ovaries of an individual diagnosed with natural death.

A 13 kg adult female was discovered on the coasts of Tenerife. Upon necropsy, a chronic multifocal fibrino-hemorrhagic oophoritis with adhesions was observed, which is defined as a fibrino-hemorrhagic ovarian inflammation affecting both ovaries.

2. <u>200/24</u>: Liver and spleen neoplasia. Spiral valve parasites.





Figure 30 and 31: Liver and spleen neoplasia in an individual diagnosed with natural death.

The adult male animal, weighing 9.3 kg, was discovered on the coast of Lanzarote, where the necropsy was conducted. Its condition was thus exceptionally fresh, allowing for the identification of well-defined, focal lesions with relief in the spleen and liver, which were determined to be neoplasms. Furthermore, parasites (cestodes) were identified within the spiral valve of the small intestine (Figure 32 and 33).



Figure 32 and 33: Presence of cestodes in the small intestinal spiral valve of an individual diagnosed with natural death.

3. <u>266/24</u>: Gastric impaction, presence of gastric ulcers and spiral valve parasites.



Figure 34: Gastric impaction in an individual diagnosed with natural death.





A 13 kg adult female was discovered stranded on the coast of Lanzarote. Upon necropsy, a gastric impaction was identified, which is defined as an excessive accumulation of food in the stomach that frequently results in severe gastric dilatation (Figure 34). In addition to the aforementioned primary finding, multifocal lesions were observed on the gastric wall, which were determined to be gastric ulcers (Figure 35 and 36).



Figure 35 and 36: Gastric ulcers in an individual diagnosed with natural death.

In the category of anthropogenic deaths, the present study shows the specific causes that resulted in the deaths of the five individuals whose etiological diagnosis of death was anthropogenic interaction (TABLE A2). As illustrated in Figure 37, two distinct etiological causes or diagnoses can be identified in 5/47 (10.64%) individuals whose death was determined to be anthropogenic. Of the five individuals, four died as a result of interaction with fishing, representing 80% of the total. One individual died as a result of trauma caused by boats, representing 20% of the total. It is important to note that not all boats are associated with fishing. In fact, many are used for recreational activities and tourism.



Figure 37: Relationship of anthropogenic deaths with their etiological entity in 5 of the Angelsharks submitted to the study, whose cause of death could be diagnosed.

A historical review of fisheries data reveals that Angelsharks have been frequently caught as bycatch. Fishing techniques such as longlines, traps, and trawls are highly dangerous and harmful to this species. It is important to note that, despite the prohibition of targeted fishing in numerous locations, bycatch remains a significant concern in the present day.

Trawl nets have a detrimental impact on Angelsharks due to their habitat preference for the seabed. Additionally, the trauma associated with being caught on fishing boats is a significant concern. Bycatch in fisheries targeting other species results in high mortality rates (Compagno *et al.*, 2005). Trawl nets have been banned in the Canary Islands and Angelsharks are protected and not commercial fishes.



**Cases of Anthropogenic Deaths.** The following section will present a series of cases in which anthropogenic mortality has been observed in Angelsharks as a consequence of interactions with fishing activities.

1. <u>494/24</u>: A 10.6 kg adult male was discovered off the coast of Lanzarote. Upon external examination, an elliptical perforating wound with inflammation, moderate to severe degeneration, and necrosis of muscle fibers was observed. These findings indicate that the perforation of a common fishing tool, a hook, was the cause of the injury (Figure 38, 39 and 40).



2. <u>233/23</u>: A 10.5 kg adult male was discovered stranded on the coast of Lanzarote. As illustrated in Figures 41 and 42, the animal exhibited erosive lesions on the skin, which may have been caused by entrapment in fishing nets.



Figure 41 and 42: Presence of erosive skin lesions due to the interaction of fishing nets with the individual.





3. <u>364/23</u>: An 11 kg adult male was discovered on the coast of Lanzarote. Upon external examination, a perforating wound of approximately 3 cm in length and elliptical morphology was observed (Figure 43 and 44). This wound exhibited moderate to severe degeneration and necrosis in muscle fibers. Furthermore, the formation of this wound resulted in the generation of secondary bacterial contamination.



Figure 43 and 44: Presence of a perforating wound of elliptical shape with well-demarcated edges in the dorsal musculature of the individual.

4. <u>565/23</u>: A female adult weighing 11.5 kg was discovered stranded on the coast of Lanzarote with a severe incised contused wound accompanied by partial evisceration of the ovarian follicles and liver (Figure 45 and 46). Additionally, the animal exhibited a complete transverse fracture of the vertebral column with marked displacement of both ends. It is likely that the trauma causing these injuries was produced by a boat.



Figure 45: Carcass of an individual common Angelshark due to collision with a boat.

Figure 46: Partial evisceration of ovarian follicles and liver.



### CONCLUSIONS

- 1. Lanzarote is the island with the most stranded Angelsharks, but the scarcity of strandings in the rest of the islands is probably due to the lack of a stable and standardized elasmobranch stranding service in those islands.
- 2. A considerable proportion of adult Angelshark (91%) are found stranded on the coast of the Canary Islands, with a similar sex ratio (53%) male vs (47%) female.
- 3. An increased trend on stranded Angelsharks is observed from November to April (95%). The maximum peak of strandings occur around February or March. This data may be attributed to the gestational period of these animals or their overall reproductive stage.
- 4. Determination of body condition of stranded Angelsharks has not been fully valid. Other parameters, such as liver weight/body weight ratio, should be recorded to contribute to an overall assessment of Angelshark body condition.
- 5. Degrees of decomposition greater than 3 (moderate autolysis) complicate the pathological diagnosis. In addition, the poor quality of the tissue of carcasses with a high degree of decomposition makes microscopic diagnosis practically unfeasible.
- 6. Fishing interactions or boat trauma were diagnosed in 11% of the stranded Angelsharks. Natural death associated to different pathological processes including: inflammatory and infectious diseases and neoplasia were diagnosed in 17%.

#### **FUTURE PROGRESS**

The establishment and reinforcement of the "Red Vigía of the Gobierno de Canarias" for the collection of dead wildlife in the archipelago has notably increased the collection of Angelshark stranded on the coasts. It would be desirable to strength a standardized system that includes all eight islands and speed up the preservation or the performance of the necropsy procedures so to notably help to increase diagnosis accuracy and consequently support the knowledge of the health status of these species and contribute to management and conservation plans in the Canary Islands.

The study of the health status of elasmobranchs found death in the Canary Islands is a brand new research line of action of the Institute of Animal Health and Food Safety of the ULPGC. Although the biological and ecological characteristics of sharks and rays have been extensively studied, there is still a huge gap in the production of knowledge associated to these species, particularly, on pathological diagnosis. Therefore, it is also a future progress to increase the number of new trained researchers and specialized educative programs to afford this challenge.



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#### **APPENDIX A: DATASET**

Table A1 shows the information corresponding to the 47 animals included in this study: shark reference (HS No.), year of appearance (year), date of finding (N.date (day/month/year)), date of reception at the faculty (N. date (day/month/year)), island (Gran Canaria, Lanzarote, Fuerteventura and Tenerife), weight (kg), liver weight (kg), PT/Liver ratio (%), length (cm), sex (female/male), age (adult/calf), body condition (BC  $\Box$  ND: not determined, 1-4), degree of decomposition (GD 1-5), morphological diagnosis (MD) and etiological diagnosis (ED).

TABLE A1: data based on strandings, and necropsies performed	on 47 angelsharks during the period	d between February 2021 and April 2024.
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Nº SA	YEAR	DATE FOUND	RECEPTION DATE	ISLAND	WEIGHT (KG)	LIVER (KG)	LIVER/ WEIGHT (%)	LENGHT	SEX	AGE	BC	GD	MD	ED
157/21	2021	-	05/02/2021	Gran Canaria	11,8	-	-	114	Female	Adult	-	4	-	
158/21	2021	-	05/02/2021	Fuerteventura	10,6	-	-	113,2	Female	Adult	-	4	-	
1445/21	2021	02/12/2021	15/12/2021	Lanzarote	12,4	-	-	109,5	Female	Adult	ND	5	ND	ND
1446/21	2021	04/12/2021	15/12/2021	Lanzarote	9,6	0,15	1,56	108	Male	Adult	3	3	ND	ND
127/22	2022	-	03/02/2022	Tenerife	13	0,48	3,68	117,3	Female	Adult	2	4	1	А
223/22	2022	07/02/2022	23/02/2022	Lanzarote	6,5	-	-	107,3	Male	Adult	ND	5	ND	ND
233/22	2022	07/02/2022	23/02/2022	Lanzarote	10,5	0,21	1,96	105,2	Male	Adult	3	4	2	В
234/22	2022	07/02/2022	23/02/2022	Lanzarote	11	0,19	1,77	104,1	Male	Adult	3	3	ND	С
387/22	2022	10/03/2022	18/03/2022	Lanzarote	13	-	-	119	Male	Adult	3	5	ND	ND
459/22	2022	-	11/04/2022	Tenerife	13,8	0,43	3,14	-	Female	Adult	3	3	ND	ND

Nº SA	YEAR	DATE FOUND	RECEPTION DATE	ISLAND	WEIGHT (KG)	LIVER (KG)	LIVER/ WEIGHT (%)	LENGHT	SEX	AGE	BC	GD	MD	ED
715/22	2022	06/06/2022	09/06/2022	Tenerife	0,1244	-	-	24	Female	Baby	ND	3	ND	ND
15/23	2023	08/01/2023	10/01/2023	Lanzarote	11	0,22	1,99	107	Male	Adult	3	5	ND	ND
16/23	2023	09/01/2023	10/01/2023	Lanzarote	13	0,27	2,11	117	Female	Adult	3	5	ND	ND
38/23	2023	11/01/2023	19/01/2022	Lanzarote	11,2	0,19	1,73	111	Female	Adult	3	4	ND	ND
68/23	2023	24/01/2023	25/01/2023	Lanzarote	9,5	0,10	1,09	110,7	Male	Adult	ND	4	ND	ND
565/23	2023	16/02/2023	23/02/2023	Lanzarote	11,5	0,30	2,63	-	Female	Adult	ND	3	3	D
569/23	2023	18/02/2023	23/02/2023	Lanzarote	13	0,28	2,13	107,5	Male	Adult	2	3	ND	ND
246/23	2023	09/03/2023	09/03/2023	Lanzarote	-	-	-	112	Male	Adult	3	1	-	-
567/23	2023	02/03/2023	22/03/2023	Lanzarote	10,8	0,15	1,38	-	Male	Adult	3	4	ND	ND
364/23	2023	05/03/2023	22/03/2023	Lanzarote	11	0,25	2,25	113	Male	Adult	3	4	4	Е
570/23	2023	15/03/2023	22/03/2023	Lanzarote	0,12	0,00	2,67	23,5	Female	Baby	3	4	5	F
571/23	2023	27/03/2023	30/03/2023	Lanzarote	14	0,22	1,56	120,5	Female	Adult	2	5	ND	ND
564/23	2023	28/03/2023	30/03/2023	Lanzarote	11,8	0,20	1,71	111	Female	Adult	3	4	6	G

Nº SA	YEAR	DATE FOUND	RECEPTION DATE	ISLAND	WEIGHT (KG)	LIVER (KG)	LIVER/ WEIGHT (%)	LENGHT	SEX	AGE	BC	GD	MD	ED
-	2023	02/04/2023	26/04/2023	Lanzarote	10,7	0,17	1,60	111	Female	Adult	-	-	-	-
566/23	2023	15/04/2023	26/04/2023	Lanzarote	0,153	0,0043	2,81	24,8	Female	Baby	3	3	ND	ND
-	2023	18/04/2023	26/04/2023	Lanzarote	10,2	0,25	2,49	112,5	Male	Adult	-	-	-	-
-	2023	19/04/2023	26/04/2023	Lanzarote	9,9	0,18	1,86	112	Male	Adult	-	-	-	-
568/23	2023	21/06/2023	22/06/2023	Lanzarote	11,9	0,21	1,79	116	Female	Adult	3	3	-	-
2/24	2023	29/11/2023	18/12/2023	Lanzarote	10,6	0,16	1,56	108	Male	Adult	-	-	-	-
3/24	2023	29/11/2023	18/12/2023	Lanzarote	13,3	0,23	1,71	118,9	Female	Adult	-	-	-	-
4/24	2023	19/11/2023	18/12/2023	Lanzarote	11,8	0,14	1,22	115	Male	Adult	-	-	-	-
200/24	2024	08/02/2024	09/02/2024	Lanzarote	9,3	0,16	1,74	108	Male	Adult	2	1	7	-
201/24	2024	09/02/2024	09/02/2024	Lanzarote	-	0,20	-	117	Male	Adult	3	1	-	-
402/24	2023	27/12/2023	07/02/2024	Lanzarote	10,6	0,19	1,83	102	Male	Adult	ND	5	ND	ND
462/24	2023	15/12/2023	07/02/2024	Lanzarote	13,2	0,26	1,97	115,4	Female	Adult	3	5	-	-
403/24	2024	08/02/2024	21/02/2024	Lanzarote	10	0,20	2,04	105,5	Male	Adult	ND	4	ND	ND

Nº SA	YEAR	DATE FOUND	RECEPTION DATE	ISLAND	WEIGHT (KG)	LIVER (KG)	LIVER/ WEIGHT (%)	LENGHT	SEX	AGE	BC	GD	MD	ED
428/24	2024	10/02/2024	21/02/2024	Lanzarote	12,7	0,18	1,40	120	Male	Adult	ND	-	-	-
463/24	2024	14/02/2024	21/02/2024	Lanzarote	8,5	0,20	2,34	103	Male	Adult	2	4	-	-
429/24	2024	04/03/2024	07/03/2024	Lanzarote	10	0,14	1,40	108	Male	Adult	ND	3	-	-
288/24	2024	07/03/2024	07/03/2024	Lanzarote	-	0,2166	-	118	Female	Adult	-	1	8	-
526/24	2024	23/03/2024	10/04/2024	Lanzarote	8,5	0,14	1,68	110,5	Male	Adult	ND	5	-	-
494/24	2024	01/04/2024	10/04/2024	Lanzarote	10,6	0,18	1,72	111	Male	Adult	ND	-	9	Н
571/24	2024	20/01/2024	12/04/2024	Fuerteventura	13,5	0,61	4,52	115,3	Female	Adult	3	3	-	-
495/24	2023	02/11/2023	12/04/2024	Fuerteventura	0,1235	-	-	-	Female	Baby	3	4	-	-
570/24	2023	01/12/2023	12/04/2024	Fuerteventura	10,2	0,12	1,13	108,5	Male	Adult	2	4	-	-
586/24	2024	25/04/2024	08/05/2024	Lanzarote	13	0,24	1,87	117,5	Female	Adult	3	4	10	-
595/24	2024	28/04/2024	08/05/2024	Lanzarote	12	0,25	2,12	114,4	Female	Adult	3	4	-	-

#### Morphological diagnosis (MD):

- 1. Chronic multifocal multifocal fibrinous-hemorrhagic oophoritis with adhesions. Moderate diffuse fibrinous cellulitis.
- 2. Hydroceloma. Mild intestinal cestodiasis. Moderate diffuse testicular hemorrhage. Bilateral gill congestion/hemorrhage.
- 3. Severe incised-contuse wound. with evisceration. Complete transverse fracture of the spine with marked displacement of both ends. Partial evisceration of ovarian follicles and liver.
- 4. Perforating wound of elliptical morphology and linear clean edges. Moderate-severe degeneration and necrosis of muscle fibers with moderate muscle hemorrhages and secondary bacterial contamination.
- 5. Partial amputation of the caudal peduncle. Loss of soft tissue in the left cephalic region.
- 6. Gravid uterus with three fetuses. Presence of a fetus in the birth canal.
- 7. Neoplasia in spleen and liver.
- 8. Gastric impaction and gastric ulcers.
- 9. Perforating wound of elliptical morphology, with inflammation. Moderate-severe degeneration and necrosis of muscle fibers with moderate muscle hemorrhages.
- 10. Gastric impaction and parasites in spiral valve.

#### **Etiological Diagnosis (ED)**:

- A) Infectious process
- B) Interaction with fishing activities
- C) Interaction with fishing activities
- D) Trauma (boat collision).
- E) Interaction with fishing activities
- F) Interespecific interaction.
- G) Dystocia.
- H) Interaction with fishing activities



Angelshark (Squatina squatina)

IA	TABLE A2. Fathological entities of death of the 47 Angelsharks included in the necropsy study from February 2021 to April 2024.											
Nº	<b>PATHOLOGICAL ENTITIES</b>	N°	<b>PATHOLOGICAL ENTITIES</b>	N°	PATHOLOGICAL ENTITIES							
157/21	ND	565/23	Anthropogenic death	200/24	Natural death							
158/21	ND	569/23	ND	201/24	ND							
1445/21	ND	246/23	ND	402/24	ND							
1446/21	ND	567/23	ND	462/24	ND							
127/22	Natural death	364/23	Anthropogenic death	403/24	ND							
223/22	ND	570/23	Natural death	428/24	ND							
233/22	Anthropogenic death	571/23	ND	463/24	ND							
234/22	Anthropogenic death	564/23	Natural death	429/24	ND							
387/22	ND	-	ND	288/24	Natural death							
459/22	ND	566/23	Natural death	526/24	ND							
715/22	Natural death	-	ND	494/24	Anthropogenic death							
-	ND	-	ND	571/24	ND							
15/23	ND	568/23	ND	495/24	ND							
16/23	ND	2//24	ND	570/24	ND							
38/23	ND	3//24	ND	586/24	Natural death							
68/23	ND	4//24	ND									

TABLE A2. Pathological entities of death of the 47 Angelsharks included in the necropsy study from February 2021 to April 2024.

\*(those individuals indicated in blue are those that were necropsied on the island of Lanzarote).

Nº	Weight (Kg)	Liver weight	% Liver/Total weight	Nº	Weight (Kg)	Liver weight	% Liver/Total weight
		(Kg)				(Kg)	
1446/21	9,6	0,15	1,56	-	9,9	0,18	1,86
127/22	13	0,48	3,68	568/23	11,9	0,21	1,79
233/22	10,5	0,21	1,96	2//24	10,6	0,16	1,56
234/22	11	0,19	1,77	3//24	13,3	0,23	1,71
459/22	13,8	0,43	3,14	4//24	11,8	0,14	1,22
15/23	11	0,22	1,99	200/24	9,3	0,16	1,74
16/23	13	0,27	2,11	402/24	10,6	0,19	1,83
38/23	11,2	0,19	1,73	462/24	13,2	0,26	1,97
68/23	9,5	0,10	1,09	403/24	10	0,20	2,04
565/23	11,5	0,30	2,63	428/24	12,7	0,18	1,40
569/23	13	0,28	2,13	463/24	8,5	0,20	2,34
567/23	10,8	0,15	1,38	429/24	10	0,14	1,40
364/23	11	0,25	2,25	526/24	8,5	0,14	1,68
570/23	0,12	0,0032	2,67	494/24	10,6	0,18	1,72
571/23	14	0,22	1,56	571/24	13,5	0,61	4,52
564/23	11,8	0,20	1,71	570/24	10,2	0,12	1,13
-	10,7	0,17	1,60	586/24	13	0,24	1,87
566/23	0,153	0,0043	2,81	-	12	0,25	2,12
-	10.2	0.25	2.49				

TABLE A3. Measurements of total weights, liver weights and % ratio (individuals marked in blue are hatchlings).

\*(Individuals marked in blue are offspring, whose weights were not included in the liver measurements).