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FROM PAST TO FUTURE: EXPLORING TOOLS FOR THE STUDY OF SHARKS' POPULATIONS OF THE MEDITERRANEAN SEA

Ph.D. candidate

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ABSTRACT

Shark conservation must become one of the priorities for several biodiversity hotspots of the world, including the Mediterranean Sea, where the decline of the populations has been documented for several species of large predatory sharks and urgent measures need to take place. The major threats that sharks are facing in the area and identified by the IUCN are bycatch, pollution, habitat loss, habitat degradation, and human disturbance. The life-history traits of most of the shark species (late maturity and low fecundity) are also factors that aggravate the effects of these threats.

In the Mediterranean Sea live nearly fifty species of sharks. In the 2016 IUCN regional assessment of the Mediterranean Sea, 57% of the species in the area are considered as threatened and 25% are listed as data deficient, which means that there is a lack of data to assess the local status of their populations. One of the most common and widespread problem in making assessments and consequential protection measures on sharks worldwide, but especially in the Mediterranean Sea, is the lack of data. Scientific campaigns and fisheries information seems not to have enough observation effort to collect data on large sharks, and especially for those species that inhabit high seas. Sharks seem to be at present time one of the rarest and elusive species in the area and new strategies need to take place. In that view, both historical ecology and genetics, as well as Citizen Science could be important tools for supporting data collection and try to make a clearer picture of the historical and present situation in terms of conservation of several species in the area.

We focused our historical and genetics studies on the species *Carcharias taurus* (Rafinesque, 1810), commonly known as sand tiger shark. In the Mediterranean Sea this species was last seen in 2008 and probably faced in the area an early strong decline due to his ecological vulnerability on coastal human impact, especially due to coastal fisheries, and the long history of exploitation of the region. Furthermore, we investigated the genetic characterization of this species in the Mediterranean Sea, using museum specimens preserved in European museums. This allowed the recognition of three haplotypes already known in the literature and present in South Africa and Brazil. We hypothesize that the climate changes that occurred during the interglacial phases of the Pleistocene have led to an attenuation of the cold Benguela current, allowing the passage of *C. taurus* specimens from the Indian Ocean to the Atlantic, and then up to the Mediterranean Sea.

Then, to support the collection of contemporary records of sharks in the area, we took part in the SharkPulse project (http:\\sharkpulse.org). SharkPulse aims to collect image-based sightings of sharks

from around the world, and works through mobile and web applications, together with the use of several social networks. Worldwide sharkPulse has aggregated over 12200 sighting records of 367 species, and for the Mediterranean Sea near 1000 records of 35 species. First explorative analyses of Mediterranean data reveal interesting patterns of distribution of the records. Rare and endangered species have been observed, with the possibility to detect last strongholds on particularly endangered species. These data are also valuable to track the presence of juveniles in order to detect possible nursery areas, and they underline the negative impact of both professional and recreational fishing activities on sharks in the area.

Furthermore, attitudes on ocean users, and especially scuba divers, to be involved in shark-related citizen science programs were tested. A global web survey was run in the most popular social networks' scuba diving pages, and then in two locations in southern Africa (Ponta Do Ouro, Mozambique, and Umkomaas, South Africa) data were collected through face-to-face interviews. Among ocean users, scuba divers are a group of people that can be a good target for citizen science programs, and sharks are one of the most wanted group of animals for them to observe underwater. This attraction holds great potential for shark-related citizen science programs. Due to the charm that sharks have on scuba divers, shark-related citizen science programs could be an opportunity for the diving industry to develop sustainable economies oriented toward conservation and educational programs. This process may benefit from professional figures that may act as a link between diving centers, scuba divers and scientists. These figures can create an organic framework involving customers and creating a relationship of trust. Scuba divers are aware of shark conservation status and are prone to contribute to scientific projects, thus their contribution could be of great scientific value.

This work put in light how the integration and coordination of different scientific fields and expertise can efficiently contribute to researches on sharks, one of the most iconic and endangered group of animals in the world, providing important outcomes to improve conservation actions.

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This Ph.D. has been possible thanks to many people who have crossed the path of their life with it in several ways in the last three years and a half.

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1. INTRODUCTION

Among all groups of animals, sharks are one of the most threatened and shark conservation seems to be one of the priorities for several biodiversity hotspots of the world, including the Mediterranean Sea (Dulvy et al. 2008, 2014). The International Union for the Conservation of Nature (IUCN) has declared that globally one-third of more than one thousand shark and ray species assessed are threatened with extinction (Cahmi et al. 2009), one of the highest proportions observed compared to other groups (Figure 1). Furthermore, for 40% of the species, it has been declared that there are not enough data to assess their conservation status and they have been labelled as data deficient. The major threats identified by the IUCN that sharks are facing in the area and are bycatch, pollution, habitat loss and degradation, and human disturbance (Cahmi et al. 2009). The life-history traits of most of shark species (late maturity and low fecundity) are also factors that aggravate the effects of the bycatch (Castro et al. 1999; Cortés 1999; Cavanagh et al. 2007; Myers et al. 2007).


More than 26500 species of plants and animals
Are threatened with extinction
That is more than 27% of all assessed species

Among sharks and rays:
- 1090 species assessed
- 1/3 threatened
- 40 % DD (Data Deficient)

Figure 1. Sharks are one of the world most threatened group of animals (source iucnredlist.org)

Above all, the Mediterranean Sea is a peculiar area where the decline of the populations has been documented for several species of large predatory sharks up to the 96-99% and urgent measure need to take place (Ferretti et al. 2008). Nearly fifty species of sharks live in the Mediterranean Sea, although for some of them the presence in the area is uncertain (Serena et al. 2014). The 2016 IUCN regional assessment of the Mediterranean Sea includes 40 species of sharks (Dulvy et al. 2016).

Between them, 12 are listed as Critically endangered, six as Endangered and five as Vulnerable. This is relevant because those three categories define the Threatened species, and it means that 23 species (57% of the total assessed) are considered as Threatened. Only seven species are Not Threatened (two Near Threatened and five Least Concern) and 10 species are listed as Data Deficient, that means that there is lack of data to assess the local status of their populations (Figure 2).

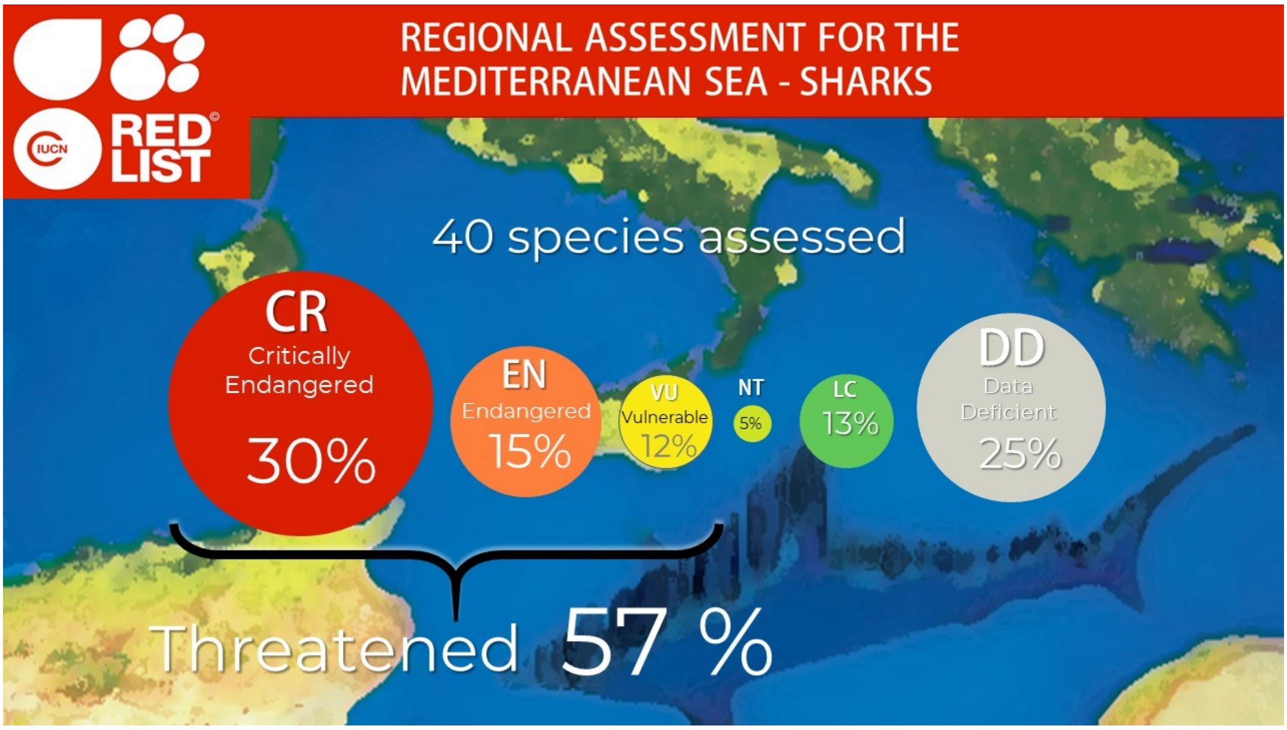

Figure 2. IUCN regional assessment of sharks for the Mediterranean Sea (Dulvy et al. 2016)

One of the most common and widespread problem in making assessments and consequential protection measures on sharks worldwide, but especially in the Mediterranean Sea, is the lack of data. Scientific campaigns and fisheries information seems not to have enough observation effort to collect data on large sharks, and especially regarding those species that inhabit high seas (Ferretti et al. 2008, 2010; Camhi et al. 2009). This is particularly important in order to set proper stock management and coordinate regional policies for the protection of these endangered species. In the last two decades, some attempts have been done to set regional conservation actions and plans, but it seems they are still not yet effective to stop the human impact on local populations (Serena et al. 2014).

1.1 REGIONAL PROTECTION POLICIES IN THE MEDITERRANEAN SEA

The concerns raised by the scientific community on the status of the Mediterranean sharks' populations and of the Mediterranean fisheries in general, have led to some important regional protection instruments. Examples include the Bern Convention on the Conservation of European

Wildlife and Natural Habitats (Bern Convention), the Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean Sea (Barcelona Convention), and the United Nations Action Plan for the Conservation of Cartilaginous Fishes in the Mediterranean Sea. The first two are agreements between states that lie on the Mediterranean coasts, and the third forms part of a United Nations program in cooperation with the International Union for Conservation of Nature (IUCN) (Cavanagh et al. 2007). The protection of sharks is not only part of these regional agreement, but also implemented in several other global agreements that have to be applied in the Mediterranean Sea, such the United Nations Convention on the law of the Sea (UNCLOS), the Bonn convention on the conservation of migratory species (CMS), and the convention on international trade in endangered species of wild fauna and flora (CITES).

Regarding the regional conventions that concerns on the Mediterranean Sea area (Table 1), the Bern Convention has the aim to protect either habitat or species where cooperation between states is required (e.g. transboundary important natural sites and migratory species). In October 2019, 45 member states of the Council of Europe (the leading human rights organization in Europe) and 5 non-member states have signed and ratified it. According to the list of strictly protected fauna species compiled by the Bern Convention, the basking shark (*Cetorhinus maximus*) and the great white shark (*Carcharodon carcharias*) are the only two listed elasmobranchs (Bern Convention, Annex II). A second list of generally protected fauna species includes the shortfin mako (*Isurus oxyrinchus*), the porbeagle (*Lamna nasus*), the blue shark (*Prionace glauca*) and the angelshark (*Squatina squatina*) (Bern Convention, Annex III). For the strictly protected species, each contracting party must adopt laws and regulations to avoid all forms of deliberate capture and killing. Any exploitation of the wild fauna mentioned in the list of generally protected species shall be regulated in order to avoid population depletions. The strictly protected species of the Bern Convention are similar as those featuring in the EU Habitats Directive's Annex II. For these species, core habitats have to be designated as sites of community importance, which must be managed in accordance with the species' ecological needs (Council Directive 92/43/EEC). The Barcelona Convention was born in 1976 as an agreement between states that face the Mediterranean Sea in order to fight marine pollution (Council Decision 77/585/EEC). In 1995, it started a second phase of the convention, and it was amended with the addition of special protocols. One of these special protocols concerns the protected areas and the biological diversity (UNEP(OCA)/MED IG.6/7). This protocol shows a list of endangered and threatened species and a list of species whose exploitation is regulated. These lists were updated in 2013 (decision IG 21/6 of the 18th meeting of the Contracting Parties of the Barcelona Convention) and contained a total of 14 either endangered or threatened shark species, and nine shark species for regulated exploitation (Table 1). The Barcelona Convention has been ratified by 22 parties, including states of Europe, Middle East and Africa. Unfortunately, only a few of them have applied legal protection to the species listed, and population declines for many species are continuing without any management (Serena et al. 2014).

Table 1. Regional conventions for the protection of sharks in the Mediterranean Sea and species listed

Convention	Annex	Listed species
Bern Convention on the Conservation of European Wildlife and Natural Habitats	Annex II (Strictly protected)	*Carcharodon carcharias* *Cetorhinus maximus*
	Annex III (Generally protected)	*Isurus oxyrinchus* *Lamna nasus* *Prionace glauca* *Squatina squatina*
Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean Sea, Protocol concerning Specially Protected Areas and Biological Diversity	Annex II (Endangered or threatened species)	*Carcharias taurus* *Carcharodon carcharias* *Cetorhinus maximus* *Galeorhinus galeus* *Isurus oxyrinchus* *Lamna nasus* *Odontaspis ferox* *Oxynotus centrina* *Sphyrna lewini* *Sphyrna mokarran* *Sphyrna zygaena* *Squatina aculeata* *Squatina oculata* *Squatina squatina*
	Annex III (Exploitation regulated)	*Alopias vulpinus* *Carcharhinus plumbeus* *Centrophorus granulosus* *Heptranchias perlo* *Mustelus asterias* *Mustelus mustelus* *Mustelus punctulatus* *Prionace glauca* *Squalus acanthias*

In parallel to conventions, international action plans have been created in the area of the Mediterranean Sea. This is because in 1999, the Food and Agriculture Organization of the United Nations (FAO) endorsed the International Plan of Action for the Conservation and Management of Sharks (IPOA–SHARKS). The objective of the IPOA-SHARKS is to ensure the conservation and management of sharks and their long-term sustainable use, with focuses on legal instruments for protection, monitoring and management of shark fisheries, landings data collection, developing scientific and educational programs. The IPOA–SHARKS recommends that all states contributing to fishing mortality on an elasmobranch species or stock should participate in its management, and should develop a National Plan of Action for the Conservation and Management of Sharks (NPOA-SHARKS). Since October 2019, only 33 states have globally produced a NPOA-SHARKS (www.fao.org). None are Mediterranean states.

However, a regional Mediterranean Plan of Action was developed in 2003, and revised and implemented in 2009, with the aim to identify specific measures for improving conservation and sustainable management of sharks in the Mediterranean Sea (UNEP-MAP RAC/SPA, 2003). This plan contains a list of measure that the states should consider in their management of sharks like, as mentioned before for the global plan, legal protection for endangered species, habitat protection and MPAs to support shark conservation, fisheries management and monitoring, scientific research and education. In the last assessment of the plan, the overall situation in the Mediterranean Sea seems to be quite far away from the recommended one, and there are still things to work on, especially in terms of regional coordination and cooperation (UNEP(DEPI)/MED WG.331/Inf.13, 2009). In the framework of the IPOA-SHARKS also the European Union ratified an Action Plan (EC Plan of Action, 2009) focused on sharks on February 2009. The aim of the EC Plan of Action is to collect data on shark fisheries and the role of sharks in the ecosystem, promote sustainable shark fisheries and regulate their by-catch, and coordination between the internal and external European Community fishery policy for sharks (EC Plan of Action, 2009). At present time sharks seem to be one of the rarest and elusive species in the area and new strategies need to take place for improving conservation and management. In that view, both historical ecology and genetics, and citizen science could be important tools for supporting data collection and try to make a clearer picture of the situation in terms of conservation of several species in the area.

1.2 HISTORICAL PRESENCE AND SHIFTING BASELINES IN SHARKS' POPULATIONS OF THE MEDITERRANEAN SEA

The presence of sharks in the Mediterranean Sea has been documented since early human presence in the area. Sharks archeological remains dating back to the Bronze age have been found in Italy, Turkey and Lebanon (Van Neer et al. 2005; Van Neer & Waelkens 2007; Martin 2013). Among the remains found in a Neolithic site of a coastal community of fishermen in Spain, sharks' discarded teeth were found together with fish remains (Roselló-Izquierdo et al. 2015). Later, elasmobranchs' representations started to appear in manufactures from Greek and Roman period: examples are a crater from Ischia dated VIII BC and mosaics from Aquileia and Pompeii (Figure 3, Mojetta et al. 2018).

Figure 3. Roman mosaics in Pompeii (Italy). Among the sea creatures represented, two dogfishes (*Schyliorhinus* spp.) and a common torpedo (*Torpedo ocellata*) are clearly recognizable (photo: pinterest.com).

Sharks sometimes also appear in the Greek mythology as man-eater monsters and Leonidas, a Greek poet, reported the death of a sponge diver after the attack of a big shark (Mojetta et al 2018). Aristoteles wrote about sharks both in his "*Historia animalum*" and "*De Generatione Animalum*", and Pliny the Elder described in the Ist century CE in his "*Naturalis Historia*" interactions between divers and sharks. From the middle age, due to the general decline of culture in the area of the Mediterranean, written documentation on nature observations become generally scarce and mixed with mysticism and superstition. However, from the 15th century with the beginning of Renaissance, interesting descriptions of marine species including sharks started again to be produced: an example is the *Historia animalum* written by Gessner (1620), that includes the description of 13 shark species.

Human presence and impact along the coasts of the Mediterranean Sea started deep in the past, and its effects on coastal and marine ecosystems are difficult to describe (Lotze et al. 2006; Bekker-Nielsen & Casasola 2007). Elasmobranches are one of the most vulnerable groups of animals to human impact due to their life-history traits and biology (Myers et al. 2007) and in the area of the Mediterranean Sea, populations have probably been affected since early times. Presence and distribution of some coastal and benthic elasmobranchs have been so strongly affected by human impact that species considered now rare or not part of the local fauna were present in the past, and sometimes widespread (Ferretti et al. 2015; Fortibuoni et al. 2016). Sawfishes (*Pristis* spp.) were not considered by some authors as part of the Mediterranean fauna, since from the modern era no sightings were available, but researches on historical records and museum specimens (Ferretti et al 2015) revealed past presence of two species in the area: large-tooth sawfish (*Pristis pristis*) and small-tooth sawfish (*Pristis pectinata*). Similarly, angelsharks (*Squatina* spp.) are very rare along the coast of the Mediterranean Sea and considered now commercially extinct and extirpated in several sectors of the area such as the Adriatic Sea, the Tyrrhenian Sea, the Sicily Strait and the Aegean Sea (Ferretti et al. 2008; Fortibuoni et al. 2016; Giovos et al. 2019). Despite that, angelsharks were very common in the coastal ecosystems of the Mediterranean, and since the 1950s' also targeted by dedicated fisheries (Fortibuoni et al. 2016). Zogaris et al. (2012) describes a wide shark presence in the Aegean Sea recorded in footages from an old documentary dated 1942 and hypothesizes that shifting baseline syndrome affects the general perceptions on the presence of sharks in the Mediterranean Sea. Marine populations may have declined before any possibility of scientific records, and modern perception of abundance and distribution of the species may be affected and distorted by this fact: this is the foundation of the theory of the shifting baseline syndrome as suggested by Pauly (1995). Since its long history of human presence that predates scientific monitoring, in the Mediterranean Sea scientific knowledge has a strong probability to be impacted by shifting baselines, and among all group of animals, due to their vulnerability to human impact (Myers et al. 2007), sharks are one of the best candidates for being affected.

1.3 THE DECLINE OF SHARK POPULATIONS IN RECENT TIMES AND EFFECTS ON ECOSYSTEMS

Fisheries reports started to be produced in the middle of the 20th century (Ferretti et al. 2008). This practice allows the production of more precise elaborations describing trends of the global shark populations. In several cases a population decline in large sharks has been observed worldwide. Oceanic whitetip shark (*Carcharhinus longimanus*) in the Gulf of Mexico between 1950s and 1990s declined by > 99 % (Baums & Myers 2004), and in the same area, large coastal elasmobranch species between 1972 and 2002 declined by 96–99% (Shepherd & Myers 2005). Decline (>75%) of several large shark species has also been observed between 1986 and 2001 in the northwestern Atlantic Ocean (Baum et al. 2003). In the Mediterranean Sea, steep declines in pelagic sharks' abundance have been documented in several sectors of the area such as Adriatic Sea, Ionian Sea, Tyrrhenian Sea, Spain, and Malta. Decline up to 99% has been observed for five big sharks that include hammerhead (*Sphyrna* spp.), blue shark (*Prionace glauca*), mackerel sharks (*Isurus oxyrhinchus* and *Lamna nasus*) and common thresher shark (*Alopias vulpinus*), indicating a loss of big marine predators in the area (Ferretti et al. 2008).

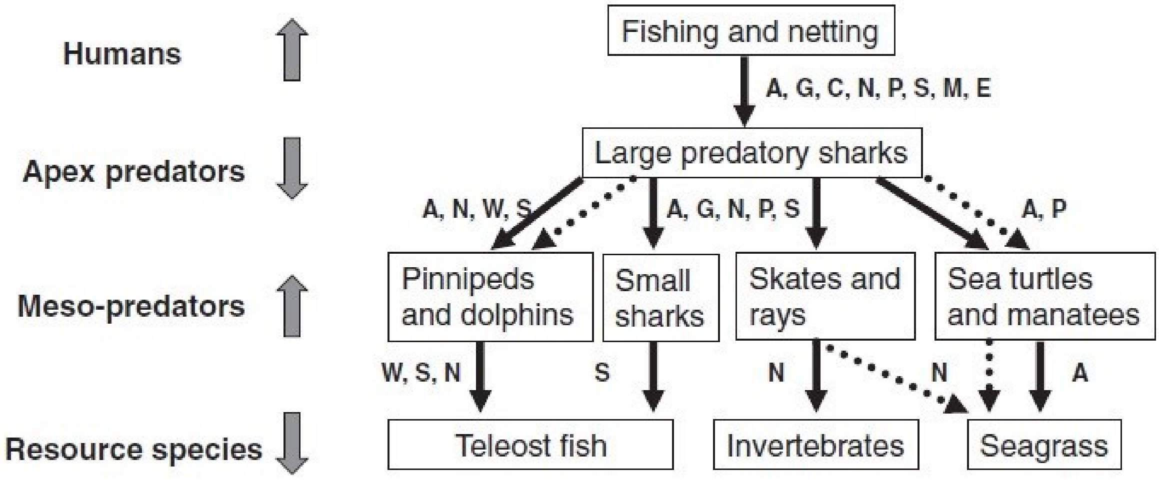

Figure 4. Ecosystem effects of depletion of large sharks. Solid arrows are trophic dynamics and dotted arrows behavioral interactions. Block arrows represent the overall population trend. Letters indicate regions in which particular interactions have been documented (A, Australia; C, Caribbean; E, Europe;

G, Gulf of Mexico; M, Mediterranean Sea; N, North American East Coast; P, Central Pacific; S, South Africa; W, North American West Coast). From Ferretti et al. (2010).

Predatory decline causes consequences in marine ecosystems. Trophic cascades are affected by predator depletion with a general "downgrading" (increase in the proportion of lower trophic level species) and with a decrease of the stability of the communities (Baum & Worms 2009; Estes et al. 2011; Britten et al. 2014). Big sharks occupy generally high trophic levels and are at the top of the trophic nets (Cortés 1990). Shark decline, and especially the decline of big species, has caused consequences in the affected marine ecosystems. It has been globally observed that depletion of big predatory sharks has caused an increase in abundance and distribution of small elasmobranchs, turtles, and marine mammals (Ferretti et al. 2010). As shown in figure 4, meso-predators may benefit by the elimination of apex-predators, with negative consequences on the lower trophic levels, where there are species that are resources for fisheries (Ferretti et al. 2010). Trophic cascade effects are not yet well understood and described (Ferretti et al. 2010), but evidence of changes in communities and ecosystems has been observed. In 50 years, from 1950 to 2000, longline surveys in the tropical Pacific has shown decline in catches of large pelagic predators (including sharks) and increase in catches of pelagic stingrays (*Pteroplatytrygon violacea*) (Ward & Myers 2005). In the Eastern coast of the US, between 1970 and 2005, Myers et al. (2007) reported a decline in large sharks and simultaneously an increase of small elasmobranchs and rays: predation on the bay scallop by the cownose ray has generate a strong local population decline. In the Adriatic Sea, a sector of the Mediterranean Sea, changes in the community has been observed since the decline of big shark in the 19th and 20th centuries that has led to a general increase of the meso-pelagic community in the early 20th century to a community dominated by smaller and less productive species in the later 20th century (Ferretti et al. 2013).

1.4 POPULATION DECLINE AND GENETICS

Since their important ecosystem role, shark conservation must be a priority, particularly for big pelagic sharks, that have shown to be the most endangered group (Ferretti et al 2008; Cahmi et al. 2009). Big pelagic sharks are high mobile species (Stevens 1990, Mancusi et al. 2005) and big sharks' populations has shown worldwide both isolation and interconnection. Genetics has a crucial role in describing this kind of relationships. It was observed that the Mediterranean blue shark (*Prionace glauca*) population has a certain grade of genetic flux with the Atlantic one (Leone et al 2017). On the other hand, the sand tiger shark (*Carcharias taurus*) has shown high isolation among its populations

(Ahonen et al. 2009). Genetics may also help in understanding how to dispersions of the species occurred: the Mediterranean white shark (*Carcharodon carcharias*) has shown genetic affinities with the indo-pacific population, suggesting a possible way of colonization from South Africa and then through the western coast of Africa, during shut down of the Benguela barrier in glacial oscillation of the Pleistocene (Gubili et al. 2010). Defining the grade of connectivity among populations is very important to set conservation plans and management actions since it allows to define if each population should be managed and monitored separately (Gubili et al. 2015). Fishing pressure and population decline have effects on genetic diversity (Spielman et al. 2004; Pinsky & Palumbi 2014), and the general decline of sharks' populations observed in the area of the Mediterranean Sea has genetic insight that needs to be investigated.

1.5 FUTURE DIRECTIONS IN SHARK SCIENCE

Despite the general increase of scientific researches and the numbers of campaigns launched in order to collect data, sharks still remain a group of animals for which is generally claimed a lack of information on distribution and abundance (Dulvy et al. 2014). This is particularly true for the Mediterranean Sea where official data collected by the biggest scientific surveys (e.g. GRUND, MEDITS) suffer a chronic lack of information on big shark species for several reasons that may include the fishing gear used (trawl) and the actual rarity of the species (Ferretti et al. 2008; Ferretti et al. 2013). On the other hand, official fisheries reports (i.e. FAO) are not enough to establish any kind of stock management and lack of taxonomic resolution (Cashion et al. 2019). In order to collect data on such rare and elusive species like big predatory sharks, new strategies must be considered. In this view, the growth of citizen science, that involve people in collecting and sharing data with scientists (Silvertown 2009), could be a great opportunity for shark science. Sharks are charismatic animals (Lucrezi et al. in review), and ocean users (e.g. fishermen, surfers, scuba divers) are prone to report encounters. The number of ocean users out on the sea every day offers to science an observation effort that has never been available before. New technologies are great candidates to play a big role in the future for creating links between people and scientists (Bonnie et al. 2014) and the growth of the smartphone and internet technologies (Orams & Lück 2014) could boost this process.

On that general overview, this dissertation explores several tools including historical ecology and genetics, and citizen science as instruments for supporting data collection on Mediterranean sharks. All together these approaches can outline a clearer picture of the past and present situation in terms of conservation for several shark species in the area of the Mediterranean Sea and provide hints for further deeper evaluations.

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2. AIM OF THE STUDY

This study aims to explore different scientific methods that can contribute to improving knowledge of Mediterranean sharks with possible conservation outcomes.

The Mediterranean Sea has a long history of exploitation, and sharks' population has been affected since early times by human presence in the area. Any study on sharks in the area can't overcome this aspect. Therefore, this study is divided into two sections based on two perspectives: historical and contemporary.

In the historical section, the studies are focused on a single species used as a model. The sand tiger shark (*Carcharias taurus*) was chosen because it is a species highly endangered in the area and possibly locally extinct. It is also a species of global interest: population decline has been observed in several parts of the world and conservation actions have been developed to protect it. Two methods were investigated: historical ecology and historical genetics. It was observed if the integration of the two methods can contribute to improving knowledge on ecological, biological and biogeographical aspects useful to make a clearer picture of the history of the species in the area, and if the information collected could be a starting point for conservation developments.

In the contemporary section, it was investigated in more general terms if new tools can be developed to collect data on sharks in the area. This because there is a general lack of information on the presence and distribution of most of the species. New technologies can play a major role in it and ocean users could be a good source of information through a citizen science process. The section is divided in a general introduction on the state of art of citizen science on shark (globally and in the area of the Mediterranean Sea), a section on a pilot project for the collection of records and first outcome, and a section where attitudes in shark conservation and participatory research were tested in a typical group of ocean users (scuba divers).

3. STUDYING SHARKS IN THE MEDITERRANEAN SEA FROM AN HISTORICAL PERSPECTIVE

3.1 RECONSTRUCTING THE HISTORY OF THE SAND TIGER SHARK (*Carcharias taurus*) IN THE MEDITERRANEAN SEA

3.1.1 Abstract

Sharks are globally exposed to several anthropogenic threats, which, in many cases, have severely reduced their distribution and have impacted populations. In the Mediterranean Sea, because of its long history of exploitation and the SPI relatively short span of scientific monitoring, reconstructing shark baselines is challenging. Many vulnerable species declined in population abundance and geographic distribution before it was possible to adequately track these changes. Consequently, for many of these species, current conservation assessments are now suffering from a severe case of shifting baseline syndrome, whereby their historical occurrence in the area is questioned.

The sand tiger shark (*Carcharias taurus*) is one of these cases. Characterized by high philopatry, restricted home range, and low interchange between populations, its Mediterranean population may have been severely impacted by the high historical fishing pressure in the region before scientific monitoring began.

In this study, the history of the abundance and distribution of *C. taurus* in the Mediterranean Sea was reconstructed through a comprehensive search of occurrence records in the literature and in museum collections. Between 1810 and 2008, 31 occurrence records and 54 publications provided information on the presence of the species in the area. These records were sparse but systematic over time and indicated occurrence hot spots in the south-western Mediterranean Sea and in the eastern Adriatic Sea.

The presence of ten sightings of juveniles from a total of 18 sightings with length information suggested local parturition. Habitat and extinction models indicate that the area has suitable oceanographic conditions for the occurrence of the species and that the species cannot be considered extinct in the Mediterranean Sea. Our study suggests that there is still hope for the recovery of the species and underlines the crucial role of historical investigations to reconstruct the history of large elasmobranchs in the Mediterranean Sea.

3.1.2 Introduction

As a result of historical and industrial fishing, sharks have shown rapid declines in many sectors of the World Ocean. This is one of the least productive groups of marine animals (Au et al. 2008) and consequently is among the most endangered taxa in the ocean. Globally, three-quarters of elasmobranch species were recently declared at high risk of extinction according to the International Union for Conservation of Nature (IUCN) Red List criteria (Dulvy et al. 2008; 2014). In the Mediterranean Sea, with its long history of exploitation, elasmobranchs are generally more endangered than elsewhere (Dulvy et al. 2016). Over the last 150–200 years, large predatory sharks have shown steep declines in indices of abundance, with some species declining by more than 99% (Ferretti et al. 2008). As a result of the heavy and long-standing historical fishing pressure that characterizes the Mediterranean Sea, marine communities and ecosystems may have been strongly affected before these changes could be detected scientifically (Fortibuoni et al. 2010). This pattern has already been reported for two species of sawfishes that may have become extinct in the Mediterranean region in the 1960s–1970s (Ferretti et al. 2016). These species of elasmobranchs lived in coastal and shallow estuarine waters, which have been characterized by high levels of historical fishing pressure, especially in the Mediterranean Sea (Ferretti et al. 2016). Similarly, occurrence records of the sand tiger shark (*Carcharias taurus*, Rafinesque, 1810) are now extremely scarce and it is unclear whether the species is still present in the area (Serena et al. 2014). The rare occurrence of the sand tiger shark has led researchers to hypothesize that the species is a vagrant from the Atlantic through the Gibraltar Strait (Fergusson et al. 2002).

The sand tiger shark of the family Odontaspididae (hereafter referred to as the STS) is the only living member of the genus *Carcharias* (Compagno 2001). The STS has a worldwide distribution in warm temperate and subtropical seas (Compagno 2001). Females can grow to a total length (TL) of up to 320 cm and reach sexual maturity at 220–230 cm, at the age of 9–10 years old. Males are smaller (300 cm TL) and mature earlier than females, at 6–7 years old and 190–195 cm (Gilmore et al. 1983; Branstetter & Musick 1994; Goldman et al. 2006). The STS is a coastal shark (Haulsee et al. 2016) that spends most of the time in shallow waters near caves and submerged reefs, between the surface and depths of up to 40 m (Otway & Ellis 2011; Smith et al. 2014). Juveniles tend to remain in shallow waters, seldom occurring at depths of greater than 80 m (Otway & Ellis 2011; Teter et al. 2015). The recorded temperature range of the species is 12–26°C, but there is high variability across populations (Lucifora et al. 2002; Smale 2002; Otway & Ellis 2011; Teter et al. 2015). The STS can also live in a wide range of salinities, from the open ocean to estuarine habitats (Gilmore 1993), and its diet is mostly composed of teleosts and other elasmobranchs, like eagle rays and skates (Lucifora et al. 2009). This shark has a biennial reproductive cycle with two pups per litter, one for each uterus; the size at birth is 90–100 cm TL and specimens grow to 120–130 cm TL by the age of 1 year (Gilmore et al. 1983; Branstetter & Musick 1994; Goldman et al. 2006; Bansemer & Bennett 2009). The gestation period is 9–12 months and pups show intrauterine cannibalism and oophagy (Gilmore et al. 1983).

Even if the STS is not a target of commercial fisheries, its biology and geographical distribution make this shark vulnerable to recreational and professional coastal fishing (Otway et al. 2004). The declining status of the species is well documented in North America (Musick et al. 2000) and Australia (Otway et al. 2004). Here, a steep decline has been observed in the local population between 1950 and 2010, and special recovery measures have been taken, including the establishment of a network of 26 marine protected areas (Otway et al. 2004; Lynch et al. 2013). STS populations still occur in Brazil, Japan, South Africa, the north-western Atlantic, and Eastern and Western Australia. They show high isolation and low genetic interchange (Ahonen et al. 2009). STSs tend to aggregate in specific and distinct habitats that the species use seasonally for feeding, mating, giving birth, and as nurseries (Smale 2002; Dicken et al. 2007; Lynch et al. 2013). Local seasonal migrations for reproductive purposes and temperature changes have been documented in the North Atlantic (Teter et al. 2015; Haulsee et al. 2016), south-western Atlantic (Lucifora et al. 2002), South Africa (Dicken et al. 2007), and Western Australia (Otway et al. 2010).

In the Mediterranean Sea, the species was first described in 1810 by the French naturalist Rafinesque-Schmaltz (Rafinesque, 1810), who observed a specimen caught off Palermo, Italy. Currently, the STS is considered part of the Mediterranean fauna but extremely rare (Serena et al. 2014). Based on a few occurrence records, the IUCN assessed the species as Critically Endangered in the region because of its low population productivity and high exposure to human impact (Walls et al. 2015). Historical ecology is a powerful tool to identify ecological changes over a long period of time (McClenachan et al. 2012). Particularly for species considered rare today, historical ecology studies have shown how severe the intergeneration shift in the perception of researchers and ocean users can be in what they consider to be the baselines of populations and ecosystems (Pauly 1995). Recent studies in the Mediterranean Sea have reported how this area may be strongly affected by this shifting baseline syndrome, especially for elasmobranch species (Ferretti et al. 2016; Fortibuoni et al. 2016). In this study, the occurrence of the STS in the Mediterranean Sea was reconstructed by making an unprecedented compilation of occurrence records in the region since the 19th century. These data were used to analyse spatial and temporal patterns of abundance, evaluate environmental suitability of the Mediterranean for the species, and test whether a local population still occurs in the basin.

3.1.3 Materials and methods

Data collections

Historical records and bibliographic accounts on STSs in the Mediterranean Sea were collected through bibliographic research on early naturalist publications, museums catalogues, old fisheries reports, and fish species checklists. Old publications related to STSs in the Mediterranean Sea were searched using several online publication databases (Google books, Biodiversity Heritage Library, and Hathi Digital Library) and using several keyword combinations of common names (e.g. 'sand tiger shark', 'ragged-tooth shark', and 'grey nurse shark'), vernacular and local names found in fish species checklists (Doderlein 1879, e.g. "pisci tauru", "requin taureau"), and the list of scientific names, including old scientific classifications (e.g. '*Eugomphodus taurus*' and '*Odontaspis taurus*'), provided by Ebert and Stehmann (2013), to detect records labelled under these classifications (Table S1). Furthermore, an overview of the ichthyology collections of the main European museums was conducted and their curators were contacted to investigate the STS specimens in their possession (Table S2). STSs could sometimes be confused with the smalltooth sand tiger shark (*Odontaspis ferox* Risso, 1810). These two species come from the same family and have similar body shapes but differ in the position of the first dorsal fin, teeth morphology, and the position and size of the eyes (Compagno 2001). In all cases (bibliographic references and museum collections), we ensured that the records were not misidentifications. For museum specimens, the species identification was cross-checked by the curators and, when possible, by personal (FB) inspection of the specimens (Table S2). For each record, the location and year of the catch, or sighting, were the minimum data collected. For museum specimens, when the collection year was not available we used the year of the published museum catalogue that first mentioned the focal items (e.g. Doderlein, 1879). Similarly, publication year was used to indicate the year of published records that did not report this information for the described sightings or catches (Table S3). When possible, the length (TL) and sex of the individual sightings were collected. A life-stage frequency distribution was then constructed by classifying specimens <120 cm TL as young-of-the-year, females <220 cm TL as immature females, males <190 cm TL as immature males, and the remaining specimens as mature (Gilmore et al. 1983; Branstetter & Musick 1994; Lucifora et al. 2002; Goldman et al. 2006). Some specimens were already reported as juvenile in the record's source publication, and we therefore left them in this class (Doderlein 1879; Fergusson et al. 2002).

Temporal analyses

From the list of reported sightings, an annual time series of the validated records was built (sighting record, SR). In some bibliographic accounts (B), the presence of the STS in the Mediterranean Sea was reported without a precise reference to individual sightings (Table S3). In these cases, the publication years were used as additional sightings and added to the time series for the records (SR + B). With this extended time series, extinction models (Solow 2005) were run to test the hypothesis that the species still exists in the Mediterranean Sea. As previously performed with sawfishes in the region (Ferretti et al. 2016), our hypothesis was tested under three different scenarios: two based on a Poisson point process with constant and exponentially declining sighting rates (i.e. testing the scenario that the population was stable or declining over the considered period, hereby referred to as stationary and non-stationary models), and a non-parametric approach based on the distribution of the most recent sightings (non-parametric model) (Solow 2005; Ferretti et al. 2016).

Spatial analysis

The geographical distribution of the records was used to identify hot and cold spots of occurrence. Areas with higher densities were expected to indicate suitable habitats. For this scope, kernel density maps (Silverman 1986) were built by using the bivariate Kernel density estimators (KDEs):

$$\frac{1}{2\pi n h^2}\sum_{i=1}^{n} exp\left(-\frac{d_i}{2h^2}\right)$$

where *n* is the number of observations at a point (*x,y*) on a plane, di is the distance between the i^{th} observation from the point (*x,y*), and *h* is the smoothing parameter (Worton 1995). The smoothing parameter gives the width of the kernels and its choice depends on the number and spatial distribution of the records (Seaman & Powell 1996; Horne & Garton 2006). There are different methods for calculating *h*. As our sampling size was small the method described by Seaman and Powell (1996) was used to minimize the spatial bias, which avoids any overestimation for sample sizes of fewer than 50 points (Seaman et al. 1999):

$$h_{x,y} = \sigma_{x,y} n^{\frac{1}{6}}$$

where $\sigma_{x,y}$ is the standard deviation for each coordinate vector and $h_{x,y}$ the smoothing parameter for each spatial direction. We caution that this density map is merely illustrative of coastal hot spots where the species has been reported, and not where it occurred, because all of our occurrence records are probably coastal projections of where the animal was caught or seen.

AquaMaps, a commonly used relative environmental suitability model (Guisan & Thuiller 2005; Kaschner et al. 2006) that estimates the presence of suitable conditions for a species from environmental parameters like depth, water temperature, salinity, primary productivity, and association with sea ice or coastal areas (Kaschner et al. 2006), was used to identify suitable habitats for the species in the region. Compared with other species distribution models (such as GARP, MAXENT, and generalized linear and additive models), AquaMaps has been shown to perform well in terms of the prediction of species range, even with limited data (Ready et al. 2010). AquaMaps has been fitted already for multiple fish species, including the STS at a global scale (Kaschner et al. 2016). We started from this global model, which used a global dataset of 942 STS occurrence records (obtained from obis.org), and added 31 new Mediterranean records to understand whether these additional records where congruent with environmental suitability patterns already available for the species, and updated the expectations for the region (Table 2). From occurrence data, AquaMaps defines half-degree latitude and longitude cells in which good environmental conditions for the species are assumed. From the characteristics of those 'good cells', for each environmental parameter considered, a preferred and absolute range is generated (*P* and *A* ranges). The P range is identified between the 25th and 75th percentile of the environmental range of the 'good cells'; the *A* range is defined as the range between the 10th and 90th percentiles. Then, using these ranges, on a half-degree latitude and longitude grid of global (or local) scale, the model identifies the cells with suitable conditions for a certain species. In cells where environmental variables fall within the P range, the probability that the habitat is suitable for the species is set to 1. Outside the *P* ranges, the habitat suitability probability is assumed to decrease linearly towards the *A* range limits. Beyond these limits, the habitat suitability probability is set to zero. The *A* and *P* ranges used for each environmental variable for the STS in the Mediterranean Sea are listed in Table 1. The overall relative habitat suitability P_c in a given half-degree cell is then the product of each individual environmental predictor:

$$P_c = P_{c,depth} \times P_{c,temperature} \times P_{c,salinity} \times P_{c,primary\ production}$$

For our analysis, depth, temperature, salinity, and primary production were considered. Ice concentration was not considered as ice does not occur in the Mediterranean Sea nor in the temperature range of the species. As mentioned before, our records are possibly coastal projections of the sightings. The STS is a very coastal species (Otway & Ellis 2011; Smith et al. 2014 and all coastal 'good cells' selected for modelling fell within the preferred depth range of the species. Environmental data for AquaMaps are listed on a Half-Degree Cells Authority File (Kaschner et al., 2016). The data source of each predictor is listed in Table 1. Ready et al. (2010) and Jones et al. (2012) provide further information on environmental data collection and elaboration.

Table 1. The *A* and *P* ranges used for each environmental predictor for the sand tiger shark (STS) in the Mediterranean Sea, and source. The values after running AquaMaps with our new Mediterranean findings are set in bold.

Environmental predictors	A-min	P-min	P-max	A-max	Data source
Depth	1	15	25	191	ETOPO 2, NOAA (2006)
	1	**15**	**25**	**191**	
Sea Surface Temperature	11	15.29	27.87	29.3	NOAA (2007), following Reynolds
	11	**15.44**	**27.87**	**32.07**	& Smith (1995)
Salinity	22.7	32.47	36.52	39.49	2001 World Ocean Atlas,
	22.7	**32.63**	**37.72**	**39.49**	Conkright et al. (2002)
Primary production	174	441	1918	3830	European Joint Research Council,
	174	**403**	**1849**	**3830**	Ready et al. (2010)

All computations were performed using r 3.4.1. KDE was computated with the mass package. AquaMaps were estimated with the raquamaps package and online resources (www.aquamaps.org).

3.1.4 Results

Data summary

Overall, 54 publications, between 1810 and 2008, were found to indicate the presence of the STS in the Mediterranean Sea (Supporting Information Table S3); 31 were actual occurrence records (Table 2 and Figure 1). Of these occurrence records, 15 came from museum collections and 16 were catches reported in scientific reports or observed in fish markets. The earliest evidence of the presence of the species in the Mediterranean Sea comes from a tooth found in a Neolithic site (10 000–4500 bc) of north-eastern Spain (Cova Fosca, Ares del Maestrat, Castelló; Roselló-Izquierdo et al. 2015). This site hosted a community of fishers near saltwater lagoons. The STS tooth was found with other fish remains related to fishing activity in the area and suggests the presence of the species in the Mediterranean coastal waters of Spain since the end of the last ice age.

Table 2. Historical records of the STS in the Mediterranean Sea.

N	Year	Location	Size (cm)	Sex	Evidence	Reference
1	1810	Palermo, Italy	300		Catch	Rafinesque 1810
2	1820	Alessandria, Egypt	85	M	Museum specimen (Berlin)	P. Bartsch (pers. comm.)
3	1842	Algeria	230	F	Museum specimen (Paris)	Guichenot 1850
4	1842	Algeria			Museum specimen (Paris)	B. Seret (pers. comm.)
5	1879	Palermo, Italy	133	M	Museum report and spec. (Palermo)	Doderlein 1879
6	1879	Palermo, Italy	118	M	Museum report and spec. (Palermo)	Doderlein 1879
7	1879	Palermo, Italy	71	F	Museum report and spec. (Palermo)	Doderlein 1879
8	1879	Palermo, Italy	Juv.		Museum report and spec. (Palermo)	Doderlein 1879
9	1879	Palermo, Italy	Juv.		Museum report and spec. (Palermo)	Doderlein 1879
10	1879	Messina, Italy	170	M	Museum specimen (Florence)	Vanni 1992
11	1881	Budve, Montenegro	100		Catch and museum report (Trieste)	De Marchesettti 1882; Brusina 1888
12	1881	Gradac, Croatia	100		Catch and museum report (Trieste)	De Marchesetti 1882; Brusina 1888
13	1881	Algeria			Museum specimen (Bruxelles)	Pers. obs.
14	1882	Algeri, Algeria			Museum specimen (Copenhagen)	M. A. Krag (pers. comm.)
15	1888	Split, Croatia			Catch	Kolombatović 1894
16	1909	Cagliari, Italy	300		Catch	Carruccio 1910
17	1916	Wied Iz-Zurrieq, Malta			Catch	Despott 1916
18	1926	Malaga, Spain			Catch	Lozano 1928
19	1933	Djerba, Tunisia			Museum specimen (Bruxelles)	Pers. Obs.
20	1945	Golfe d'Aigues Mortes, France			Catch	Granier 1964
21	1967	Lampedusa, Italy			Catch, photo	Carletti 1971
22	1969	Cyprus North Coast			Sighting	Demetropoulos & Neocleous 1969
23	1969	Bay of Izmir, Turkey			Catch	Geldiay, 1969
24	1971	Gulf of Tunis, Tunisia	228	F	Catch	Quignard & Capabé, 1972
25	1976	Sidi Daoud, Tunisia	250	F	Catch	Capabé et al. 1976
26	1977	Mazara del Vallo, Italy	Juv.		Catch	Fergusson et al. 2002
27	1985	Fano, Italy			Catch	Poggiani, 2009
28	1999	Molat, Croatia	380		Catch, photo	Lipej et al. 2004
29	2002	Split, Croatia			Head in a fish market	Lipej et al. 2004
30	2003	Molat, Croatia			Catch	Walls et al. 2015
31	2008	Sorat Bay, Turkey	99.7	F	Catch	Ismen et al. 2009

The first scientific account of the species in historical times comes from Rafinesque in 1810, however, from a specimen collected off Sicily, near Palermo. The species was mentioned several times in the 19th century as part of the Sicilian fauna (Rafinesque 1810; Doderlein 1881; 1879), where it appeared mostly in winter and spring (Doderlein 1879).

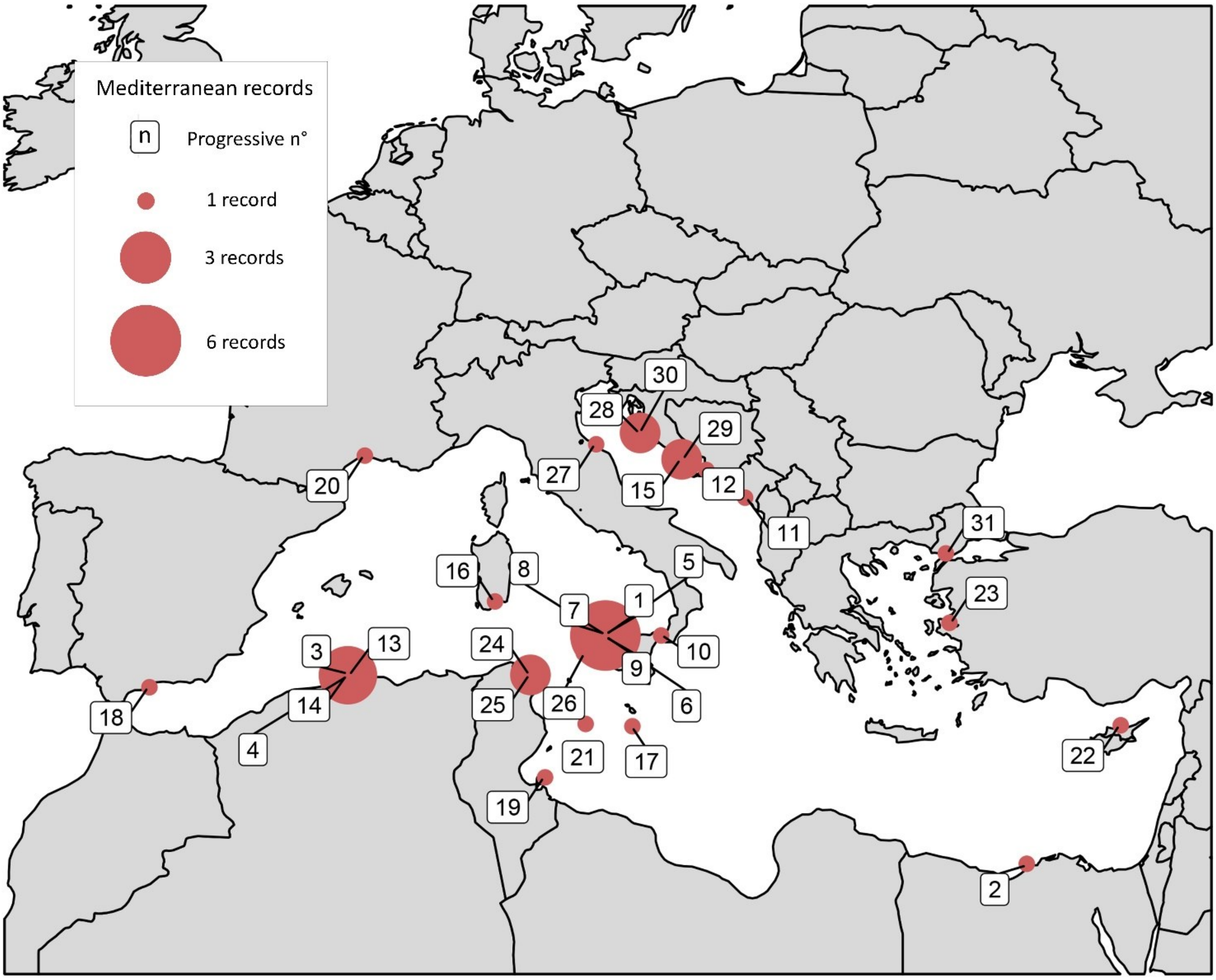

Figure 1. Distribution of the Mediterranean sand tiger shark (STS) records and locations mentioned in the text. The circle size is proportional to the number of records found at each location. Record numbers refer to Table 1.

In this period, Doderlain was very active in the collection of fish specimens from Sicilian waters and some specimens are still preserved in Palermo at the Doderlein Museum (Doderlein 1879; E. Bellia, pers. comm., June 2016; pers. obs.). In 1820, a specimen was collected near Alexandria in Egypt (P. Bartsch, pers. comm., July 2018), and several authors reported the presence of the species in the Mediterranean Sea in the 19th century with increasing frequency (Figure 2; Bonaparte 1846; Müller & Henle 1841; Guichenot 1850; Duméril 1856; Cisternas 1867; Canestrini 1872, 1869; Gervais 1877;

Moreau 1881; Faber 1883; Navarrete 1898). Cisternas (1867) mentioned the species as quite rare in Valencia but more frequent further south in Spain, along the coasts of Cullera, especially near the Jucar river estuary. Unfortunately, the author did not report any specific account of individuals seen or caught. The species was reported four times from Algeria, all from 19th century scientific surveys investigating biodiversity in northern Africa (Guichenot 1850; B. Seret, pers. comm., March 2017; M. Krag, pers. comm., April 2018; O. Pauwels, pers. comm., March 2018). In the Adriatic Sea, De Marchesetti (1882) was the first to report the species with the catch of two juveniles STSs in July 1881 (100 cm TL; Brusina 1888). They were brought to the Trieste Natural Museum, after the Austro-Hungarian Maritime Government set a reward with the aim to reduce the number of dangerous sharks in the Adriatic Sea. A further STS was reported from the area in 1888 (Kolombatović 1894) and Ninni (1912) mentioned the species as part of the Adriatic fauna in the early 20th century.

Whereas in the 19th century, records generally came from museum reports and collections, in the 20th century, the species records are in general more related to catches or sightings (Table 2). At the beginning of the century, two more records were observed in sectors surrounding Sicily, namely southern Sardinia, where the species had never been observed before (Carruccio 1910), and Malta (Despott 1916), where the species was already recorded as present but rare (Giulia 1862). Subsequently, the species was recorded in several zones of the Mediterranean Sea: Spain (Lozano 1928), France (Granier 1964), Turkey (Geldiay 1969), Cyprus (Demetropoulos & Neocleous 1969), Tunisia (Quignard & Capapé 1972) and Italy (Fergusson et al. 2002; Poggiani 2009). All authors reported the species as very rare. Geldiay (1969) recorded STSs in the Bay of Izmir and the Aegean Sea in the late 1960s, and there is also an anecdotal record of a specimen caught in the Dodecanese; however, there is no written documentation for this specimen (M. Corsini, pers. comm., November 2017). Some records appeared again from the Sicilian channel until the 1970s: Lampedusa, Gulf of Tunis, and Mazara del Vallo (Carletti 1971; Capapé et al. 1976; Fergusson et al. 2002;). Carletti (1971) provided the first photographic documentation from Lampedusa: a specimen caught before 1967 between 15 and 65 m in depth on the ‘Secca di Levante’ by purse-seine fishers (S. Carletti, pers. comm., October 2018). This is one of only two photographic proofs of occurrence of the species; the other is more recent from Croatia (Lipej et al. 2004), a specimen of approximately 380 cm TL caught in 1999 off the island of Molat (Croatia).

Only three occurrences have been reported in the 21st century: two from Croatia in 2002 and 2003 (Lipej et al. 2004; Walls et al. 2015), and a juvenile female of 99.7 cm from Sorat Bay (Turkey) in 2008. This is the last sighting of the species in the region (Ismen et al. 2009).

Temporal patterns

The question of whether the species could be considered still present in the Mediterranean Sea was tested based on this time series of records (Figure 2a).

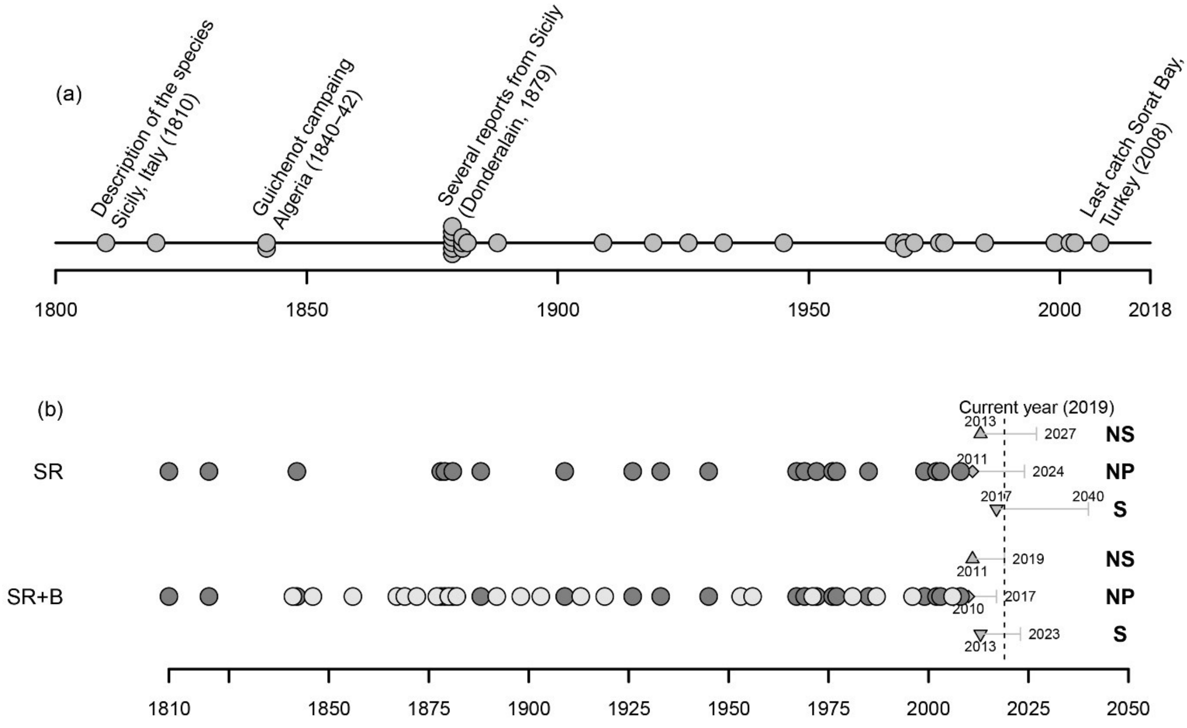

Figure 2. Timeline of records and extinction analysis. (a) Time series of sand tiger shark (STS) records in the Mediterranean Sea. Records occurring in the same year are stacked on top of each other. (b) Sighting records together with the associated estimates of extinction years and upper bounds of their 95% confidence interval. ◇, estimation making no assumption on population (non-parametric, NP); △, estimation under the assumption of a declining population (non-stationary, NS); ▽, estimation under the assumption of a stationary (S) population; SR, modelling the time distribution of sighting records; SR + B, modelling sighting records plus significant bibliographic accounts (for details, see Materials and methods).

These analyses suggest that it was not possible to reject the hypothesis that the species still occurs in the area in 2018; however, there are several publications on the presence of the species in the Mediterranean Sea that do not provide evidence for any specific catch or sightings in terms of year and location, but generally mention the occurrence of the STS in a given area (Supporting Information Table S3). The extinction analysis run with these additional bibliographic accounts suggests that the STS became extinct in the Mediterranean Sea in 2011 on the assumption of a declining population, and in 2010 under the non-parametric assumption, based on the distribution of the most recent sightings (Figure 2b, Table 3).

Table 3. *P* value of the null-hypothesis that the sand tiger shark (STS) is still present in the Mediterranean Sea in 2019. SR, modelling time distribution of sighting records; SR + B, modelling sightings records plus significant bibliographic account (for details, see Materials and methods).

Data	Statistical assumption	p-value
SR	Stationary	0.34
	Non-stationary	0.16
	Non-Parametric	0.15
SR + B	Stationary	0.09
	Non-stationary	0.03
	Non-Parametric	0.02

Spatial patterns

The geographical distribution of the historical records is mostly concentrated in the north-east Adriatic, around Sicily, and the Algerian coast (Figure 3b).

The habitat suitability analysis suggested that the Mediterranean Sea could offer the species suitable conditions of temperature, salinity, and primary production. The global AquaMaps model indicated several Mediterranean sectors with suitable environmental conditions for the STS (Figure 3c). By updating this analysis with our Mediterranean records (Figure 3d), the distribution of suitable habitat changed, with an increase in the absolute probability of habitat suitability, but with a similar profile of relative change across different areas. Two records from the eastern Mediterranean Sea (from Egypt and Cyprus) were in a cold spot of estimated habitat suitability for the species.

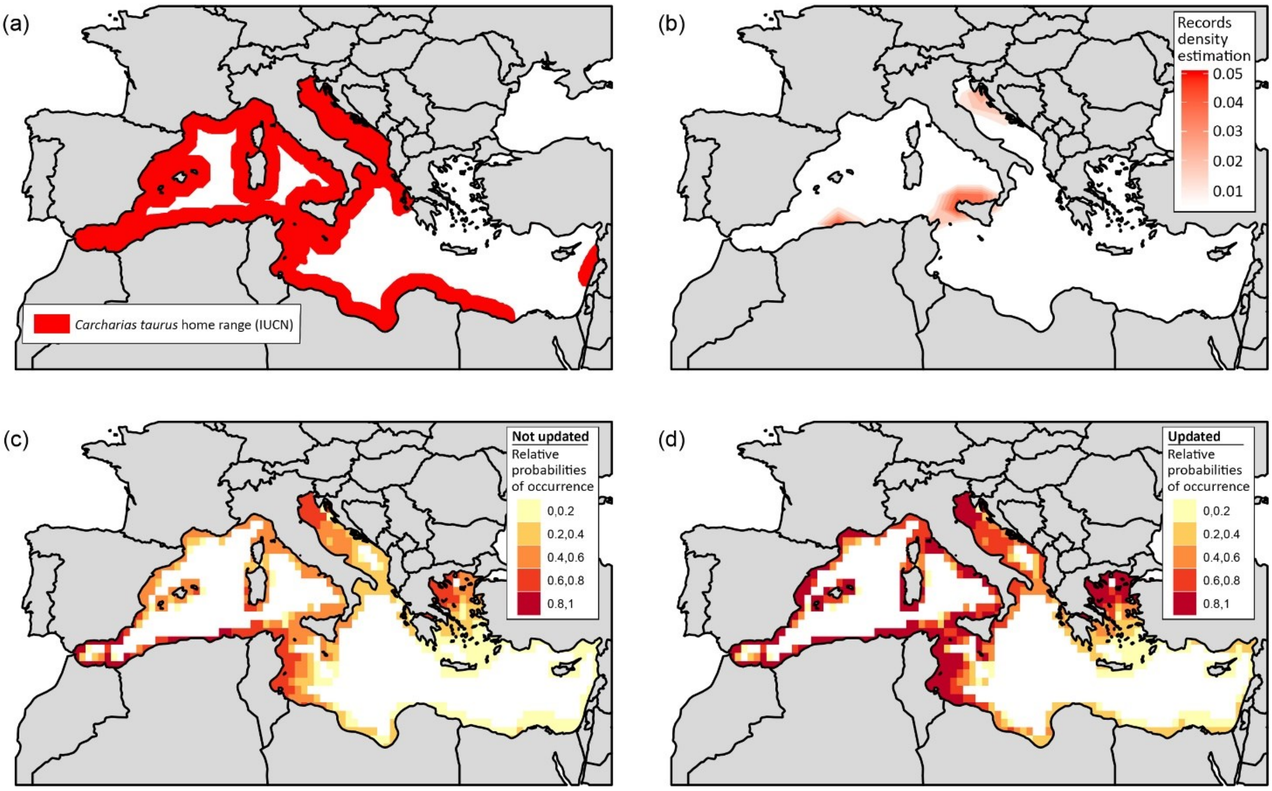

Figure 3. (a) International Union for Conservation of Nature (IUCN) distribution range of sand tiger shark (STS) in the Mediterranean Sea as indicated in the Red List assessment of the Mediterranean Sea (Walls et al. 2015). (b) Density estimation of the geographical distribution of STS historical records in the Mediterranean Sea. The density estimation was performed with a fixed kernel densities model ($h = h_{ref}$). (c) Relative habitat suitability for the STS in the Mediterranean Sea based on AquaMaps run with global data (Kaschner et al. 2016). (d) Updating these AquaMaps with our new findings extends the range of suitable conditions for the STS in the Mediterranean Sea.

3.1.5 Discussion

The reconstruction of the occurrence of the STS in the Mediterranean Sea over the last two centuries provided a detailed list of 31 occurrence records and 54 publications, giving information on the presence of the species in the region and the possible role of the Mediterranean Sea for its life history. These records suggest that at least in the last 200 years the regional presence of the species has been occasional but systematic. Simple habitat models indicated that the Mediterranean Sea was environmentally suitable to host a local population of STSs, although the species has always been described as rare in its waters.

The rarity of the STS in the Mediterranean Sea is possibly the result of a combination of the intrinsic vulnerability of the species to fishing and habitat degradation, especially in coastal ecosystems (e.g.

pollution and coastal urbanization). A long history of exploitation and other human impacts in the region and the lack of ichthyological expertise necessary to monitor the species in historical times may have all contributed to an undetected historical decline of this shark species and its current rarity. The STS is a coastal shark inhabiting relatively shallow waters. This ecology makes the species extremely vulnerable, especially in the Mediterranean Sea, which has been exploited by intensive coastal fishing for millennia (Lotze et al. 2006). Already in medieval times the fishing gear used in the Mediterranean Sea impacted coastal ecosystems, such as the ganguy and oyster dredges and trawls (sometimes banned for their known impact on coastal ecosystems; De Nicolò 2018). Potentially, Roman fishing gear, such as longlines with multiple hooks, dredges, and seine and stationary nets (Bekker-Nielsen 2007), was capable of catching STSs when congregating in shallow waters (Smale 2002; Otway & Ellis 2011; Smith et al. 2014). Unfortunately, fishing gears were seldom reported in the catch records. Only in four cases were fishing gear mentioned: two catches were from trawl nets (Ismen et al. 2009; Poggiani 2009), one from a trammel net (Quignard & Capapé 1972) and another one from a purse seine (S. Carletti, pers. comm., October 2018). Yet historical records of this species may have been sporadic, because the species is easily confused with the congeneric smalltooth sand tiger shark and other large sharks occurring in the Mediterranean Sea, including the white shark. This aspect was already recognized by the ichthyologists of the early 20th century, who noted that the rarity of the STS had more to do with the lack of ichthyological knowledge of the people who may have seen and reported this shark than to its actual level of abundance (Lozano, 1928). In the Northern Adriatic, for example, catches originally reported as white sharks were, on scientific inspection, re-classified as *C. taurus* (De Marchesetti 1882; Kolombatović 1894) (Table 1 and Supporting Information Table S3).

As a result of its rarity and occasional presence, more recent researchers have started to question whether the STS was truly residential in the Mediterranean Sea (Walls et al. 2015; Fergusson et al. 2002). The STS was historically detected in the eastern Atlantic Ocean, from Senegal to Morocco (Cadenat & Blache 1981), and so it is possible that occasional stray individuals entered the Mediterranean Sea from the Atlantic; however, this present study compiled a list of a high number of bibliographic accounts that report the species as part of the marine fauna of the Mediterranean Sea (Supporting Information Table S3) from both the Western (Bonaparte 1846; Duméril 1856; Cisternas 1867; Canestrini 1872, 1869; Gervais 1877; Moreau 1881; Faber 1883; Navarrete 1898; Despott 1916; Lozano 1928; Tortonese 1956) and Eastern Mediterranean (Papakonstantinou 1988; Golani 2006; Hadjichristophoru 2006; Bilecenoğlu et al. 2014). Some of these accounts were supported by evidence of actual catches or sightings (Table 2), and included new-born specimens, which are unlikely to have travelled long distances before capture, and records from the Eastern Mediterranean Sea, prior to the opening of the Suez Canal (another possible gateway from outside the Mediterranean Sea, P. Bartsch, pers. comm., July 2018). Yet, it is unclear whether the Mediterranean STSs are a distinct population or part of a larger north-east Atlantic population also occurring in western Africa.

Historical researchers also reported clues on STS abundance and seasonality, and the specific distribution of sightings is helpful to frame hypotheses on the life history of the species in the region. Together with reported catches, these data informed simple habitat models that identified landscapes of suitable habitats for the species in the basin. The STS is a coastal littoral species preferentially occurring in water depths of 20–40 m (Otway & Ellis 2011; Smith et al. 2014). Thus, even though the catches reported were landings (i.e. essentially coastal projections of the true catch locations, when geolocated), the records were in the locations and depth range typical for this species (for details, see Materials and methods) and thus could be used to fit the AquaMaps model. This analysis suggested that the Mediterranean Sea has temperature and salinity regimes that are within the ranges identified for the species (Lucifora et al. 2002; Smale 2002; Otway & Ellis 2011). Similarly, the Mediterranean coastal morphology is in line with the environmental requirements of the species. Rocky reef shores, caves, and sandy bottoms are frequent along Mediterranean shores, and in particular in the Sicilian channel, along with the North African coasts, in the Eastern Adriatic Sea, and in the Ionian and the Aegean Sea, where our habitat suitability model suggested the highest probability of occurrence of the species. The Eastern Mediterranean Sea was instead considered a cold spot for the species, with the exception of the Aegean Sea in the Dodecanese where a few records were detected. Here, a lower observation effort, low propensity to report sightings because of a lower historical scientific effort, or lower chance to detect finds because of linguistic barriers (Ferretti et al. 2016; Moro et al. 2019).

The density hot spots identified by these spatial analyses may have been important aggregation areas for the species (Figure 3b). Of the 18 records for which we had information on size (Figure 4), seven were juveniles and four were less than 1 year of age (for details, see Materials and methods).

Most of the specimens recorded in Sicily were reported as juveniles or can be identified as newborns or younger than 1 year of age (Table 2). This is also true for two of the six specimens reported from the Adriatic Sea and for two of the records from the Eastern Mediterranean (Table 2). The presence of juveniles would suggest local parturition, especially for a species whose juveniles rarely travel > 50 km (Dicken et al. 2007), and, because the average travel distance of adult STS is 342 km (Dicken et al. 2007), parturition by vagrant specimens is highly improbable. The Gibraltar Strait is 1800 km away from Palermo, 2500 km from the Eastern Adriatic Sea, 3200 km from Saros Bay and 3300 km from Egypt. Travelling these long distances is not expected for a large number of vagrant pregnant females (Dicken et al. 2007).

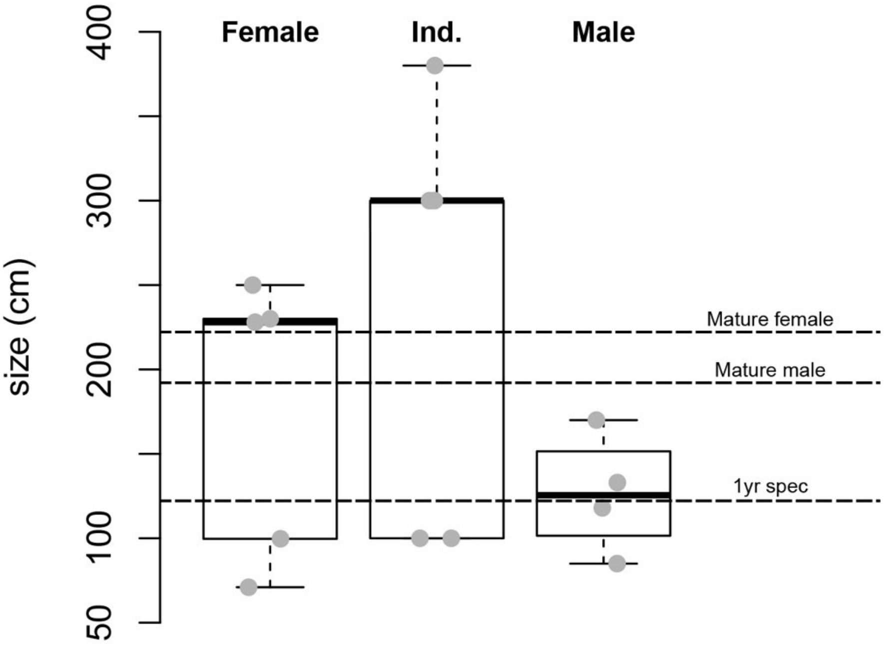

Figure 4. Box plot of size distribution related to the sex of sand tiger shark (STS) Mediterranean records (where size and sex were reported; for details, see Materials and methods). Ind., specimens in which a sex determination was not possible).

By describing STS specimens collected in Sicily (Table 2), the ichthyologist Doderlein (1879) suggested an increasing presence of the species in Sicilian coastal waters and a seasonal occurrence, specifically between winter and spring. It is unclear whether these perceived trends were real or the result of changes in fishing practice (across years and seasons) and observation effort. In South Africa, STSs are known to move to mating sites in winter and then give birth in the spring (Smale 2002; Dicken et al. 2007). This pattern matches Doderlein's seasonal observations and his collection of juveniles (five specimens, still preserved in Palermo Doderlein Museum, pers. obs.). Cisternas (1867) mentioned that the species was frequent along the coast of Cullera (Spain), and especially next to the mouth of the Jucar river. No details of catches, sightings, sizes, or sexes were given, but STS young-of-the-year and juveniles are usually observed in estuarine areas of South Africa (Dicken et al. 2006) and North America, where the species use these habitats as nurseries (Gilmore 1993).

Our analysis on the sighting record time series suggests that we cannot consider the species extinct from the area. Adding relevant bibliographic accounts on the presence of the species in the Mediterranean Sea (SR + B; for details, see Materials and methods) to the time series of records would move the upper confidence limits of the models, as well as the estimates of 2010, as the year of extinction under the assumption of a greater number of unreported sightings in the past (non-parametric analysis), and 2011, under the assumption of a declining population (non-stationary analysis). These bibliographic accounts did not report specific catches, however, and were vague about the actual period when the species was observed (Table S3). Nonetheless, the small number of these sightings supports the IUCN Red List assessment of Critically Endangered for the species in the Mediterranean (Walls et al., 2015). It is extremely important that more dedicated monitoring is employed to ascertain whether the species still persists and, if so, where the last population strongholds are. Opportunistic observations from ocean users (e.g. fishermen, sailors, and divers) could be very useful to detect the remaining specimens still present in the area. Unfortunately, from programmes that are aggregating such data in the Mediterranean Sea, e.g. sharkPulse (http://sharkpulse.org) and Medlem (F. Serena, pers. comm., 2018), no recent observation has been reported, but continuing monitoring is essential.

As for other Mediterranean elasmobranchs, our perception of what is natural for the STS has been changing over time (Pauly 1995; Ferretti et al. 2016; Mojetta et al. 2018). This species was probably confused with other similar large sharks before it was described scientifically, and when taxonomic knowledge was consolidated on this species, the records increased in frequency. The history of the STS in the Mediterranean is very similar to that of the sawfishes in the region (Ferretti et al. 2016). This easily detectable species was considered endemic in the Mediterranean in the late 18th and early 19th century. Then, in the late 19th century and early 20th century, information on its abundance and distribution became contradictory and variable. Eventually, there was increasing concern to protect the last individuals left in the area and finally researchers started to question whether residential populations ever existed in the area (Ferretti et al. 2016). In the northern Adriatic Sea the common angelshark (*Squatina squatina*) has also had a similar history. Data from dedicated fisheries suggest that the species was very common in the 19th and early 20th century. Then in the 1960s its populations collapsed, and currently young fishers in the area do not even know that this species existed at all (Fortibuoni et al. 2016). These shifting baselines (Pauly 1995) occur because species declined in abundance and contracted in distribution before the beginning of scientific monitoring and the available data on their occurrence were properly organized, described and maintained over time

(Michener 2006; Ferretti et al. 2016). These aspects have implications for setting expectations on the occurrence of the species when reconstructing their history. The ecological and biological parameters that we observe and use for habitat models are restricted to the short period of observation and the contracted spatial extent from which historical records are available. This aspect is even more relevant for a species like the STS, which shows a wide range of adaptation to environmental parameters and variability in the optimal temperature range among populations across the world (Gilmore 1993; Lucifora et al. 2002; Otway & Ellis 2011; Teter et al. 2015). Declines of vulnerable elasmobranch species due to habitat loss because of human impacts are probably more common than it is known, especially in areas with a long history and high intensity of human impact, such the Mediterranean Sea (Mojetta et al. 2018). And even in the early stages of scientific observation, early ichthyological accounts were less concerned with abundance than biodiversity or other aspects of fish biology, making historical abundance inferences from these accounts challenging.

Clarifying the history of the STS in the Mediterranean Sea is challenging but has implications for reconstructing baseline and pathways of change of the whole marine ecosystem (Ferretti et al. 2008; Coll et al. 2010). The Mediterranean Sea is a model of how large marine ecosystems can change when subjected to centuries of human impact (De Nicolò 2018). Reconstructing this history is very useful to make predictions in other large marine ecosystems with a shorter history of human impact, but it is challenged by the scant information that we have for periods pre-dating scientific monitoring (Fortibuoni et al. 2010). In-depth historical investigations, consolidation of historical datasets, integrative analyses of multiple datasets, the use of forensic methods on biological remains, and the analysis of patterns detected in other regions can help us to reconstruct this long history (Ferretti et al. 2018). Furthermore, as many species of sharks are becoming increasingly rare in the region, this effort has to be coupled with a close monitoring of fisheries and other biodiversity records available for the region, including strengthening the efforts of citizen science (Bargnesi et al. 2018). This approach is fundamental to reconstruct the baselines of animal populations, communities, and life histories for eventually constructing proper conservation and recovery plans. The recovery of STS populations in the Mediterranean Sea remains possible. In south-eastern Australia, over the last 60 years, the same species went through steep declines through the combined effect of targeted and incidental fishing and beach protective shark meshing. The population abundance was reduced to near extinction levels (as low as 300 individuals in some estimates). Eventually, after the establishment of a network of marine protected areas (Otway et al. 2004; Lynch et al. 2013) recovery was promoted at historical aggregation sites. A similar conservation approach may be proposed for the Mediterranean

Sea. Crucial for the Mediterranean population would be identifying and closely monitoring the remaining individuals and suitable habitats that may still exist in the region. These remnant populations may eventually act as new sources for recovery. Citizen science and other monitoring approaches using new technologies (e.g. social media and smartphone apps) may help in more efficient data collection for identifying these possible strongholds and eventually promote conservation actions (Bargnesi et al. 2019).

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3.1.7 Supporting information

Table S1. List of common, local, vernacular names and old scientific classifications for *C. taurus*.

Type	Names	References
Scientific names	*Carcharias taurus* (Rafinesque, 1810; accepted), *Triglochis taurus* (Muller and Henle, 1837), *Eugomphodus taurus* (Gill 1862), *Odontaspis taurus* (Agassiz, 1838)	Ebert & Stehman (2013)
Old local and vernacular name for Mediterranean Sea	Odontaspe tauro, carcharia tauro, triglochide tauro, pisci cani, pisci tauru, odontaspide taureau, lamio, verdoun, taurus shark	Doderlein (1879)
Actual common names (with the geographic area of use)	Sand tiger shark (global), grey nurse shark (Australia), ragged-tooth shark (South Africa), sand shark (USA), pez toro (Spain), squalo toro (Italy)	Compagno (2001)

Table S2. *C. taurus* specimens preserved in European museums with certain or possible Mediterranean origin. In bold specimens personally inspected by the main author in this study.

N	Museum	Cat. n.	Origin of spec.	Reference	Notes
1	Museo di Scienze Naturali di Trieste	None	Probably Med. (Dalmatia)	De Marchesetti (1982) G. Tomasin pers. comm.	Found in the museum deposit without origin. From literature, in the XIX cent. two specimens where given to the museum (De Marchesetti, 1982)
2	Museo di Storia Naturale "La Specola", Florence	**6373**	Possibly Med.	Vanni (1992)	Jaws from old museum collections
3	-	**6136**	Messina	Vanni (1992)	Dry specimens
4	Museo di Zoologia P. Donderain, Palermo	**AN 68**	Palermo	Doderlein (1879)	Jaws. Sample collected by Doderalain himself from Palermo or surrounding area
5	-	**AN 60**	Palermo	Doderlein (1879)	Jaws. Sample collected by Doderalain himself from Palermo or surrounding area
6	-	**AN 38**	Palermo	Doderlein (1879)	Skeleton. Sample collected by Doderalain himself from Palermo or surrounding area
7	-	**AN 548**	Palermo	Doderlein (1879)	Digestive tract. Sample collected by Doderalain himself from Palermo or surrounding area
8	-	**P 522**	Palermo	Doderlein (1879)	Dry specimens. Sample collected by Doderalain himself from Palermo or surrounding area
9	Muséum national d'histoire naturelle, Paris	A-9685	Algeria	Guichenot (1850) B. Seret pers. comm.	Dry specimens. Collected by Guichenot (1840-42)
10	-	A-9696	Possibly Med.	B. Seret pers. comm.	Dry specimens. Probably form Guichenot campaign (1840-42) since consecutive catalog number (B. Seret pers. comm.)
11	-	AB-0038	Algeria	B. Seret pers. comm.	Jaws. Collected by Guichenot (1840-42)
12	-	AB-0037	Possibly Med.	B. Seret pers. comm.	Jaws. Probably form Guichenot campaign (1840-42)

					since consecutive catalog number (B. Seret pers. comm.)
13	-	AB-0039	Possibly Med.	B. Seret pers. comm.	Jaws. Probably form Guichenot campaign (1840-42) since consecutive catalog number (B. Seret pers. comm.)
14	Stazione Zoologica A. Dohrn, Naples	CHON080	Unknown	A. Travaglini pers. comm.	Jaws. No documentation found.
15	Hydrobiological Station of Rhodes	None	Possibly Med. (Dodecanese)	M. Corsini pers. comm.	Jaws. Old employed at the Rhodes station refer it as from Dodecanese region but any documentation found
16	Royal Belgian institute of natural Sciences, Bruxelles	**507 β**	Algeria	O. Pauwels pers. comm.	Jaws. Donation from Goyen de Hausch, 1881
17	-	**1386 β**	Djerba	O. Pauwlels pers. comm.	Teeth. Misidentified as *Odontaspis ferox* and collected on 1st October 1933
18	Museum fuer Naturkunde, Berlin	ZMB 4532	Alessandria (Egypt)	P. Bartsch pers. comm.	Dry specimen. Collected in Hemprich&Ehrenberg expedition
19	Natural History Museum of Denmark	P2395608	Algeri (Algeria)	M. A. Krag pers. comm.	Jaws. Collected by A. Brun on 1st October 1882

Table S3. Bibliographic reports of STS in the Mediterranean Sea. Year related to the publication of the document. In bold years of significant bibliographic accounts used as additional records with high uncertainty (see methods for details).

N	Year	Document (original title)	Autor(s)	Content related to STS
1	1810	Caratteri di alcuni nuovi generi e nuove specie di animali e piante della Sicilia	Rafinesque S.C.	Description of a ten feet specimen collected off Sicily. First description of the species.
2	1810	Indice d'Ittiologia Siciliana	Rafinesque S.C.	Present in a list of fishes from Sicilian seas and freshwaters.
3	**1841**	Systematische Beschreibung der Plagiostomen	Müller J. & Henle J.	Present in the Mediterranean Sea. Preserved specimens in Berlin, Lyden, London, Paris (no mention on origin).
4	**1846**	Catalogo metodico dei pesci europei	Bonaparte C.L.	Present in a list of fishes from European waters.
5	1850	Exploration Scientifique de l'Algérie: Pendant les Années 1840, 1841, 1842. Histoire naturelle des reptiles et des poisson (Vol. 5).	Guichenot A.A.	Description of a specimen collected in Algeria and sent to Paris at National Museum of Natural History. The author identified it as *O. ferox*. The specimen is still in the collection of the museum and afterward re-identified as STS (*C. taurus*).
6	**1856**	Ichthyologie analytique ou essai d'une classification naturelle des Poissons, à l'aide de tableaux synoptiques	Dumeril A.C.	Morphological description. Present in the Mediterranean Sea.
7	**1867**	Catalogo de los peces comestibles que se crian en las costas españolas de Mediterráneo y en los rios y lagos de la provincia de Valencia	Cisternas R.	Mentioned as rare in Valencia, but more frequent along the coast of Cullera, especially next to the mouth of the Jucar river. Same observation reported both for STS (*C. taurus*) and *O. ferox*.
8	**1869**	Compendio di zoologia ed anatomia comparata	Canestrini G.	Morphological description of the two species of the genus *Odontaspis spp.*, both Present in the Mediterranean Sea.
9	**1872**	Fauna d'Italia; parte terza Pesci	Canestrini G.	Live in the seas around Sicily.
10	1862	Fauna Maltese – Plagiostomi	Giulia G.	Present in Maltese waters but rare.
11	**1877**	Les poisson de mer	Gervais P.	Morphological description. Present in the Mediterranean Sea but quite rare.
12	1878	Prospetto metodico delle varie specie di	Doderlein P.	Present in Sicilian seas.

		pesci riscontrate sinora nelle acque marine e fluviali della Sicilia		
13	1879	Manuale ittiologico del Mediterraneo: ossia sinossi metodica delle varie specie di pesci riscontrate nel Mediterraneo ed in particolare nei mari di Sicilia (Vol. 1).	Doderlein P.	Extensive description of the species, with a list of local and scientific names, and morphological characters. Present in Nice, Algerian coasts and Sicily. Rare. In Sicily recorded mostly in winter and spring. At Palermo museum mentioned as present three dry specimens (one female 71 cm, two males 133 and 188 cm), a skeleton, jaws, two dry digestive systems, a dry brain, and in alcohol a digestive system, one heart, two brains, and one reproductive apparatus.
14	1880	Elenco dei Mamiferi, degli Uccelli e dei Rettili ittiofagi appartenento alla fauna italica e catalogo degli anfibi e dei Pesci italiani	Giglioli E.H.	Present but rare in Italian seas. Specimen in Palermo museum.
15	1881	Alcune generalità intorno la fauna sicula dei vertebrati	Doderlein P.	Sometimes fished in the Mediterranean Sea.
16	1881	Rivista della fauna sicula dei vertebrati	Doderlein P.	Mentioned in a list of rare species sometimes found in Sicilian seas.
17	1881	Histoire naturelle des poisson de la France (Vol. 2)	Moreau È.	Morphological description. Present in the Mediterranean Sea, Nice, very rare. One specimen present in Paris museum and collected by Guichenot campaign.
18	1882	La pesca lungo le coste orientali dell'Adria	De Marchesetti C.	Two *C. taurus* mentioned in a list of fifty-three sharks caught between April 1872 and July 1882 and provided to Trieste museum after the Maritime Government set a reward with the aim to low the number of dangerous sharks in the Adriatic Sea.
19	1882	Essai sur L'histoire Naturelle des Vertebres de la Province et des Departements Circonvoisins: Vertebres Anallantantoidiens (Poissons et Batraciens)	Reguis J.M.F.	Morphological description. Present in the Mediterranean Sea, Nice, very rare.
20	1883	The fisheries of the Adriatic and the fish thereof: a report of the Austro-Hungarian Sea-fisheries, with a detailed description of the Marine fauna of the Adriatic Gulf	Faber G.L.	Two *C. taurus* mentioned in a list of fifty-three sharks caught between April 1872 and July 1882. Same information as from the report of De Marchesetti mentioned above.
21	1888	Morski psi Sredozemnoga I Crljenog mora	Brusina S.	Species quite rare in the Mediterranean Sea. Further information reported on the two specimens mentioned by De Marchesetti and Faber, they were caught respectively on 19th July in Budve (Montenegro) and 25th July in Gradac (Croatia), both around 100 cm.
22	1892	Manuel d'ichthyologie française	Moreau È	Morphological description. Present in the Mediterranean Sea, Nice, very rare.
23	1894	O navodima vrsti meći i kralješnjaka jadranskoga mora	Kolombatović J.	Report of a specimen caught in 1888 around Split.
24	1898	Manual de ictiología marina concretado á las especies alimenticias conocidas en las costas de España é islas Baleares	Navarrete A.	Morphological description. Rare in the Mediterranean Sea but not in the Atlantic Ocean.
25	1903	Ittiologia italiana: descrizione dei pesci di mare e d'acqua dolce	Griffini A.	Morphological description. Rare, found in Nice and Sicily seas, mostly in winter and spring.
26	1910	Sovra un raro Odontaspis taurus (Müll.) Catturato presso il Golfo di Cagliari ed acquistato dal Museo Zoologico della R. Università di Roma.	Carruccio A.	Report of a specimen of about 3 m caught in the Gulf of Cagliari (Sardinia) in January 1909.
27	1912	Catalogo dei pesci del mare Adriatico	Ninni E.	Mention of the two specimens caught in 1881 in the Adriatic Sea and previously reported by De Marchesetti (1882).

28	**1913**	The Plagiostomia: Sharks, skates, and rays	Garman S.	Morphological description. Present in the Mediterranean Sea and the Atlantic Ocean.
29	1916	The Ichthyology of Malta	Despott G.	The authors mention the jaws of a specimen caught some year before 1916 by some of the fishermen of Wied iz-Zurriek
30	1928	Fauna ibérica: Peces	Lozano Rey L.	Extensive description of the morphology of the species based on a specimen caught in Malaga in June 1926. The species is mentioned as present in several locations of the Mediterranean Sea, both in the African and on the European side. Then the author says "It is likely that the species is not so much rare in the Mediterranean Sea as it is claimed and the reason for the low number of citations is easily due to the lack of ichthyological knowledge by the people who may report its presence"
31	**1953**	Catalogue des poisson des côtes Algériennes	Dieuzeide R. & Novella M.P.B.	Present but quite rare in Algeria.
32	**1956**	Fauna d'Italia (Vol. 2)	Tortonese E.	Present in the Mediterranean Sea. It seems rare in Italian waters.
33	1964	Les Euselaciens dans le golfe d'Aigues-Mortes	Granier J.	The species is rare in the area (Aigues-Mortes gulf). Report of one specimen caught on November 1945.
34	**1969**	The fishes and crustaceans of Cyprus	Demetropoulos A. & Neocleous D.	Present but rare in Cyprus. Reported in the North Coast by Andreas Keleshis.
35	**1969**	İzmir Körfezinin başlıca balıkları ve muhtemel invasionları	Geldiay R.	Found in the Mediterranean Sea and collected in the Bay of Izmir and the Aegean Sea
36	**1971**	A list of the fresh and sea water fishes of Greece	Ondrias J.C.	Present in a list of fishes that have been recorded in Greece.
37	1971	Naumachos	Carletti S.	In the book are present two photos of a specimen caught near Lampedusa (Secca di Levante, between 13 and 65 m depth) some years before 1967 (author pers. comm.). The specimen is misidentified as a great white shark, but body color pattern, teeth shape, and relative fins position clearly identify the specimen as STS.
38	1972	Complément à la liste commentée des Sélaciens de Tunisie	Quignard J.P. & Capapé C.	Report of a 2.28 cm female caught by a trammel net near Ras Fartas (Gulf of Tunis)
39	1976	Les Sélaciens dangereux des côtes Tunisiennes	Capapé C. et al.	Report of a 2.50 cm specimen caught off Sidi Daoud (Gulf of Tunis). Catches of the species periodical and rare.
40	**1981**	Requins de Méditerranée et d'Atlantique (plus particulièrement de la Côte Occidentale d'Afrique)	Cadenat J.	The species is known in the Mediterranean Sea and not rare in both Western and Eastern Atlantic Ocean. In the Eastern Atlantic Ocean, the species is observed from Morocco until Southern Africa. In tropical waters it is observed from December to May north of the Equator, and from June to November in the Southern Hemisphere, that may imply some kind of migrations. Report of 111 specimens observed in Joal (Senegal) between December 1948 and May 1949.
41	**1987**	Guide Fao d'Identification des Espèces pour les Besoins de la Pêche Méditerranée et Mer Noire - Zone de Pêche 37 Volume 2: Vertébrés	Fischer et al.	Species mentioned as present in the Mediterranean except for southern France and North Aegian Sea. Occasionally present in fish markets in Morocco, rarely elsewhere, used as bait in Morocco.
42	1989	Les Sélaciens des côtes méditerranéennes: aspects généraux de leur écologie et exemples de peuplements	Capapé C.	The species is a post-glacial immigrant in the Mediterranean Sea, subtropical origin. Very rare and mostly found in the Western Mediterranean.
43	1992	Cataloghi del Museo di Storia Naturale dell'Università di Firenze	Vanni. S.	One specimen mentioned in the collection of Firenze museum, dated 15th November 1979, from Messina (Sicily). Coming from the Zoological Museum of the University of Palermo after an exchange.
44	1996	Commercial landings of sharks within Maltese fisheries 1982-1992	Fergusson I.K. & Marks M.A.	The species should be considered extremely rare in the central Mediterranean and possibly declining in numbers since the 19th Century. The authors report "Franco Cigala-Fulgosi saw only a single *C. taurus* (a juvenile, in 1977) in his monitoring, between 1977 and 1990 of sharks caught within the Mazara del

				Vallo offshore fisheries."
45	1996	The marine ichthyofauna of the Eastern Levant - history, inventory, and characterization	Golani D.	Present in the checklist of Eastern Mediterranean Sea.
46	1999	The Chondrichthyans of the Adriatic Sea	Bello G.	Presence in the Adriatic of the species based on the information provided by Soljan (1975) and Tortonese (1956).
47	2002	Note on the declining status of the sand tiger shark *Carcharias taurus* in the Mediterranean Sea	Fergusson I.K. et al.	Brief note on the presence of the species in the Mediterranean Sea. The species is mentioned extremely rare with few examples of catches. Discarded teeth reported from reef flats off north-west Beirut (Libano).
48	2003	Revision of the records of shark and ray species from the Maltese Islands (Chordata: Chondrichthyes).	Schembri T. et al.	The presence of the species cannot be confirmed in Maltese waters. It must be considered exceptionally rare. Report of a specimen recorded in 2003 off Croatia.
49	2004	Sharks of the Adriatic Sea	Lipej L. et al.	Regarding the Adriatic Sea, the species is nowadays considered to be very rare. Report of a head of *C. taurus* found at a fish market in Split in summer 2002. In the photo plates: photos of an approx. 380 cm *C.taurus* caught in the waters off the Island of Molat (Croatia) in September 1999.
50	2006	Cartilaginous fishes of the Mediterranean coast of Israel	Golani D.	Species present in the list and mentioned as rare.
51	2006	Chondrichthyes in Cyprus	Hadjichristo-phorou M.	Species present in Cyprus with unconfirmed reports from local sources.
52	2009	Length-weight relationships for ten shark species from Saros Bay	Ismen A. et al.	Between 2005 and 2008 a 99.7 cm female was caught in Saros Bay (Turkey)
53	2009	I pesci del mare di Fano	Poggiani L.	A specimen caught in Fano (Italy, Adriatic Sea) between 1985-1987 by a trawl net.
54	2015	*Carcharias taurus*. The IUCN Red List of Threatened Species	Walls R.H.L. & Soldo A.	Following the authors, the last known record from the Mediterranean Sea was a specimen caught in 2003 in waters off the Island of Molat.

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3.2 HISTORICAL DNA AS A TOOL TO GENETICALLY CHARACTERIZE THE MEDITERRANEAN SAND TIGER SHARK (*Carcharias taurus*, LAMNIFORMES: ODONTASPIDIDAE): A SPECIES PROBABLY DISAPPEARED FROM THIS BASIN

3.2.1 Abstract

The sand tiger shark (*Carcharias taurus*) is a coastal species distributed in temperate and sub-tropical waters, classified as "Vulnerable" at global level and "Critically endangered" in Eastern Australia, Southwestern Atlantic Ocean and Mediterranean Sea. Six populations (Northwestern Atlantic, Brazil, South Africa, Japan, Eastern Australia and Western Australia) with low genetic diversity and limited gene flow were identified worldwide, but genetic information for many other geographic areas are still missing. Specifically, this species is listed in several reports as part of the Mediterranean fauna, even if there is a lack of catches and sightings in recent years in this basin. In order to clarify the origin of *C. taurus* individuals caught in the past in the Mediterranean Sea, historical samples were genetically analysed.

Nine samples with a certain Mediterranean origin were collected from different European museums. Genomic DNA was extracted and ~ 600 bp of the mitochondrial DNA control region was amplified using eight overlapping species-specific primer pairs. Sequences obtained were aligned with all the haplotypes globally known so far.

Genetic analysis revealed the misidentification of one museum specimen. Among the remaining Mediterranean historical samples, three different haplotypes were recovered. Two of them previously observed only in South Africa and one described in both South African and Brazilian populations.

Results suggest a genetic relationship between Mediterranean sand tiger sharks and those from the Western Indian Ocean. According to previous studies, we hypothesized that during the Pleistocene the cold Benguela upwelling barrier was temporarily reduced allowing the passage of *C. taurus* individuals from the Indian to Atlantic Ocean. After the restoration of this phylogeographic barrier some individuals were trapped in the Atlantic Ocean and probably migrated northward colonizing the Western African coasts and the Mediterranean Sea.

3.2.2 Introduction

The sand tiger shark (*Carcharias taurus* Rafinesque, 1810) is a lamniform shark characterized by a burly body and protruding teeth. It can be found in coastal temperate and sub-tropical areas, except in the Eastern Pacific Ocean, usually swimming in shallow waters close to sandy or rocky bottoms or submerged reefs (Compagno 2001). Tracking and tagging studies in Australia, South Africa, and Northwestern Atlantic have demonstrated that, despite the presence of some differences depending on the geographic area examined, this is a phylopatric species and undertakes north-south seasonal migrations (Lucifora et al. 2002; Dicken et al. 2007; Bansemer & Bennett 2011; Kneebone et al. 2014; Teter et al. 2015; Haulsee et al. 2018). *C. taurus* reaches sexual maturity at the age of six-seven years in males and nine-ten years in females (Goldman et al., 2006). Gestation lasts between nine-twelve months and, together with intra-uterine cannibalism, leads to the birth of only two newborns every two years (Gilmore 1993). As for many other sharks, the features of its life cycle (i.e. late sexual maturity, long gestation, low fecundity) make it extremely prone to the risk of extinction (García et al. 2008). This risk is exacerbated by the drastic population decline observed in some areas as a direct consequence of coastal habitat degradation and overexploitation, due to by-catch and intentional fisheries (Pollard et al. 1996; Otway et al. 2004). For these reasons, in 2000, the IUCN classified the sand tiger shark as "Vulnerable" at global level (Pollard & Smith 2000) and it is currently considered "Critically endangered" in Eastern Australia (Pollard et al. 2003), Southwestern Atlantic Ocean (Chiaramonte et al. 2007) and Mediterranean Sea (Walls & Soldo 2016).

It is well-known that a reduction in size of wild populations leads to a loss of genetic diversity (Frankham 1996), with a consequent decrease in the ability to adapt to future environmental changes and an increased probability of extinction (Frankham 2005). In this context, to shed light on the conservation status of threatened sharks, such as *C. taurus*, genetic population analyses are necessary (Dudgeon et al. 2012). Currently, there are a limited number of studies describing levels of genetic variation and connectivity between different populations of this species. The first was performed at regional scale (South Africa, Eastern and Western Australia) by Stow et al. (2006) using AFLP loci and the mitochondrial DNA control region (mtDNA CR) as molecular markers. The second one was performed at global level using a longer sequence of the mtDNA CR and six microsatellite loci (Ahonen et al. 2009). Low levels of genetic diversity were demonstrated, probably related to historical processes rather than recent human-mediated bottleneck events (Stow et al. 2006; Ahonen et al. 2009). In addition, a genetic structure with six distinct populations corresponding to different geographic areas (Northwestern Atlantic, Brazil, South Africa, Japan, Eastern Australia and Western Australia) was revealed, with a low gene flow shown only between Southern Africa and Brazilian populations. These results highlighted the necessity to manage the populations of this shark as distinct Evolutionary Significant Units (ESUs; Waples 1991) for a better conservation of this species (Ahonen et al. 2009).

Unfortunately, the genetic characterization of *C. taurus* populations seems to be still incomplete because for some geographic areas the current presence and abundance of this species is unknown, even if their existence has been historically well documented. This is the case of the Mediterranean Sea where the occurrence of this species was known in the past. Since the 1970s, records of *C. taurus* have become more and more sporadic (Fergusson et al. 2002) until they ceased in the last decade (Chapter 3.1). Some of the last catches were made in Sicily (Fergusson et al. 2002), Tunisia (Quignard & Capapé 1972; Capapé et al. 1976), Croatia (Lipej et al. 2004) and Aegean Sea (Ismen et al. 2009). The lack of contemporary records makes the sampling of individuals for genetic studies impossible, however, the analysis of historical samples of *C. taurus* from the Mediterranean area could be very useful to improve the phylogeography of this species.

A first attempt to extract good quality DNA from historical shark jaws and teeth, including from *C. taurus* specimens, was made by Ahonen & Stow (2008). Two different DNA extraction methods were successfully tested. As expected, a lower amplification success of historical DNA compared to a contemporary one was observed. In fact, the PCR amplification of DNA from ancient samples is usually difficult due to the high degradation and small concentration of DNA extracted and/or by the presence of PCR inhibitors (Pääbo et al. 2004). Subsequently, DNA from historical tissue and jaw cartilage was analysed to confirm the previous hypothesized Indo-Pacific origin of Mediterranean white sharks (*Carcharodon carcharias* Linnaeus, 1758) (Gubili et al. 2011, 2015). In this paper, the mtDNA CR of historical samples of Mediterranean *C. taurus* was amplified and sequenced with the aim to genetically characterize sand tiger sharks observed and caught in the past in the Mediterranean Sea. The Mediterranean haplotypes found were then compared with haplotypes known from the literature in order to assess the presence of haplotypes endemic to the Mediterranean Sea and therefore to understand if the extinction of *C. taurus* in this basin may have affected the global genetic variability of the species.

3.2.3 Materials and Methods

Precautions to work on historical DNA

Genetic analyses on ancient and historical samples are subject to a high risk of contamination by exogenous DNA. In order to avoid this problem, pre- and post-PCR work phases were performed in two separate laboratories located in different buildings (Pääbo et al. 2004; Knapp et al. 2012). In particular, the pre-PCR laboratory was equipped with two hoods provided with UV lamps, the first one dedicated only to DNA extraction and the second one to reagents and PCR preparation (Knapp et al. 2012). The entrance to the pre-PCR area was allowed only to qualified staff equipped with total body coverall, laboratory shoes, safety glasses, face mask and two pairs of gloves (Knapp et al. 2012). All laboratory surfaces were daily cleaned with 10% bleach and wiped with ethanol 70%. In addition, they were UV irradiated for 20-30 min before and after every work session. Laboratory equipment (micropipettes, glassware, plasticware, etc...) was exposed to UV light for 20-30 min before and after their use. In contrast, the post-PCR area was dedicated only to thermocycling, electrophoretic analysis of amplicons on agarose gel and preparation of samples for sequencing. The thermocycler placed in this area was dedicated only to the amplification of ancient or historical DNA and, after each PCR cycle, was decontaminated with UV light for 30 min. Moreover, each sample was analysed separated from others to avoid cross-contamination and, extraction and PCR controls were always added to detect if contamination occurred during work phases (Pääbo et al. 2004).

Sampling and DNA extraction

An overview of the ichthyological collections of the main European museums was done through on-line resources and personal contact with curators in search of *Carcharias taurus* Mediterranean specimens. A total of nine historical samples of *C. taurus* with a certain Mediterranean origin (Table 1) were found and collected. Five samples were powder from jaw cartilage, two were pieces of cartilage and two were teeth (Table 1). All samples were decontaminated prior to DNA extraction to reduce the presence of exogenous DNA and inhibitors from their surface, thus reducing the risk of contamination and the probability of PCR failure (Rohland & Hofreiter 2007). In the case of cartilage powder, the decontamination phase was performed before sampling. Specifically, the sampling area was chosen from an internal portion of the jaws and was previously scratched using sandpaper, washed with bleach and then rinsed with ultrapure sterile water. When the surface was perfectly dry, the cartilage powder was obtained using a drill equipped with a sterile drill bit at very low speed to avoid overheating and additional damage to DNA (Rohland & Hofreiter 2007). The powder obtained was recovered in a sterile 1.5 ml microcentrifuge tube and the hole produced on the jaws was closed with dental restoration paste to make them invisible for museum visitors. For pieces of cartilage, the decontamination phase was the same as described above for the jaw surface, while teeth were decontaminated using the protocol proposed by Rohland and Hofreiter (2007) with an additional final step consisting in the exposure to UV light for 30 min for each side of the tooth. After decontamination, small pieces of the root were cut using a serrated blade previously washed with DNA AWAY™ Surface Decontaminant (Thermo Scientific) and UV irradiated for 30 min per side. The root was chosen for DNA extraction because it was more accessible than the inner part. In addition, *C.*

taurus teeth do not contain a pulp cavity, that usually has a higher quantity of DNA, but both the root and the inside of the tooth are made of osteodentine (Whitenack et al. 2010).

Table 1. Information about museum specimens of *Carcharias taurus* sampled and analysed in the present study.

Genetic code	Institution	Institution code	Description	Sampling location and date	Reference	Sample type
FI002	Museo di Storia Naturale, Sezione di Zoologia "La Specola", Florence, Italy	INV6136	Taxidermied specimen, male, 170 cm length	Messina, Sicily, 15th November 1879	Vanni, 1992	Cartilage powder
PA001	Museo di zoologia "Pietro Doderlein", Palermo, Italy	ID AN 68	Jaws	Sicily, second half of the XIX century	Doderlein, 1979	Cartilage powder
PA002	Museo di zoologia "Pietro Doderlein", Palermo, Italy	ID AN 94	Jaws	Sicily, second half of the XIX century	Doderlein, 1979	Cartilage powder
PA003	Museo di zoologia "Pietro Doderlein", Palermo, Italy	ID AN 60	Jaws	Sicily, second half of the XIX century	Doderlein, 1979	Cartilage powder
PA004	Museo di zoologia "Pietro Doderlein", Palermo, Italy	ID AN 38	Skeleton	Sicily, second half of the XIX century	Doderlein, 1979	Cartilage powder
PR001	Muséum National d'Histoire Naturelle, Paris, France	A-9685	Taxidermied specimen, female	Algeria, ~ 1840	Guichenot, 1850 B. Seret, personal communication	Piece of cartilage
PR004	Muséum National d'Histoire Naturelle, Paris, France	AB-0038	Jaws	Algeria, ~ 1840	Guichenot, 1850 B. Seret, personal communication	Piece of cartilage
XL001	Royal Belgian Institute of Natural Sciences, Brussels, Belgium	507β	Jaws	Algeria, end of the XIX century	O. Pauwels, personal communication	Tooth
XL002	Royal Belgian Institute of Natural Sciences, Brussels, Belgium	1386β	Teeth collection, erroneously classified as *Odontaspis ferox*	Tunisia, 1st October 1933	O. Pauwels, personal communication	Tooth

Genomic DNA was extracted using the protocol developed for ancient bones by Yang, Cannon, and Saunders (2004) with some modifications. Samples were put in 4 ml of lysis buffer (0.5 M EDTA pH 8.0, 0.5% SDS, 0.5 mg/ml proteinase K) and were incubated overnight at 50°C in a washing bath with gentle orbital oscillation. After incubation, samples were centrifuged to facilitate the deposition of undigested materials, 3 ml of supernatant were recovered and transferred on Amicon Ultra-15 centrifugal filter units (MWCO 30kDa, Merck Millipore) to concentrate samples up to 125 µl. Finally, the recovered volume was purified using QIAquick PCR purification kit (Qiagen) and DNA was eluted in 100 µl of ultrapure sterile water.

Amplification and Sanger sequencing

A fragment of ~ 600 bp of the mitochondrial control region (D-loop) (Ahonen et al. 2009), was analysed in this study. In order to avoid amplification problems related to the low quality and quantity of DNA extracted from historical samples, eight overlapping primer pairs were designed (Table 2, Fig.

1) using the software Primer3Plus (Untergasser et al. 2012) and the complete mtDNA genome of *C. taurus* deposited in GenBank (Accession number: KF569943, Chang et al. 2015) as reference sequence.

Table 2. Primer pairs designed and used to amplify a portion of the mtDNA CR of *Carcharias taurus* historical samples.

Primer name	Sequence 5' to 3'		Product length
CtCR1	F	CTTCAATCCTTGATCGCGTCA	135 bp
	R	CTTCCGGGGAATAGCGATGG	
CtCR2	F	TGGCATTTTCGTCCTTGATCG	146 bp
	R	TGAGTATGTTAGATAGATGTCGAGGA	
CtCR3	F	GGCTGAACTGGGACACTGAG	146 bp
	R	TCGAAACTTGCCGACTATGG	
CtCR4	F	TGTCAAGTTGACCAAAACTGAAA	118 bp
	R	CCGGATGGGGGTTAAGAGAG	
CtCR5	F	CCATAGTCGGCAAGTTTCGA	148 bp
	R	TGCCAGATAAAGTGAAGAATGTGT	
CtCR6	F	CTCTCTTAACCCCCATCCGG	213 bp
	R	GGGTTTTTCGAGGAGTCCGT	
CtCR7	F	ACACATTCTTCACTTTATCTGGCA	172 bp
	R	ATGTCCGGCCCTCGTTTTAG	
CtCR8	F	ACGGACTCCTCGAAAAACCC	141 bp
	R	TCATCTTAGCATCTTCAGTGCCA	

PCRs were performed in a 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgCl2, 0.08 mM of each dNTP, 0.48 µM of each primer, 4U of Platinum Taq DNA Polymerase (Invitrogen) and 3 µl of genomic DNA. All amplifications were performed in a BioRad T100™ Thermal Cycler (BioRad) with an initial denaturation step at 94°C for 7 min, followed by 60 cycles of 20 s at 94°C, 30 s at 54°C and 40 s at 72°C, with a final extension at 72°C for 7 min.

PCR products were checked on 2% agarose gel stained with GelRed™ (Biotium). All amplicons were sent to BMR Genomics (Padua, Italy) for Sanger sequencing, purified by exoSAP-IT™ (Thermo Scientific) and sequenced in both directions using an automated sequencer, ABIPRISM 3730XL (Applied Biosystems).

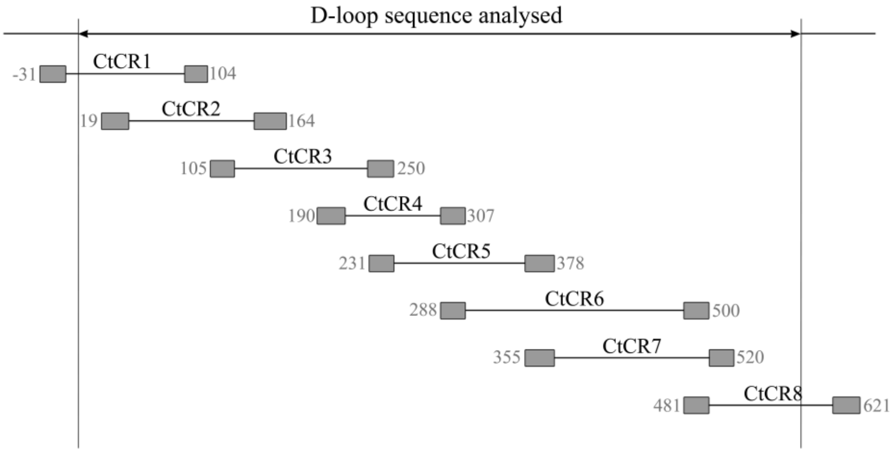

Figure 1. Graphic representation of the eight overlapping primer pairs designed to amplify a portion of the mtDNA CR sequence in Mediterranean historical samples of *Carcharias taurus*. The numeration of the mitochondrial DNA started from the first base of the region studied by Ahonen et al. (2009)

Alignment and data analysis

For all the samples, sequences obtained using each primer pair were checked by eye and assembled to have the complete sequence of interest. All historical sequences were checked with BLAST (Altschul et al. 1990) and aligned using CLUSTALW (Larkin et al. 2007) with the 11 haplotypes described so far at the global level (Ahonen et al. 2009; Chang et al. 2015; Wynne & Wilding 2018). When necessary, the alignment was manually edited on BioEdit (Hall 1999). For the sample PA002, the very low amplification success and the lack of a correspondence after the alignment with *C. taurus* sequences suggested a mislabelling of the museum specimen. For this reason, the short and not contiguous sequences obtained from this sample were checked using BLAST (Altschul et al. 1990) and a morphological analysis of the teeth on the jaws was carried out (Compagno 2001) using pictures taken during the sampling phase.

Excluding the PA002 sample, evolutionary relationships between all haplotypes were shown on a Median-Joining Network (Bandelt et al. 1999) using Network 5 (Fluxus Technology Ltd., www.fluxus-engineering.com), considering also gaps and missing nucleotides. The **ε** parameter was set to zero and information from previous studies about sampled individuals and sampling locations (Ahonen et al. 2009; Chang et al. 2015; Wynne & Wilding 2018) were added to the analysis.

3.2.4 Results

DNA was successfully extracted and amplified from all the historical samples of *Carcharias taurus* (Table 1). The complete mtDNA CR sequence of 574 bp in length, previously analysed also by Ahonen et al. (2009), was obtained for five samples (FI002, PA001, PA003, PR004, XL002). Amplification failures produced 550 bp for PA004, 507 bp for PR001, 495 bp for XL001 and only 198 bp for PA002. Specifically, the primer pairs CtCR2 and CtCR3 failed the amplification of the samples PR001 and XL001, respectively. The sequence produced by the primer pair CtCR6 was not obtained for two samples, PA004 and PR001. For PA002, only CtCR4, CtCR7 and CtCR8 provided a PCR product.

Table 3. Polymorphic sites obtained after the alignment.

	Polymorphic sites																	
	42	131	182	318	330	335	337	339	356	407	408	420	421	427	444	445	562	572
HapA	T	C	A	C	A	G	G	G	T	A	G	-	-	G	G	A	G	A
FI002	.	.	.	.	.	.	.	.	.	.	.	-	-	.	.	.	.	.
PA001	.	.	.	.	.	.	.	.	.	.	.	-	-	.	.	.	.	.
PA003	.	.	.	.	.	.	.	.	.	.	.	-	-	.	.	.	.	.
PR004	.	.	.	.	.	.	.	.	.	.	.	-	-	.	.	.	.	.
PA004	.	.	.	.	.	.	.	.	?	.	.	-	-	.	.	.	.	.
HapB	.	.	.	.	.	A	.	.	.	.	.	-	-	.	.	.	.	.
XL001	.	.	?	.	.	A	.	.	.	.	.	-	-	.	.	.	.	.
PR001	.	.	.	.	.	A	.	.	?	.	.	-	-	.	.	.	.	.
HapJ	.	.	.	.	.	A	.	.	C	.	.	-	-	.	.	.	.	.
HapD	.	.	.	.	G	A	.	.	.	.	.	-	-	.	.	.	.	.
HapI	.	.	.	.	.	A	A	.	.	.	.	-	-	.	.	.	.	.
XL002	.	.	.	.	.	A	A	.	.	.	.	-	-	.	.	.	.	.
HapC	.	.	.	.	.	A	A	.	.	.	.	-	-	.	.	.	.	G
HapH	.	T	G	.	G	A	A	A	.	.	.	-	-	.	.	.	.	G
HapE	.	.	G	.	G	A	A	A	.	.	.	-	-	.	.	.	.	G
HapG	.	.	G	.	G	A	A	A	.	G	A	A	T	A	-	-	T	.
HapF	.	.	G	T	G	A	A	A	.	G	A	A	T	A	-	-	T	.
HapK	A	.	G	.	G	A	A	A	.	G	A	A	T	A	-	-	?	?

Haplotypes from previous studies were highlighted in grey (Ahonen et al., 2009; Chang et al., 2015; Wynne & Wilding, 2018). Haplotype A was used as a reference sequence. All identical nucleotides in other sequences are indicated as full stops (.), indels as dashes (-) and missing nucleotides as question marks (?). In the case of historical samples, missing data are due to amplification failures, whereas for Haplotype K (Wynne & Wilding, 2018) they are present because the sequence is shorter than the others (518 bp vs 574 bp).

Undoubtedly, the primer pair and the sample with the worst amplification success were CtCR6 and PA002, respectively. The comparison of the short not contiguous sequences obtained from PA002 with those of *C. taurus* and with all the sequences deposited in data banks did not show any perfect match. The morphological analysis of the jaws showed a probable misidentification of the museum specimen; teeth on the museum jaws have two lateral cusplets on each side of the main cusp, a characteristic of the small-tooth sand tiger shark (*Odontaspis ferox* Risso, 1810) (Compagno 2001). The lack of the complete mitochondrial genome and/or the mtDNA CR sequence of this species in data banks makes the corroboration of morphological observations impossible and this sample was precautionarily excluded from the following analysis.

All sequences obtained from historical samples have been submitted to the GenBank database under accession numbers: MK434273-MK434280. The alignment of all *C. taurus* sequences known so far and those obtained in this study have allowed, on the basis of 18 polymorphic sites, the classification of Mediterranean historical samples into three previously described haplotypes: Haplotypes A, B and I (Table 3). Of the five samples for which the complete sequence of interest was obtained, four belonged to Haplotype A (FI002, PA001, PA003, PR004) and one to Haplotype I (XL002) (Table 3). The affinity to a specific haplotype was also clearly defined for two of the three Mediterranean incomplete sequences. Sample PA004 seems to belong to Haplotype A also in absence of the diagnostic site in 356 and, sample XL001 to Haplotype B also in absence of the diagnostic site 182 (Table 3). The classification of the sample PR001 was more difficult. The amplification failure of the primer pair CtCR2 did not mask any known polymorphic sites (Table 3), while the failure of the primer pair CtCR6 did not allow us to obtain the diagnostic site 356. This latter failure prevented us from understanding if sample PR001 belonged to Haplotype B or Haplotype J (Table 3).

The alignment result was also confirmed by the Median Joining network performed to visualize haplotypes relationships (Figure 2). In addition, the inclusion of information about sampling locations from other previous studies (Ahonen et al. 2009; Chang et al. 2015; Wynne & Wilding 2018) was very useful because it showed that Mediterranean historical samples have the same haplotypes as *C. taurus* individuals sampled in South Africa (Western Indian Ocean) and Brazil (Western Atlantic Ocean). Specifically, five Mediterranean samples (FI002, PA001, PA003, PR004, PA004) belonged to Haplotype A and one (XL002) to Haplotype I, previously observed only in individuals sampled in South Africa (Figure 2). The sample XL001 was identified as Haplotype B, which was found in both South Africa and Brazil (Figure 2). The Network 5 software also included the PR001 sample within Haplotype B cluster, on the basis of the maximum parsimony principle (Figure 2).

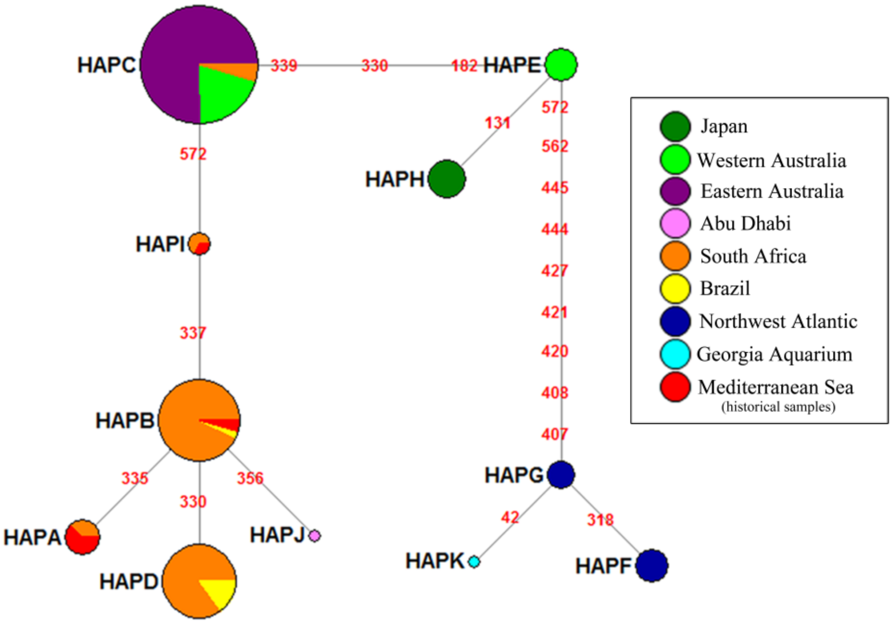

Figure 2. Median Joining network showing the relationship between mtDNA CR haplotypes of *Carcharias taurus*. The circle size is related to the number of individuals sampled worldwide for each haplotype. Each colour indicates a different sampling location

3.2.5 Discussion

The sand tiger shark (*Carcharias taurus*) is considered as "Critically endangered" within the Mediterranean Sea by the IUCN (Walls & Soldo 2016). However, its presence in this basin is currently uncertain due to the lack of sightings and catches over the last decade, which suggest a probable extinction at regional scale (Chapter 3.1; Fergusson et al. 2002; Walls & Soldo 2016). The use of DNA extracted from historical samples has allowed us to genetically characterize, for the first time, *C. taurus* individuals that inhabited the Mediterranean waters in the past and to suggest a possible route of colonization of this basin. Only eight specimens of certain Mediterranean origin were sampled and analysed. It was not possible to obtain a larger sample mainly because of the lack of information about the original catch location for most museum specimens and because some institutions do not allow samples to be taken from their collections.

MtDNA was successfully extracted from all the nine historical samples using a protocol developed for ancient bones (Yang et al., 2004) and, in contrast to Ahonen and Stow (2008), a higher amplification success was achieved. Ahonen and Stow (2008) tried the DNA extraction and PCR amplification on 34 historical samples (20-40 years old) from different shark species, including of *C. taurus* (cartilage and teeth). The PCR amplification failed for 19 of them highlighting that the use of a single primer pair to amplify a region of ~ 700 bp of the mtDNA CR (Stow et al. 2006) is unsuitable to analyse historical DNA. Instead, the use of overlapping primer pairs delimiting a region of 150-200 bp in length has been able to improve the amplification success of both historical and ancient DNA (Barnett et al. 2014; Splendiani et al. 2016, 2017; Cole et al. 2018) and was successful also in this study. However, for three samples, an incomplete sequence was obtained probably due to the degradation of DNA extracted and/or to the presence of PCR inhibitors (Pääbo et al. 2004). The primer pairs with the lowest amplification success was CtCR6 because it failed the amplification in two *C. taurus* samples. It was designed to amplify a sequence of 213 bp in length, while it is widely known that DNA molecules extracted from ancient samples rarely exceed 200 bp (Pääbo et al. 2004). The presence of repeated motifs and a high AT content in the region encompassed by these primers have limited us in primer design. The repetition of a single base or dinucleotide motifs for many times in a DNA sequence can cause the incorrect pairing of the primers on the DNA template. In addition, the presence of AT rich sequences leads to primers with a very low melting temperature (Tm). A low Tm is responsible for pairing of the primers even in regions with several mismatches, thus leading to the amplification of aspecific PCR products (Dieffenbach et al. 1993).

Excluding the sample PA002, due to the probable misidentification of the museum specimen, all the other historical jaws and teeth undoubtedly belonged to *C. taurus* individuals. The mtDNA CR sequences obtained here were attributed to two different haplotypes (Haplotype A and I) previously reported only for South Africa and one (Haplotype B) shared by both South Africa and Brazil (Ahonen et al. 2009). The incomplete sequence of PR001 could be attributed to two distinct haplotypes (Haplotype B and J) however, the presence of another Haplotype B among the Mediterranean historical samples (XL001) and the distribution of the Haplotype J only in Abu Dhabi waters (Chang et al. 2015) suggest that the sample PR001 bears Haplotype B, as indicated also by the Median Joining network. The lack of new haplotypes from Mediterranean historical samples was probably due to the limited number of samples analysed or to the low rate of molecular evolution estimated for this species (Stow et al. 2006; Ahonen et al. 2009). Instead, the observation of haplotypes mainly described for South African individuals suggests a genetic relationship between Mediterranean sand tiger sharks and those from the Western Indian Ocean.

Ahonen et al. (2009) observed the deepest genetic divergence between the Northwest Atlantic population and all the others, while the lowest divergence was identified between South Africa and Brazil, which also share some haplotypes. In the first case, the major divergence was traced back to the formation of the Isthmus of Panama (~ 3 million years ago), which has definitively separated Atlantic and Pacific Oceans (Toonen et al. 2016). On the other hand, the low differentiation between South African and Brazilian populations indicates a relatively recent connection (Ahonen et al. 2009). The belonging of historical samples analysed here to haplotypes already described in the Western Indian Ocean highlights a recent origin also in the case of the Mediterranean sand tiger sharks excluding an ancient origin due to the separation between the Mediterranean Sea and the Indo-Pacific Ocean by the rising of the Isthmus of Suez (11-18 million years ago) (Toonen et al. 2016). The Mediterranean Sea was separated many years before the formation of the Isthmus of Panama indicating that if the Mediterranean *C. taurus* are descendant from those trapped after the raising of the Isthmus of Suez, they should have a greater genetic divergence than observed.

The connection between the Red and Mediterranean seas was re-established in 1876, after the opening of the Suez Canal, and promoted the entry of Indo-Pacific species into the Mediterranean basin, a phenomenon known as "Lessepsian migration" (Por 1978). However, this route for colonization by Lessepsian migrants of *C. taurus* is rejected as several evidences indicate that this species was already present in the Mediterranean Sea before the opening of the Suez Canal: i) the species was described for the first time by Rafinesque in 1810, based on an individual caught in Sicilian waters (Compagno 2001; Fergusson et al. 2002), ii) other catches and sightings were reported in the Mediterranean basin before the 1876 (Fergusson et al. 2002) and iii) our historical samples were mainly from the Western Mediterranean and the collection dates are earlier or close to the date of the opening of the Suez Canal opening. A migration through the Red Sea can also be hypothesized in the opposite direction (anti-Lessepsian migration), from the Mediterranean Sea to the Western Indian Ocean, but anti-Lessepsian migrants are very rare (Por 1978). In addition, the low genetic diversity observed in the Mediterranean historical samples could be due to a "founder effect" suggesting that the South Africa, characterized by the highest genetic diversity (Ahonen et al. 2009), was probably the origin of the Mediterranean population.

Thus, the most probable biogeographic way used by the sand tiger sharks to colonize the Mediterranean Sea is along the Western African coasts. Ahonen et al. (2009) explained the low rate of genetic differentiation and the gene flow observed between South African and Brazilian populations by the establishment of a recent connection between Indian and Atlantic Ocean. The Southwestern African coast is characterised by the presence of an upwelling zone, caused by the northward flow of the cold Benguela Current, that acts as a phylogeographic barrier (Benguela barrier) (Dudgeon et al. 2012; Toonen et al. 2016). During Pleistocene interglacial periods, the northward cold Benguela current was reduced with a simultaneous expansion of the south-westward warm Agulhas current (Peeters et al. 2004) that seems to have promoted the passage of *C. taurus* individuals from the

Western Indian to Atlantic Ocean (Ahonen et al. 2009). A similar pattern of dispersion was also proposed to explain the genetic similarities observed for South Atlantic and Indo-Pacific populations of other shark species such as *Carcharinus limbatus* (Keeney & Heist 2006), *Carcharhinus longimanus* (Camargo et al. 2016) and *Carcharhinus falciformis* (Domingues et al. 2018).

A relatively recent colonization of the Mediterranean Sea by individuals of Indo-Pacific origin was also suggested for the white shark *Carcharodon carcharias* (Gubili et al. 2011) and confirmed by the analysis of historical samples (Gubili et al. 2015). Contrary to what observed for the Mediterranean sand tiger shark, the great white shark haplotypes from the Mediterranean Sea were more similar to North-Eastern Pacific/Australia/New Zealand haplotypes and not to South African (Western Indian Ocean) ones (Gubili et al. 2011, 2015). This discrepancy is probably related to the life history characteristics of the two species. Both species are characterized by natal philopatry but shows a different migratory behaviour. *C. taurus* is a coastal species that usually accomplish short migration, for example in the South-eastern coast of South Africa a seasonal north-south migration between mating, gestating and parturition areas was observed (Dicken et al. 2006). *C. carcharias* instead has a high migratory capacity as documented by the observation of a trans-oceanic migration from South Africa to Western Australia (Bonfil et al. 2005). Gubili et al. (2011) estimated that the separation between Mediterranean and Indo-Pacific white shark populations occurred during the Late Pleistocene, a period characterized by climate instability. During a trans-oceanic migration some Indo-Pacific white sharks reached South Africa and, following the expansion of the Agulhas current, were driven to the Eastern Atlantic Ocean. The chase of prey, such as Atlantic bluefin tuna and swordfish, that showed a similar dispersion pattern (Alvarado Bremer et al., 2005) and the propensity to swim eastward to return to natal areas have forced them within the Mediterranean Sea.

In the case of *C. taurus*, an immediate colonization of the Mediterranean area seems unlikely because this species usually undertakes short migrations, only in one case a distance travelled of ~ 2000 km was observed (Dicken et al. 2007). We propose that South African individuals have reached the Atlantic Ocean during the Pleistocene, when the cold Benguela Current was temporarily attenuated and the Agulhas current enhanced. The restoration of the cold Benguela upwelling barrier probably trapped some individuals of sand tiger shark along the Southeast African coasts from which they migrated northward to reach warmer habitats. In fact, *C. taurus* rarely tolerates temperature lower than 15°C (Lucifora et al. 2002; Otway & Ellis 2011; Smale et al. 2012; Kneebone et al. 2014; Teter et al. 2015). The coastal behaviour of this species together with the propensity to accomplish north-south seasonal migrations probably allowed, following a stepping stone model of dispersion, the colonization of Western African coasts and finally entry into the Mediterranean basin. However, the lack of unique haplotypes among the Mediterranean historical samples and the lack of genetic data for Western Atlantic Ocean do not allow us to understand if Mediterranean sand tiger sharks belonged to a distinct population or if they were visitors from African Atlantic coasts (Fergusson et al. 2002).

3.2.6 Conclusions

The decline of chondrichthyan species recorded at global scale and in particular in the Mediterranean Sea as a consequence of human activities is alarming (Ferretti et al. 2008; Dulvy et al. 2014). In this context, the importance of genetic tools to develop beneficial management and conservation strategies has been largely demonstrated (Dudgeon et al. 2012). However, the difficulty in collecting shark specimens poses a serious limit to conservation genetic studies. This limit can be overcome by the use of historical shark jaws and teeth that represent an alternative source of DNA (Ahonen & Stow 2008; Gubili et al. 2015; Nielsen et al. 2017). In this study, the genetic analysis of historical samples helped us to genetically characterize Mediterranean sand tiger sharks using historical DNA and to hypothesize a biogeographic scenario for the colonization of the Mediterranean Sea by individuals coming from Western Indian Ocean. However, the limited number of samples and the complete lack of genetic information for some geographic areas (e.g. Eastern Atlantic Ocean) did not allow us to clarify if Mediterranean individuals belonged to a distinct population currently extinct or if they were vagrants from the African Atlantic coast (Fergusson et al. 2002). The identification of previously described haplotypes among historical Mediterranean samples suggests that, if a Mediterranean *C. taurus* population had been lost, there would have not been a loss in terms of global genetic variability. Regarding individuals from African Atlantic coasts, a conservation planning to reduce the threats for this species could allow the recolonization of the Eastern Atlantic coast and probably of the Mediterranean Sea. Shark species of Western Africa have long been subjected to over-exploitation by fishing activities (Diop & Dossa 2011), this could have led to the reduction of *C. taurus* populations also in this area. Further studies are therefore necessary to clarify the status of the Mediterranean sand tiger shark and to improve the global knowledge on this species. Following the last IUCN assessment for the sand tiger shark (Walls & Soldo 2016), trends and dynamics in the world populations of this species are still unknown. Data about its distribution range and conservation status are absent or incomplete for several geographic area, as observed for the Mediterranean Sea and Eastern Atlantic Ocean. Fragmentation and isolation are known as factors that may weak subpopulations, and in the case of a species as the sand tiger sharks such vulnerable to coastal human impact (i.e. by-catch, commercial fisheries, habitat degradation), they can strengthen a declining process. Additional information about the distribution range, size of populations, levels of genetic diversity and gene flow between different geographic areas, also by the analysis of historical samples, must be obtained. These data could favour the development of regional and inter-regional conservation policies to prevent the extinction of *C. taurus* at local and global level and, if possible, to encourage the recolonization of areas from which it seems to have disappeared.

3.2.7 References

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4. STUDYING SHARKS IN THE MEDITERRANEAN SEA IN THE MODERN ERA

4.1 OPPORTUNITIES FROM CITIZEN SCIENCE FOR SHARK CONSERVATION, WITH A FOCUS ON THE MEDITERRANEAN SEA

4.1.1 Abstract

The Mediterranean Sea is a hotspot for shark conservation. A decline in large pelagic shark populations has been observed in this vast region over the last 50 years and a lack of data on the local population status of various species has been pointed out. Throughout history, the relation between people and sharks has been revolving around a mixture of mystery, fear, and attraction. Recently, however, a remunerative ecotourism industry has been growing in areas of shark aggregation globally. This growth has been accompanied by the establishment of a citizen science (CS) movement aimed to engage and recruit ecotourists in data collection for shark research. Several CS projects have generated interesting results in terms of scientific findings and public engagement. In the Mediterranean Sea, shark aggregations are not as relevant to support locally-focused CS actions on shark diving sites as in other parts of the world. However, a series of other initiatives are taking place and CS could offer an excellent opportunity for shark conservation in the Mediterranean Sea. The dramatic decline of shark populations shown in the region calls for alternative ways to collect data on species distributions and abundance. Obtaining such data to set proper conservation and management plans for sharks in the Mediterranean Sea will be possible if existing CS initiatives collaborate and coordinate, and CS is widely acknowledged and deployed as a valuable tool for public education, engagement, and scientific discovery. After providing an overview of multiple facets of the relationship between humans and sharks, we focus on the possibility of exploiting new technologies and attitudes toward sharks among some groups of ocean users to boost participatory research. CS is a great opportunity for shark science, especially for areas such as the Mediterranean Sea and for large pelagic sharks whose populations are highly impacted.

4.1.2 Introduction

Sharks are among the most threatened vertebrates in the ocean. Rapid and steep population depletions have been shown in several ocean regions (Dulvy et al. 2016), and the Mediterranean Sea has presented some of the most extreme population declines. Here, many species of large predatory sharks have declined by up to 96–99%, calling for urgent conservation measures (Ferretti et al. 2008).

IUCN (International Union for Conservation of Nature) assessments indicated that bycatch, pollution, habitat loss and degradation, and human disturbance are the major threats affecting sharks in the Mediterranean Sea (Cavanagh & Gibson 2007; Bonanomi et al. 2017). These stressors combined with the slow population dynamics of most shark species (e.g. late maturity and low fecundity) are making the Mediterranean Sea one of the most dangerous places for sharks in the ocean (Cavanagh & Gibson 2007).

In the Mediterranean Sea, a few species of sharks are still fished to be commercially used. Examples include smooth-hounds (*Mustelus* spp.), catsharks (*Scyliorhinus* spp.), and dogfishes (*Squalus* spp.). Species that are directly targeted by fisheries such as the common smooth-hound (*Mustelus mustelus*) and the spiny dogfish (*Squalus achantias*) are listed as vulnerable and endangered respectively by the IUCN (Cavanagh & Gibson 2007). At the moment, there are no management measures in place for sharks in the region and some options (e.g. fishing closure in critical habitats of the northern Adriatic Sea) are urgently required to restore depleted populations (Bonanomi et al. 2018). Several other shark species, however, are part of fisheries' bycatch (Cavanagh & Gibson 2007). Bottom trawling is a widespread fishing activity in the Mediterranean Sea (Kroodsma et al. 2018) that produces abundant elasmobranch bycatch, especially demersal species, impacting their abundance, distribution and suitable habitats (Ferretti et al. 2013, 2016a). Among these species, angelsharks (*Squatina* spp.) have shown steep declines, and are now considered commercially extinct in areas where they were previously abundant and supporting dedicated fisheries (Ferretti et al. 2016a), such as the Adriatic Sea (Ferretti et al. 2013; Fortibuoni et al. 2016), the Marmara Sea (Kabasakal & Kabasakal 2014) and the Alboran Sea (Muñoz-Chapuli 1985).

In the Mediterranean Sea, there are no fisheries that officially target pelagic sharks, which, nevertheless, form part of the bycatch of fisheries targeting tuna and swordfish with pelagic longlines, and small pelagic fishes with pelagic trawls (Fortuna et al. 2010). In longline fisheries, the predominant shark bycatch includes blue sharks (*Prionace glauca*), thresher sharks (*Alopias vulpinus*) and shortfin makos (*Isurus oxyrinchus*) (Megalofonou 2005). Various types of driftnet could intercept as bycatch species such as the blue shark, the thresher shark and the basking shark (*Cetorhinus maximus*) (Cavanagh & Gibson 2007). Large-scale drift netting, which is prohibited by European Union Member States, though still used illegally by EU and non-EU fishing nations, could affect a wide range of species (Camhi et al. 2009). Since sharks are top predators or high-level consumers (Cortés 1999), bycatch of these species not only affects distribution and abundance of sharks' populations but also the structure and function of marine communities (Ferretti et al. 2010).

Table 1. IUCN assessments for Mediterranean shark species (Dulvy et al. 2016). CR = critically endangered, EN = endangered, VU = vulnerable, NT = near threatened, LC = least concern, DD = data deficient. Asterisks (*) mark species considered as threatened following IUCN criteria.

Species name (and family)	Common name	IUCN
Lamniformes		
Alopias vulpinus	Thresher shark	*EN
Alopias superciliosus	Bigeye thresher	*EN
Carcharias taurus	Sand tiger shark	*CR
Carcharodon carcharias	Great white shark	*CR
Cethorhinus maximus	Basking shark	*EN
Isurus oxyrinchus	Shortfin mako	*CR
Isurus paucus	Longfin mako	DD
Lamna nasus	Porbeagle shark	*CR
Odontaspis ferox	Smalltooth sand tiger	*CR
Hexanchiformes		
Hexanchus griseus	Bluntnose sixgill shark	LC
Hexanchus nakamurai	Bigeye sixgill shark	DD
Heptranchias perlo	Sharpnose sevengill shark	DD
Squaliformes		
Centroscymnus coelolepis	Portuguese dogfish	LC
Centrophorus granulosus	Gulper shark	*CR
Dalatias licha	Kitefin shark	*VU
Echinorhinus brucus	Bramble shark	*EN
Etmopterus spinax	Velvet belly lanternshark	LC
Oxynotus centrina	Angular roughshark	*CR
Somniosus rostratus	Little sleeper shark	DD
Squalus acanthias	Spiny dogfish	*EN
Squalus blainvillei	Longnose spurdog	DD
Squalus megalops	Shortnose spurdog	DD
Carcharhiniformes		
Carcharhinus altimus	Bignose shark	DD
Carcharhinus brachyurus	Bronze whaler shark	DD
Carcharhinus limbatus	Blacktip shark	DD
Carcharhinus obscurus	Dusky shark	DD
Carcharhinus plumbeus	Sandbar shark	*EN
Galeorhinus galeus	Tope shark	*VU
Galeus atlanticus	Atlantic catshark	NT
Galeus melastomus	Blackmouth catshark	LC
Mustelus asterias	Starry smoothhound	*VU
Mustelus mustelus	Smoothhound	*VU
Mustelus punctulatus	Blackspot smoothhound	*VU
Prionace glauca	Blue shark	*CR
Scyliorhinus canicula	Smallspotted catshark	LC
Scyliorhinus stellaris	Nursehound	NT
Sphyrna zygaena	Smooth hammerhead	*CR
Squatiniformes		
Squatina aculeata	Sawback angelshark	*CR
Squatina oculata	Smoothback angelshark	*CR
Squatina squatina	Angelshark	*CR

To date, nearly 50 species of sharks have been recorded in the Mediterranean Sea, although the presence of some is now uncertain (Serena 2005). The 2016 IUCN Red List regional assessment of Mediterranean elasmobranchs includes 40 species of sharks for which the occurrence in the area has been verified (Table 1; Dulvy et al. 2016). Among these, 12 are listed as Critically Endangered, six as Endangered and five as Vulnerable. Hence, 23 species (57% of the total) in the Mediterranean Sea are considered at risk of extinction. Of the remaining species, seven are either Near Threatened (2) or Least Concern (5).

One of the most common and widespread problems in assessing the conservation status of and implementing important protection measures on sharks worldwide is the lack of data on the local status of shark populations. To date, ten species (25% of the total) are listed as data deficient in the Mediterranean Sea (Dulvy et al. 2016). Currently, sharks appear to be among the rarest and most elusive species in the Mediterranean Sea. Scientific surveys and fisheries information are often incomplete, inadequate or absent on large sharks, especially those species that inhabit the high seas (Ferretti et al. 2008; Camhi et al. 2009). Hence new approaches are needed to obtain information on shark population abundance and distribution. Citizen science (CS), the involvement of non-professional volunteers in generating scientific knowledge (Bonney et al. 2009), is increasingly seen as a valuable option (Thiel et al. 2014).

Globally, CS has already supported research on climate change, landscape ecology, rare and invasive species, disease, populations, communities and ecosystems (Dickinson et al. 2012). In the ecological sciences, CS has a long history of application, and in the last decades, information technology has facilitated the participation of a high number of people (Kobori et al. 2016). Although several CS initiatives for shark research are taking place worldwide both with a global and local scope (www.sharkpulse.org, www.eoceans.org), and focused on single species or broader taxonomic groups (Davies et al. 2013; Andrzejaczek et al. 2016; Araujo et al. 2017; Meyers et al. 2017; Norman et al. 2017), these initiatives are lagging in terms of scientific output in comparison with other similar projects on other groups of animals (Figure 1).

In the Mediterranean Sea, 33 CS initiatives on sharks have been launched since the 1980s (Table 2).

By reviewing these and other initiatives, here we build a case for the use of CS as an effective tool for shark monitoring and conservation in the Mediterranean Sea.

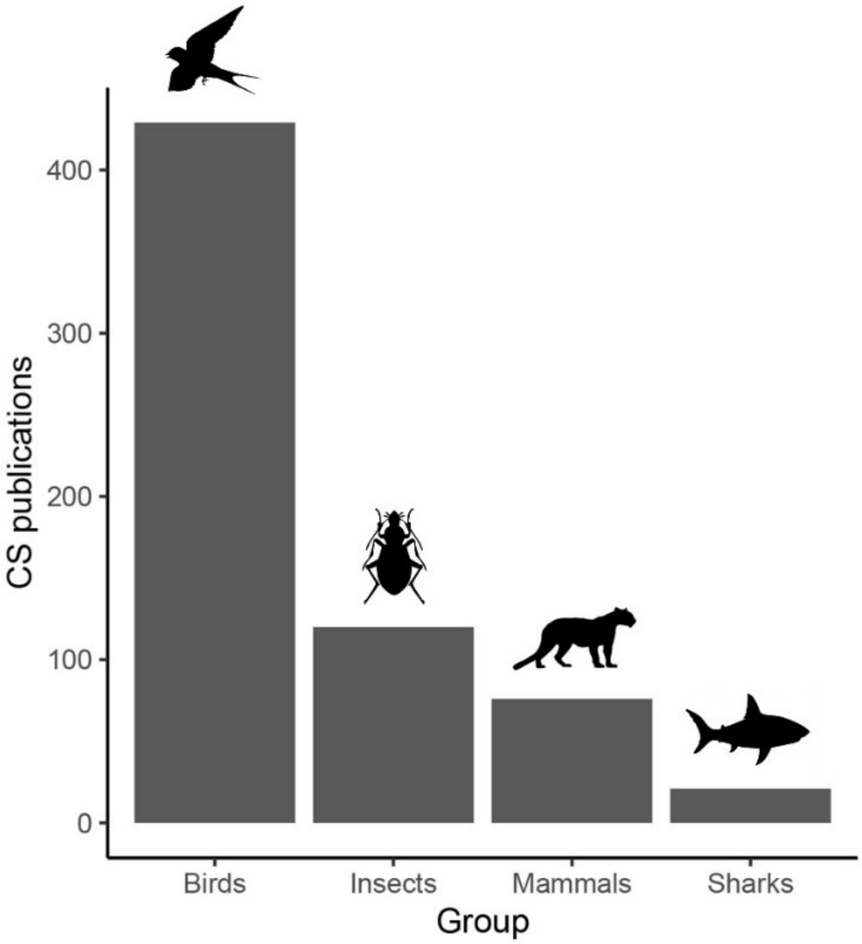

Figure 1. Number of scientific publications based on CS focusing on different groups of animals.

We start with a description of the historical and contemporary interactions between humans and sharks; then discuss shark CS initiatives globally; describe the status and perspectives of shark CS in the Mediterranean Sea; and finally, summarize key elements of effective shark CS, providing advice for filling the gaps of data deficiency on shark species in the Mediterranean Sea.

Table 2. Groups and initiatives related to shark CS in the Mediterranean Sea, including country where initiatives are based and media types deployed to reach users and collect data (SN = social network, WP = web page, MA = mobile app).

nº	Name	Country	Media	Brief description
1	Angel Shark Project	Spain	SN, WP	Collection of data on angelsharks
2	Associaciò Lamna	Spain	SN, WP	Association that aims to promote research and conservation on sharks
3	Elasmocat	Spain		Collection of photos or recordings of sharks from Spain
4	Expedition Grands Requins Du Bassin Algerien	Algerie	SN	Sharks research project in Algeria
5	Ailerons	France	SN, WP	Association to protect Mediterranean sharks
6	A.P.E.C.S.	France	SN, WP	Association for the promotion of shark research and conservation
7	Corsica-Groupe de Recherche sur les Requins de Méditerranée	France	SN, WP	Research and conservation of sharks in Corse
8	Groupe Phocéen d'Etude des Requins	France	SN	Research on sharks and rays of the Mediterranean
9	Longitude 181	France	SN, WP	Shark conservation program, see *Program Requin*
10	Shark Citizen	France	SN, WP	Association promoting protection and public scientific dissemination on sharks
11	Centro Studi Squali	Italy	SN, WP	Italian research institute on sharks
12	Guppo Ricerca Italiano Squali Razze Chimere	Italy	SN, WP	Group of researchers, part of the Italian marine biology association (SIBM)
13	MEDLEM	Italy	-	Project with the aim to collect data on large Mediterranean sharks. See text for details
14	Medsharks	Italy	SN, WP	Association for research, conservation and public scientific dissemination on sharks
15	Operazione Squalo Elefante	Italy	SN	Focused on *C. maximus*
16	Progetto Stellaris	Italy	SN	Focused on *S. stellaris*
17	sharkPulse Italia	Italy	SN, WP, MA	Crowdsourcing platform collecting shark sightings from images
18	Tracking sharks for Conservation	Italy	SN, WP	Tagging program
19	WWF Italia	Italy	SN, WP	Shark conservation program, see *Safe Sharks*
20	Libyan sharks	Libya	SN	Focused on sightings collection in Libya
21	Sharklab Malta	Malta	SN, WP	Shark research center in Malta
22	Sharks and Rays in Albania *	Albania	SN	Focused on sightings collection in Albania
23	iSea	Greece	SN, WP, MA	Protection of aquatic ecosystems. Project on sharks
24	Sharks in Greece	Greece	SN, WP	Focused on sightings' collection in Greece
25	Sharks and Rays in Gr and Cy *	Greece, Cyprus	SN	Focused on sightings' collection in Greece and Cyprus
26	Sharks and Rays in Turkey *	Turkey	SN	Focused on sightings' collection in Turkey
27	Sharks in Isreael *	Israel	SN	Focused on sightings' collection in Israel
28	CIESM Most Wanted Shark	-	WP	Focused on a list of rare sharks
29	Eastern Mediterranean Shark Club	-	SN	Focused on the eastern Mediterranean
30	Hai-Sichtungen Mittelmeer/Sharks of the Mediterranean *	-	SN	Group in German on sharks of the Mediterranean Sea
31	Reef Check Med	-	SN, WP	Generic CS marine project, but with data on sharks
32	Seawhatchers	-	WP	Generic CS marine project, but with data on sharks
33	The MECO project	-	SN, MA	Sightings' collection in the Mediterranean Sea. Related initiatives marked with asterisks (*)

4.1.3 Sharks and human society

Conservation is above all a matter of people, as laws and regulations are promulgated and managed by people, and ultimately affect people (Brown 2003). Shark CS is real people-centered action, and the relationship between humans and sharks strongly affects its future perspective. This relationship, however, is multifaceted and has a complex history. Historically, sharks have mainly been viewed negatively by the public. In ancient times, the sea was a source of myths and legends, especially in the Mediterranean area. Lamia was a shark-like children-eating sea monster in ancient Greece. Several Greek and Roman authors (e.g. Aristotele in "*Historia animalum*", Pliny the Elder in "*Naturalis Historia*", Oppians in "*Halieutica*") also reported evidence of interactions between people and big sharks, which were mostly seen as fearsome and dangerous creatures (Mojetta et al. 2018). Coastal fisheries were important activities of ancient Mediterranean populations, and evidence of the presence of sharks in the catches can be found in mosaics of roman archaeological sites (Mojetta et al. 2018). Later, the vivid imagination of the people of the Middle Ages (from 5th to 15th century) continued to populate the sea with fantastic beasts like the basilisk, tritons, and sirens (Van Duzer 2013). Often, negative connotations including fearsomeness, terror, and death, were ascribed to such creatures, thus instilling in people dread towards the sea (Gessner 1620; Aldovrandi 1642).

Medieval people often observed strange animals stranded along the coasts, or while sailing, and misidentified sharks and other marine animals with those fantastic creatures. Those encounters were often reported with imaginative descriptions and drawings (Figure 2; Jonstonius 1649), and sometimes had religious connotations. An example is the story of a large sawfish (*Pristis pristis*) rostrum preserved as a relic in the Basilica del Carmine Maggiore (Naples, Italy). This is a rostrum of sawfish found stuck on a ship hull after the vessel was rescued from a storm in 1573. The sawfish blade became a relic as the fishers believed the animal prevented the ship from sinking during the storm, a sign of the Virgin Mary's intercession to the fishers who had prayed (Ferretti et al. 2016b). Throughout history, sharks were not only perceived as mysterious and sometimes dangerous creatures but also as pests and therefore were the object of persecution. In the 19th century, the Austro-Hungarian government rewarded fishers in the North Adriatic for killing great white sharks (*Carcharodon carcharias*) seen as competitors of their local fisheries (Faber 1883).

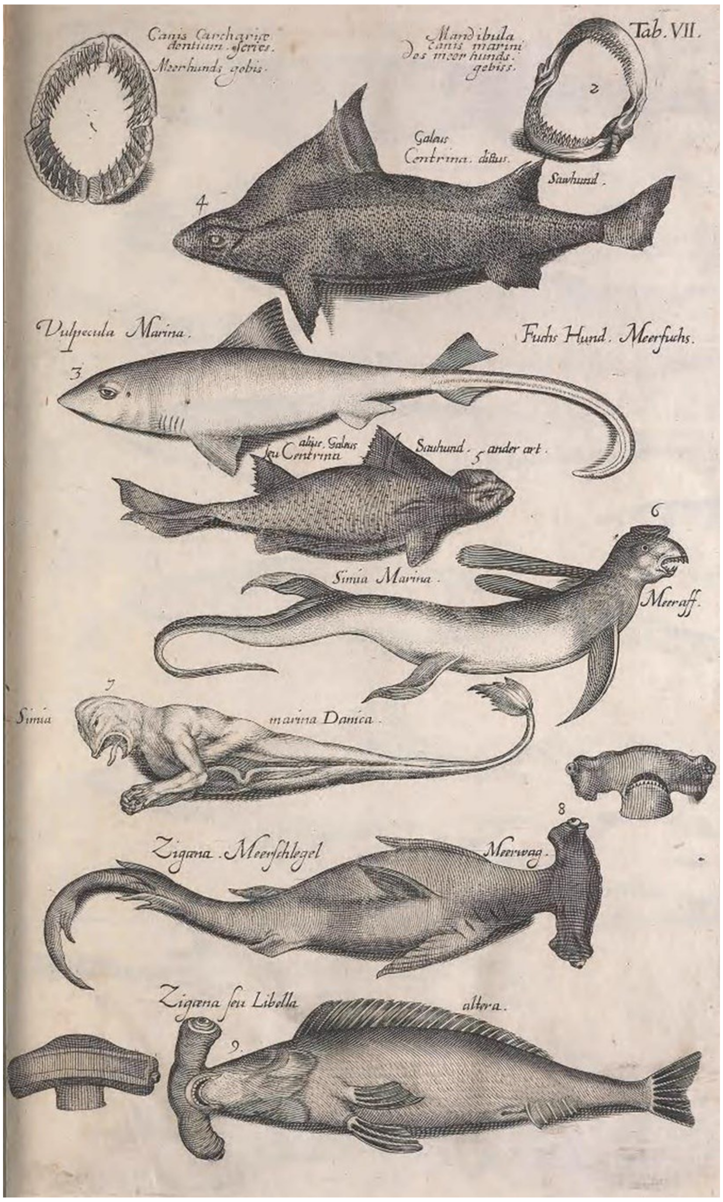

Figure 2. Drawings of sharks from the XVI century including imaginative details such as of fantastic creatures (Jonstonius 1649).

In the last century, the image of sharks has remained mostly negative, with detrimental implications for their conservation (Gibbs & Warren 2015; McCagh et al. 2015; Neff 2015). Shark bite incidents are low-probability high-consequence incidents with a high value for news, and thus their coverage continues to prevail over more positive pro-shark stories (Sabatier & Huveneers 2018). In movies, sharks have often been depicted as villains (Neff 2015). These negative and sensationalized narratives have been high-grossing for the movie industry but have also misinformed the public about sharks' biology and human-shark interactions (Neff & Hueter 2013). For example, the use of expressions like "shark attack", "man-eater", "man-killer", "rogue", "monster" and "jaws", together with dramatized headlines and images in news reports and movies, have created a negative framing and provided sharks with a negative public image (Philpott 2002; Jacques 2010; Neff 2012, 2015; Muter et al. 2013; Neff

& Hueter 2013; McCagh et al. 2015). Through an analysis of media content, Neff and Hueter (2013) concluded that using “shark attack” for describing different types of human-shark interactions has been highly inappropriate, as it has also been used for describing human-shark interactions without physical contacts with sharks like sightings and encounters. In Florida waters, out of 637 reported “shark attacks”, only 11 represented fatal shark bites (Neff & Hueter 2013). Ultimately, misinformation and negative media framing of sharks and human-shark interactions are held responsible for inducing fear among the general public, thus reducing popular concern for sharks, government action to protect sharks, and proper conservation efforts for shark species. Governments often respond to reported “shark attacks” with knee-jerk policy responses (Neff & Hueter 2013). Examples include governments’ decision to launch shark culling campaigns after a series of shark bite incidents which happened in 2001 in the southeastern United States during the so-called “Summer of the Shark”, and after several episodes of shark bites in Western Australia between 2000 and 2014, and New South Whales in 2009 (Philpott 2002; Lynch et al. 2010; Crossley et al. 2014; Neff 2015). A recent study by Pepin-Neff and Wynter (2018) has demonstrated that perceptions that sharks intentionally “attack” people, which is a narrative typical of Jaws and other movies, are directly related to public fear of sharks and public support for lethal shark control policies. However, in recent times, sharks have become more popular, and there is an increasing trend in public concern for the conservation of sharks. Today, public awareness of the declining status of shark populations and of the threats that sharks are facing seems to be high, at least among people with a clear interest in the marine environment (Friedrich et al. 2014).

4.1.4 Changing the tide on the public opinion of sharks: focus on some users of the sea

Based on the analysis of the status quo regarding contemporary shark framing and its potential effects on shark conservation, a change in the public perception of sharks is a critical step for any future conservation actions. In particular, a shift from a “protect human from shark” to a “protect shark from human” perspective is necessary in order to gain public support for shark conservation, which can influence positive political decisions (Simpfendorfer et al. 2011; Muter et al. 2013). In this regard, there is evidence of a reduction in the trend of shark fin sales partly due to campaigns that aim to increase the popular concern for sharks (Dell’Apa et al. 2014). In similar initiatives, special attention ought to be paid to any group of people who have a higher chance to interact with sharks and can play a significant role in shark population dynamics. Two such groups include fishers (recreational and commercial) and ecotourists.

Fishers are one of the main groups of people who interact with sharks. Recreational and commercial fishers often catch sharks, both as target and unintentional catch. Attitude towards shark conservation in fishers has different facets. In Florida, a study on an online anglers' forum reveals that some anglers are aware that fishing certain shark species is illegal, although they believe that this practice has no effect on shark populations and therefore requires no regulation (Shiffman et al. 2017). However, another Florida-based study has demonstrated that personal knowledge of shark conservation issues positively influences anglers' willingness to act in favour of shark conservation, particularly of endangered species (Gallagher et al. 2015). In the Mediterranean Sea, sharks, including vulnerable species, have been catch and bycatch of many fisheries, with destructive consequences (Ferretti et al. 2008, 2010; Font & Lloret 2014). However, in several situations, fishers have been willing to contribute to scientific research with verbal and media-based information on their catches and sightings (Maynou et al. 2011; McClenachan et al. 2012; Fortibuoni et al. 2016).

Shark-based ecotourism can provide significant conservation and educational benefits (Kimmel 1999). It can have high economic value in several parts of the world, especially developing countries, and can be an essential resource of support to local communities, both in terms of job provision and in terms of conservation and education (Brunnschweiler 2010; Cisneros-Montemayor et al. 2013). Coastal communities in Fiji, Palau, Maldives and the Philippines have realized the more sustainable perspective of exploiting shark species as non-consumptive tourism products rather than consumptive fishing products (Pine et al. 2007; Brunnschweiler 2010; Vianna et al. 2011; Gallagher et al. 2015). Ecotourism, however, can also have negative impacts on species, on public safety, and on the management of activities in marine areas, for example, due to feeding, chumming and excessive disturbance (Apps et al. 2015; Bradley et al. 2017; Brunnschweiler et al. 2018; Huveneers et al. 2018). In Australia, cage diving has been observed to influence the swimming behavior of white sharks, possibly impairing their fitness levels (Huveneers et al. 2018). Concerns about public safety have also been raised concerning cage diving, but no evidence of an increase in shark bite incidents has been observed related to this activity (Meyer et al. 2009).

In the Mediterranean Sea, there are a few places where large pelagic sharks can be observed in the wild, and some shark diving activities have been reported from the area (Figure 3). A shark (Small-tooth sand tiger shark, *Odontaspis ferox*) diving hotspot is operating in Beirut, Lebanon, (Gallagher & Hammerschlag 2011). Seasonal aggregations of dusky sharks (*Carcharhinus obscurus*) and sandbar sharks (*Carcharhinus plumbeus*) can be seen in Hadera (Israel) near the Orot Rabin power plant (Barash et al. 2018) where shark-diving activities have recently developed (Zemah Shamir et al. 2019). Similarly, in Lampedusa (Italy), sandbar sharks are often observed from August to September in a diving site near the small rock of Lampione (www.pelagoslampedusa.it). Bluntnose sixgill sharks (*Hexanchus griseus*) are sometimes observed in night dives near deep wrecks (www.oloturiasub.it) in the Messina strait, Italy. Several underwater encounters with blue sharks (*Prionace glauca*) have occurred in Corse (France) (www.legallais.net), but no commercial diving activity has been reported from the area. Shark diving ecotourism has development capacity mainly in the eastern Mediterranean Sea, where relatively less depleted populations of large coastal sharks still exist. Despite the economic potential of shark ecotourism in the region, the touristic intensity may cross the sustainability threshold, requiring proper control (Zemah Shamir et al. 2019).

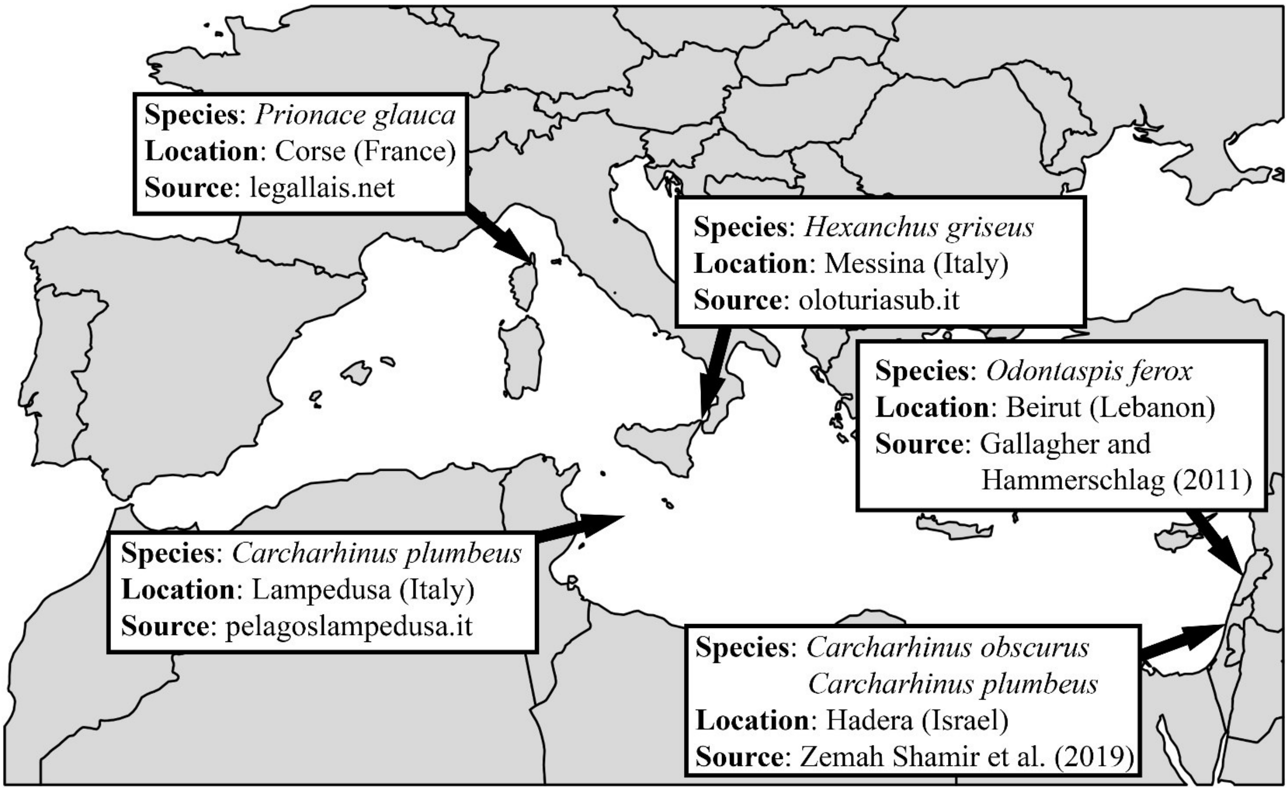

Figure 3. Diving spots in the Mediterranean Sea where shark encounters have been reported.

4.1.5 Sharks and citizen science

Shark research has been steadily growing over the last decades. A scholarly search (webofknowledge.com) using the keyword "shark" as a topic has yielded a total of 13,066 publications. In the last five years, these publications have increased by 48% (R2 = 0.905; Figure 4). Recently, laypeople have become increasingly committed to participating in the scientific process (Silvertown, 2009). A scholarly search with the keyword "Citizen Science" has yielded a total of 7,563 publications. An increment of 135% has been observed over the last five years (R2 = 0.937; Figure 4).

Despite the increasing trend in shark research, the recent expansion of CS has not resulted in a similar trend for shark CS. Among the published CS literature, only 20 scientific publications deal with sharks (Table 3). Two of these have a global scope, and the remaining are more geographically restricted, focusing on specific regions of the world, particularly in the tropical areas of the Indo-Pacific Ocean, and the Eastern Pacific Ocean. The kind of scientific involvement citizens show in these publications is varied. For example, it can be based on observations and counts of shark individuals by scuba divers or on providing media material (e.g. photos, videos), information and knowledge on shark species by divers, fishers, and wildlife watchers. It can also be more opportunistic and based on the access and use of data accessible through databases.

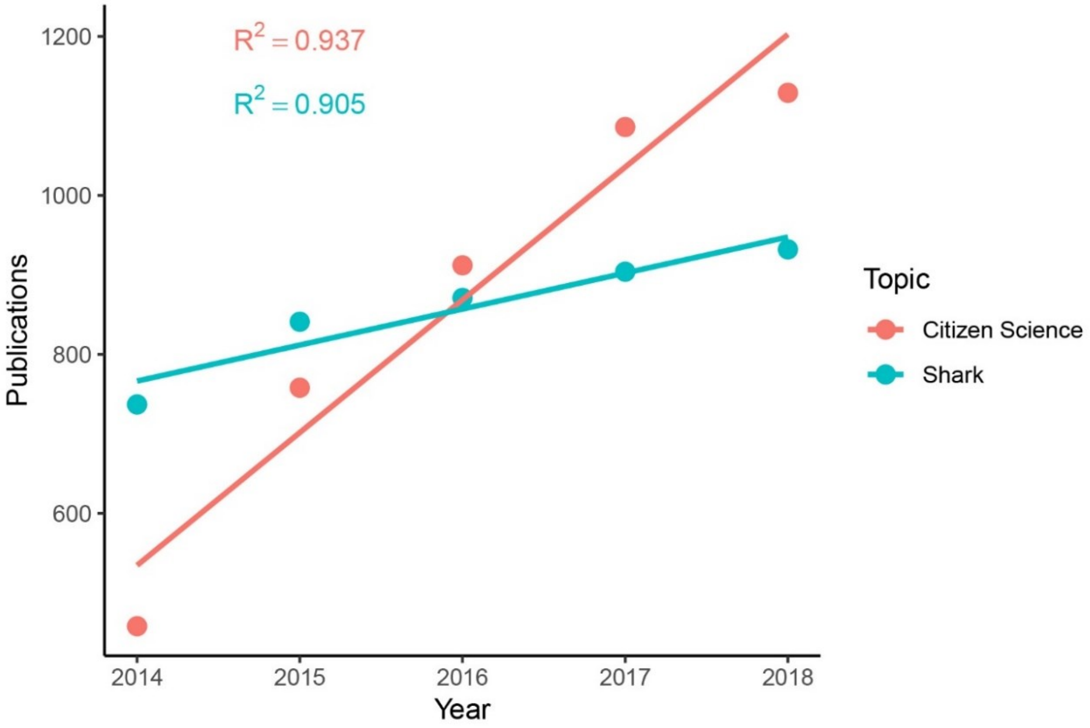

Figure 4. Trends of scientific publications on the topic “citizen science” and “shark” between 2014 and 2018.

CS processes can result in the capture of data regarding a wide variety of shark species across geographies, through the contributions of various groups of ocean users. Trends of shark CS, however, show that this field is not being fully exploited. A large proportion (25%) of the published shark CS tends to revolve around photo identification of one species, the whale shark (*Rhincodon typus*), by scuba divers. Whale sharks are a preferred species in sharks CS initiatives because of their charisma, size, tame nature, ease of monitoring aggregations and identifying morphological characteristics, and value for the tourism industry (Andrzejaczek et al. 2016; Araujo et al. 2017; Norman et al. 2017). Among ocean users, scuba divers are the greatest contributors to shark CS; 60% of shark CS publications are from scuba divers. Scuba divers have successfully contributed to a variety of CS projects, including the study of endangered shark species such as the angelshark (*Squatina squatina*) for zoning (Meyers et al. 2017) and valid investigations on long-term distributions of the whitetip reef shark (*Triaenodon obesus*) and the grey reef shark (*Carcharhinus amblyrhynchos*) (Whitney et al. 2012; Vianna et al. 2014). Scuba divers are generally considered ideal citizen scientists, thanks to some key characteristics such as the ability to access and monitor underwater environments, a general commitment to protect the ecosystems scuba diving depends on, and a desire to grow and to learn (Lucrezi et al. 2018b).

Table 3. List of contributions in shark CS studies by users including fishers and divers. Asterisks (*) mark contribution from the Mediterranean Sea.

n°	Species	Study Type	Area	User Type	Reference
1	*Cethorhinus maximus**	Distribution, size	Mediterranean Sea	Fishers, others	Mancusi et al. 2005
2	*Cethorhinus maximus*	Temporal dynamics	UK	Fishers, others	Witt et al. 2012
3	*Manta alfredi*	Temporal abundance	Lady Elliot Island (AUS)	Divers	Jaine et al. 2012
4	*Triaenodon obesus*	Habitat use	Hawaii	Divers	Whitney et al. 2012
5	*Rhincodon typus*	Mark-recapture	Maldives	Divers	Davies et al. 2013
6	*Carcharhinus amblyrhynchos*	CS data validation	Palau	Divers	Vianna et al. 2014
7	Several species	Temporal trends	Cocos Islands	Divers	White et al. 2015
8	*Rhincodon typus*	Connectivity	Indian Ocean	Divers	Andrejaczek et al 2016
9	*Rhincodon typus*	Demographics, distribution	Philippines	Divers	Araujo et al. 2017
10	*Prionace glauca**	Pollution report	Spain	Fishers	Colmenero et al. 2017
11	*Squatina squatina**	Sightings	Croatia	Fishers	Holcer & Lazar 2017
12	*Rhincodon typus*	Demographics, distribution	Global	Divers	
13	*Squatina squatina*	Demographics, distribution	Canary Islands	Divers	Meyers et al. 2017
14	Sharks in general	Evaluation of shark sanctuaries	Global	Divers	Ward-Paige et al 2017
15	*Hexanchus nakamurai**	Sighting	Albania	Fishers	Bakiu et al. 2018
16	*R. Rhinobatos; G. cemiculus**	Distribution	Aegean Sea	Fishers	Giovos et al. 2018
17	*Rhincodon typus*	Population dynamics, habitat use	Philippines	Divers	McCoy et al. 2018
18	*Oxynotus centrina**	Sighting	Malta	Fishers	Koehler 2018
19	Several species	Distribution	Thailand	Divers	Ward-Paige et al. 2018
20	*Squatina sp.**	Distribution	Mediterranean Sea	Fishers	Giovos et al. 2019

Fishers also contribute to shark CS (eight papers published), while CS initiatives involving other ocean users tend to be scarce (two papers published). An example pertains to the UK initiative of the Marine Conservation Society for monitoring the basking shark (*Cetorhinus maximus*). The initiative began in 1987 involving several kinds of ocean users (e.g. sailors, nature watchers, fishers) and led to the creation of an extensive database. This database later merged with other data collected by the Cornwall Wildlife Trust, making it possible to evaluate the seasonality of shark sightings and its correlation with climatic oscillations (Witt et al. 2012). Another interesting example of fishers involved in CS comes from an Italian monitoring program of bycatch on species of conservation concern: Tracking Sharks for Conservation (http://www.tshark.org/). Within this action, fishers located in different areas of the Adriatic Sea host observers on board in order to gather as much data as possible. Sharks and skates caught during fishing operations are marked by observers on board (tagging) and then, in agreement with the captain, released. Fishers are finally requested to record and communicate the recapture of a tagged specimen.

Global shark CS tends to be based on the collection of data from public and open-access databases. While these initiatives currently represent a minor proportion (2 published papers) of the overall published shark CS, they possess enormous potential to contribute to CS and more importantly, to shark science. One instance is sharkPulse (sharkpulse.org). Launched in 2014 by researchers at Stanford University, the initiative has the aim of creating a global database of image-based sightings to gain information on distributions and abundance of shark species. Through the use of mobile and web applications, data are collected from a variety of ocean users (e.g. scuba divers, sailors, surfers, fishers and beachgoers) and outsourced from other online initiatives and repositories. These data are then organized, validated and curated by shark experts and made available online (http://sharkpulse.org). To date, sharkPulse aggregates over 12,200 records of 367 species of elasmobranchs. Another instance is eOceans (www.eoceans.org), which aims to use CS to describe social, ecological, environmental, policy and economic trends of several marine animal populations (including sharks) and human use patterns. Primarily targeting divers through structured questionnaire surveys, eOceans recently tested the importance of shark sanctuaries for shark conservation, but also the importance of creating programs that can increase public understanding and awareness of sharks, while simultaneously providing an instrument to collect baseline information (Ward-Paige & Worm 2017).

Intending to collect information on shark presence and diversity from egg cases stranded on beaches or found underwater, the Shark Trust launched in 2003 the Great Eggcase Hunt. It started from a beach in Devon, and now it is a global initiative with more than 200,000 records from 22 countries (www.sharktrust.org). iNaturalist is a more general CS initiative that also involves sharks (www.inaturalist.org). Launched in 2008 and currently owned by the California Academy of Science, iNaturalist is a social network entirely dedicated to CS and naturalists and focused on all biodiversity records across taxa. It counts over 10 million observations of species (around 6,000 are on sharks). These data are public and shared with the Global Biodiversity Information Facility (GBIF).

4.1.6 The perspective of shark-related Citizen Science in the Mediterranean Sea

The Mediterranean Sea is in great need of scientific efforts to establish the current trends in distribution and abundance of shark species, and CS has great potential to fulfil this role. There are some challenges to the effective implementation of shark CS in the region, such as the limited availability of shark-based ecotourism activities, and therefore of potential ecotourist volunteers in shark CS (Figure 3). Nevertheless, the Mediterranean Sea is one of the most densely populated regions on the planet and a tourism hot spot. Hence shark CS in the Mediterranean Sea can rely on a very large and diverse suite of users (such as sailors, fishers, and beachgoers) and data collection methods, resulting in initiatives holding educational and political weight. A web search of all the existing shark CS initiatives in the Mediterranean Sea has yielded a total of 33 ongoing projects (Table 2). All initiatives are based on crowdsourcing of sightings and accounts on the occurrence of sharks. Nearly all initiatives use social networks to reach out to ocean users and recruit potential participants, although they have a dedicated webpage to showcase ongoing research activities and data already collected (Table 2).

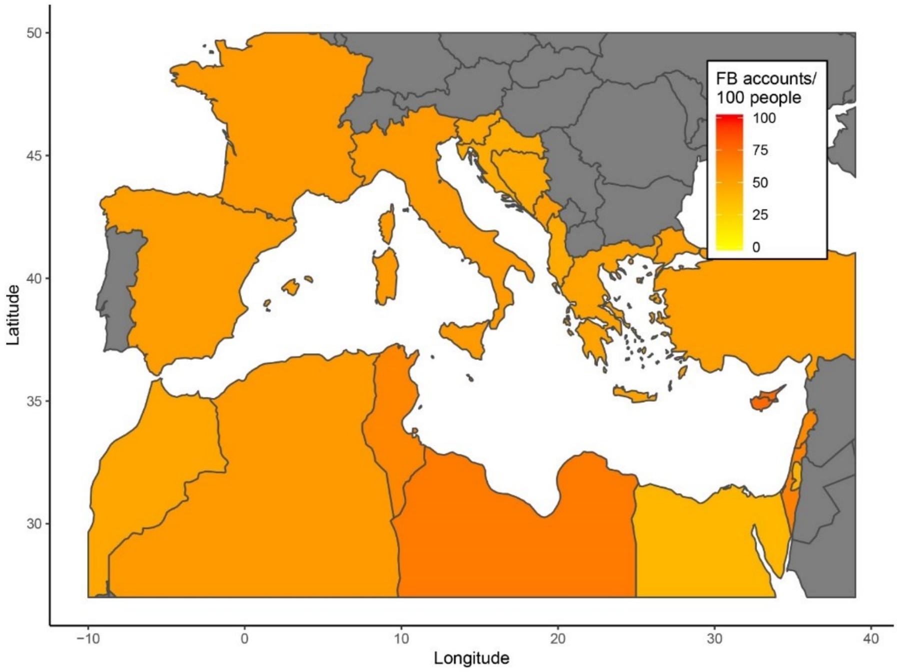

Figure 5. Pattern of Facebook accounts in the countries facing the Mediterranean Sea (source napoleoncat.com), showing that public access to new technology is uniform in the area, with good opportunities for CS to reach users.

Only seven shark CS papers have been published for the Mediterranean Sea (Table 3). This number underrepresents the actual CS effort towards shark research in the region (Table 3). Reasons behind this mismatch are unclear but may include: the difficulty in managing large databases originated from crowdsourcing projects; the distrust of some scientists in the data generated through CS efforts; lack of time, people and resources for processing and publishing data, and no coordination between initiatives. These are problems generally associated with CS projects across disciplines and focal species (Lucrezi et al. 2018b). Two research papers cover the Mediterranean Sea as a whole: a study on the basking shark (*Cetorhinus maximus*), conducted with data originated from the "Large Elasmobranch Monitoring" program (MEDLEM) database (Mancusi et al. 2005), and a study on the presence and distribution of angelsharks (Chondrichthyes: Squatinidae; Giovos et al. 2019). Both studies mixed CS contribution with other research methods. MEDLEM is a survey on the presence of large elasmobranchs commenced in Italian waters in 1985 and later enlarged to other Mediterranean countries: major data have been provided by the collaboration of military authorities and research institutes, but the program also allows the contribution of professional and recreational fishers (Serena et al. 2014). The study on angelsharks was conducted mixing CS photographic reports with targeted interviews, fisheries data and bibliographic accounts (Giovos et al. 2019). The remaining five papers focus on more localized research primarily in the eastern Mediterranean Sea, with only one study in the western Mediterranean (Spain) (Table 3). These papers highlight the crucial role of social networks for obtaining information on species' occurrence. Examples include records of individuals of the locally rare and endangered angelshark (*Squatina squatina*) in the North Adriatic Sea (Holcer & Lazar 2017); guitarfishes (Chondrichthyes: Rhinobatidae) in Greece (Giovos et al. 2018); angular rough shark (*Oxinotus centrina*) in Maltese waters (Koehler 2018); and bigeye sixgill shark (*Hexanchus nakamurai*), considered rare in the Mediterranean area and possibly misidentified with the bluntnose sixgill shark (*Hexanchus griseus*) (Bakiu et al. 2018). Last, published shark CS has contributed to the growing collection of evidence on the effects of plastic pollution in the Mediterranean Sea on juvenile blue sharks (Colmenero et al. 2017).

While most of the Mediterranean CS initiatives on sharks have yet to publish their data, their activities are already contributing significantly to our understanding of the distribution, abundance and behavior of shark species through their ongoing outreach effort. Videos are particularly useful. An example is a video showing the predation of a giant devil ray (*Mobula mobular*) by a shortfin mako in the Messina Strait, which was shared by several shark CS Facebook groups and projects. The video was made available online and shared by several local web news services (e.g. la Sicilia.it, letteraemme.it), often with misidentification of the shortfin mako with a great white shark (*Carcharodon carcharias*), belonging to the same family. Video shared by ocean users could also report interesting and uncommon events: a stranded pregnant blue shark female was filmed giving birth to near 50 pups in Villapiana (Italy), and the video was published and shared online (gazzettadelsud.it).

Fifty species of sharks occur in the Mediterranean Sea, and 57% are endangered according to the IUCN. However, only ten have been the focus of investigations using CS approaches. These investigations had limited geographical scope, focusing mostly on local rather than regional scales (Table 3). Local CS projects can be useful in identifying rare and uncommon species as they may have a more intense and effective focus in a given area. However, large CS networks are necessary in order to reach a consistent number of observations, as it happens for example on reef CS, where Reef Check (https://reefcheck.org) acts as aggregator of several regional and local initiatives creating a global network of local projects and succeeding in effectively creating a global snapshot on the status of tropical and temperate reefs. In order to properly analyze and assess the regional status of sharks in the Mediterranean Sea, networks are desirable since they would promote connections between various existing initiatives and between stakeholders. Global networks of local initiatives can breach linguistic barriers and thus reach more ocean users. This is an aspect particularly important in the Mediterranean Sea as the region has 22 coastal nations and 12 languages. It is also an aspect that the sharkPulse initiative is implementing through the creation of national focal points.

CS networks for shark science in the Mediterranean Sea would greatly benefit from the use of new technologies, which offer the opportunity to share detailed information quickly and effectively. Smartphones and social networks are widespread. There are 6.5 billion smartphone users around the world (Orams & Lück 2014), and the use of social networks has been rapidly increasing in the last decade. As shown in Figure 5, in coastal Mediterranean countries, the percentage of Facebook users goes between 40% and 68% of the population with a total of 250 million users (data from napoleoncat.com). This pattern reveals how new technologies are uniformly spread among people around the Mediterranean Sea, offering a tremendous opportunity for CS in the region. The use of new technologies, however, needs to be accompanied by connections with experts who can validate the records provided by volunteers. The previously mentioned case of the misidentified shortfin mako with a great white shark is just one instance of imprecise or incorrect shark sightings often shared online. Hence it is extremely important that the surge of new observations becoming available through social networks and other online platforms are carefully validated by scientists before being used for research and management.

CS networks for shark research in the Mediterranean Sea can become more effective when educational efforts accompany them. Although shark-based ecotourism in the area is uncommon, marine tourism offers ample opportunities for interpretation and education on threats affecting shark populations, from overfishing to climate change and pollution. The Ocean Literacy movement, which originated in the United States, characterizes an important component of public education on the connection between humans and the ocean, including descriptions of marine food webs and predator-prey interactions (Steel et al. 2005). Similarly, institutions including museums and aquaria would represent important partners supporting CS projects as they play a critical role in public education and stimulate the public's interest in the ocean (Lucrezi et al. 2018a). Aquaria also allow the public to gain high-impact firsthand knowledge of shark biology and ecology, through the direct observation of individuals and interpretation programs run by staff and researchers (Friedrich et al. 2014; Grassmann et al. 2017; Pepin-Neff & Wynter 2018). Ultimately, museums and aquaria are capable of casting a wide promotional net for several shark CS projects. For example, iNaturalist is managed by the California Academy of Science. Similarly, the Monterey Bay Aquarium in California, USA, is collaborating in the sharkPulse initiative.

While there are cases of successful CS promotion and management by museums and aquaria, the number of institutes of this kind engaging in CS remains limited globally. Let alone in the Mediterranean Sea, the Cattolica Aquarium, in Italy, actively collaborates with sharkPulse and is currently the only Mediterranean aquarium involved in a shark CS initiative (Bargnesi et al. 2018). Europe counts a total of 107 aquaria having shark exhibitions, and 12 of them lie along the coasts of the Mediterranean Sea. These structures have been playing a crucial role in shark conservation, through the improvement of husbandries and captive management techniques, reaching the important goal of captive reproduction for several endangered species (Janse et al. 2017). A future commitment to CS by these structures would increase the opportunities of ocean education for visitors, enable visitors to participate in shark CS actively, and ultimately increase the capacity of these initiatives in thus gathering information on focal shark species.

4.1.7 Conclusions

The relationship between humans and sharks has been historically characterized by a mixture of mystery, fear, and respect. Especially in the last few decades, the attitude of people toward sharks changed from having a negative connotation to a strong attraction, fascination and awareness of their conservation status as probably never happened before. Sharks have become more important in management and conservation agendas, and this has produced a beneficial effect on the attitude of people toward these animals. This attraction offers an opportunity to engage citizens in shark science through CS, especially to counteract the negative effects that human activities are having on shark species and populations. Overall, shark CS has successfully gathered important data for the mapping of distributions and abundance of shark species at several locations. In the Mediterranean Sea, despite the limited capacity of shark-based ecotourism, several shark CS projects are undergoing, thanks to the coordination of associations and nongovernmental organizations, and the contributions of ocean users. These projects can potentially aggregate a large amount of data on the occurrence and distribution of endangered and extremely rare species, which require research and protection. New technologies such as mobile phone apps, together with social network initiatives, are fundamental to reach and recruit a large number of people and create a large, diverse CS community, including both regular and occasional ocean users. Although multiple CS initiatives for shark research are ongoing in the Mediterranean Sea, coordination, networking and collaboration are needed for effective data collection, and for informing spatial and temporal analysis of shark species' distribution and abundance. These elements can ensure that useful and up to date data are provided to decision-makers for developing effective conservation measures for threatened shark species. CS projects can also stimulate public awareness of marine issues and active participation in shark conservation. Several institutions can be involved in this process and, among all, aquaria are the best candidates, considering their role in connecting people and the ocean through the direct observation of species and other experiential learning programs. Networking and new technologies are key for the future of CS (Newman et al. 2012), and this is particularly true for the Mediterranean Sea, where CS efforts of scientists, institutions, communities, organizations, and volunteers, are not yet efficiently coordinated and integrated for the common goal of promoting effective shark conservation measures.

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4.2 NEW TECHNOLOGIES CAN SUPPORT DATA COLLECTION ON ENDANGERED SHARK SPECIES IN THE MEDITERRANEAN SEA

4.2.1 Abstract

In the last fifty years, Mediterranean sharks' populations showed steep declines and the recent IUCN regional assessment listed 57% of the species as endangered, while 25% of them are still considered data deficient. Nevertheless, scientific surveys do not provide enough observation effort to collect relevant data on the presence and distribution of sharks in this area, where sharks are currently one of the rarest and more elusive groups of animals. In light of this situation, new technologies can create a link between people and scientists, supporting the development of new monitoring strategies that will improve data collection in the Mediterranean Sea. Here we use data collected in the framework of sharkPulse, an international collaborative project aimed to create the biggest world database of sharks' image-based sightings. SharkPulse is a modular crowdsourcing platform created to mine and aggregate shark sightings from images available on the web, social network, and private archives.

From 2017, the systematic collection of sharks' photo records in the Mediterranean Sea provided 967 photographic records for 36 shark species. Exploratory data analysis revealed interesting insights, as the detection of possible strongholds for rare and endangered species, seasonal changes in the spatial distribution of the sightings, and, especially for pelagic species, presence of pups and immature specimens with four cases of documented parturition. Most of the pelagic species registers are related to fishing activities, sometimes identified as both professional and recreational. Opportunistic data collection and citizen science are growing fields in the Mediterranean area, with several initiatives targeting sharks that are emerging in the lasts years. As our preliminary analysis showed, these kinds of data could really have an important role in setting management and conservation plans, but it is important to think and act at a regional level, and an integrated and coordinated network among initiatives for the collection and analysis of these kinds of data is mandatory to face the serious conservation issues that sharks are facing today in the Mediterranea Sea.

4.2.2 Introduction

The sharks' conservation status is a crucial and particularly delicated point in the Mediterranean Sea (Cashion et al. 2019). Following the last IUCN regional assessment, 23 species (57 %) are listed as endangered, while for 10 of them (25 %), data collected are not enough consistent to assess their status and, hence, are still listed as data deficient. This means that only 7 (18 %) of the overall 40 assessed species in the area are considered not threatened by IUCN (Dulvy et al. 2016). This makes the Mediterranean Sea one of the areas with the worlds' higher percentage of threatened shark species (Dulvy et al. 2014). Furthermore, local pelagic shark populations showed declines up to 98-99 % in the second half of the last century (Ferretti et al. 2008), albeit historical studies provide clues of a wider presence of species that now are very rare (i.e. the angelsharks, Squatina spp. , Fortibuoni et al. 2016) and the sand tiger shark, Carcharias taurus (Bargnesi et al. 2020b). Fisheries targeting sharks are present in the Mediterreanean Sea and normally target some specific species such as: smoothounds (*Mustelus* spp.), catsharks (*Scyliorhinus* spp.) and dogfishes (*Squalus* spp.). However, sharks represent also part of the bycatch of several fisheries such as trawling and longlines (Cavanagh & Gibson 2007). Official data on sharks bycatch (provided by FAO) in the area are not consistent enough to carry out any stock management, and, in addition, this data are often collected with a very low taxonomic resolution (Cashion et al. 2019). Furthermore, data on threatened species are insufficient, especially for the most endangered ones (Dulvy et al. 2016).

Collecting data on the sharks presence in the area is a pivotal need and new strategies need to take place. Involving non-scientist people in the collection of data for scientific purposes is a growing field of science, known as citizen science (Bonney et al. 2009). New tecnhologies are a great opportunity since they can build an easy and quick connection between scientists and people (Kobori et al. 2016). Web platforms and mobile applications are part of the opportunities provided by the emerging technologies and the use of these tools in citizen science processes has already been done with good results (Sullivan et al. 2014). Opportunistic data on Mediterranean sharks gained from citizen science processes were already collected and used, but this attempts were focusing on few species in specific areas (Bargnesi et al. 2020a). In the area, the collection of data through the use of mixed methods, which includes both citizen science and traditional science tools, has been already carried out, permitting to gather important information on endangered species such as the angelsharks in the Eastern Mediterranean Sea (Giovos et al. 2019) and the basking shark (*Cethorhinus maximus*, Mancusi et al. 2005). However, an exstensive data collection on sharks' presence for the whole Mediterranean area, supported by an integrated network and new technologies, does not exist. Here we present data elaborations from the first global citizen science project focused on sharks' opportunistic data collection, and we explore the opportunities brought by this kinds of data in terms of shark conservation and management in the Mediterranean Sea. Opportunistic data can provide useful support to gain information on the biology and the ecology of the species, they can contribute in the identification of strongholds for particularly endangered species, and they can provide information to identify the major threats that sharks are facing in the area.

4.2.3 Materials and Methods

Study area

The area considered in this study is the whole Mediterranean Sea. Although a few records from the Black Sea were collected and stored in the main database, they were not included in the analysis concernig this publication.

Data collection

Mediterranean sharks' opportunistic records were collected within the shakPulse project. SharkPulse is a crowdsourcing platform created and managed by the Stanford University with the aim to store in one place the world biggest database of shark image-based sightings. To be part of the database each shark image has to be accompanied at least with date and location of the shot. Species identification is checked and validated by a team of researchers for each record. Several strategies were used to build up the biggest image-based shark sightings database, from citizen science to web scraping. Ocean users (e.g. scuba divers, fishermen, surfers) are involved in the research through both a dedicated web page (http:\\sharkpulse.org) and a mobile app (iOS and Android), that permit to directly submit new sightings. Moreover, new sightings are published on the project dedicated pages and profiles existing on the most widespread social networks (Facebook, Twitter, Instagram). In addition, scripting (Unix, Phyton, R) was used to automatically aggregate images identified or flagged as sharks in some web platforms (e.g. iNaturalist, Flickr) with the available APIs. SharkPulse is a global project, hence, in order to reach the maximum number of ocean users and to breach linguistic barriers, national focal points were created. Since February 2017, the Italian national focal point is collecting records and sightings from the Mediterranean Sea, in coordination with several Italian universities (Marche Polytechnic University, Sapienza University of Rome, University of Bologna), and in collaboration with local research bodies. The data presented in this paper where collected in a three year period, between February 2017 and January 2020.

Data storage and management

SharkPulse data are stored in a PostgreSQL (version 9.5.19) table, an open-source object-relational database system. Each record is archived with the related information. Required information are: date, latitude, longitude, image name, and source. Some additional information such as time, email or contact of the source, device type, common name, and notes could eventually be included. A progressive and unique ID is attributed to each record. Images are stored in a dedicated folder with a .jpeg format and both the database and all the images are saved in a central server (Linux 4.4.0).

We also recorded the observation type, dividing our records among fishing observation (indicating where possible if coming from a professional fishing, recreational fishing, or record from a local fish market), observation while diving, stranded specimens, and surface observation of a free-swimming animal (i.e. while sailing or from the shore). In some cases, picture details (e.g. person handling the animal, boat engine) allowed us to estimate the class size of the shark in the shoot. This process was applied for two species: the shortfin mako (*Isurus oxyrinchus*) and the blue shark (*Prionace glauca*). For the shortfin mako, we considered as immature all the specimens showing a TL (Total Length) objectively < 200 cm (Kabasakal 2015). Similarly, a 120 cm threshold was set for immature blue sharks. In some cases, blue shark specimens looked very young (TL << 70 cm) and, hence, they were classified as newborn, or young-of-the-year (YOY) following Megalofonou et al. (2009). For both species, some images were classified as ND (not detected) since the size class could not be inferred from the picture.

Data analysis

Exploratory data analysis, mapping, and plotting were performed with Rstudio software (version 1.0.153), with the support of several related packages ("tidyverse", "ggpubr", "mapdata", "raster", "sp"). IUCN categories were assigned according to the last regional assessment for the Mediterranean Sea (Dulvy et al. 2016). For Critically Endangered species, we overlapped a 0.5° cell size grid to the entire basin and calculated the number of records belonging to each cell in order to identify critical areas. In addition, rare and endangered species occurrence locations were used to create spatial polygons identifying possible species' last strongholds. Then these polygons were plotted on a map in order to identify geographical zones with the presence of particularly endangered and rare species. We tested this method in two demersal species: *Squatina squatina* and *Odontaspis ferox*.

In order to estimate seasonal variations in the point density, a bivariate Kernel density (Silverman 1986) was applied to sightings locations. In this way, we identified areas with a higher density of records within the study area. A bivariate kernel density function is estimated as:

$$\frac{1}{2\pi n h^2}\sum_{i=1}^{n} exp\left(-\frac{d_i}{2h^2}\right)$$

where n is the number of location points on a surface (x,y), d_i is the distance between the i^{th} observation and the point (x,y) and h is the smoothing parameter (Worton 1995). There are several methods for calculating h, which gives the width of the kernels. Since our sampling size is not large (< 50 points), to minimize the spatial bias, the method proposed by Seaman & Powell (1996) is generally suggested to avoid an overestimation of the kernels' width (Seaman et al. 1999):

$$h_{x,y} = \sigma_{x,y} n^{\frac{1}{6}}$$

where $\sigma_{x,y}$ is the standard deviation for each coordinate vector and $h_{x,y}$ the smoothing parameter for each spatial direction.

4.2.4 Results

A total of 967 image-based records belonging to 36 different shark species were collected from the Mediterranean Sea in this study (Table 1). The five most reported species are: the bluntnose sixgill shark (*Hexanchus griseus*, 22%, 217 pictures), the blue shark (*Prionace glauca*, 20%, 198 pictures), the shortfin mako (*Isurus oxyrichus*, 9%, 86 pictures), the basking shark (*Cethorhinus maximus*, 8%, 81 pictures), and the thresher shark (*Alopias vulpinus*, 7%, 70 pictures). The most-reported order is the Lamniformes with a total of 339 pictures (35%). For 81 pictures (8%) it was not possible to identify the shark to the species level, but the specimens were classified to the genus (73 records, 7%) or family level (8 records, 0.8%). The most challenging group in image classification was the genus *Carcharhinus* spp., where it was not possible to classify records to the species level in 42 cases (57% of all the *Carcharhinus* spp. records). Among the seven records classified to the family level, five belongs to the family Lamnidae, and two belongs to the family Odontaspididae. In the Mediterranean sea, two species of Odontaspididae are found, *Carcharias taurus* and *Odontaspis ferox*, and in our study we recorded 11 times the presence of *O. ferox* in the area, but we did not found any evidence of *C. taurus*.

Following the IUCN criteria for endangered species and the regional assessment for the Mediterranean Sea (Dulvy et al. 2016), among the 15 most recorded species, seven are listed as critically endangered, four as endangered, two as least concern, one is listed as vulnerable and the last is data deficient (Figure 1A).

Seventy-three percent of our photo records belong to threatened or data deficient species, and 44% of the total records are referred to species listed as critically endangered (Figure 1B). More than an half (60%) of the recorded species are threatened and 26% are data deficient or not yet evaluated (Figure 1C). Areas with a high number of records belonging to critically endangered species were identified around Corsica, in the Gulf of Lion, around Malta and near the Strait of Messina in Sicily, in

Southern Italy; hot spots are also present in the Adriatic Sea, in Gulf of Gabes, in the central Aegean Sea and in the Marmara Sea (Figure 1D).

Table 1. Summary of the sharks' records from sharkPulse in the Mediterranean Sea.

Records	Counts	IUCN
Lamniformes	339	
Cethorhinus maximus	86	EN
Isurus oxyrinchus	81	CR
Alopias vulpinus	70	EN
Carcharodon carcharias	43	CR
Alopias superciliosus	25	EN
Odontaspis ferox	13	CR
Lamna nasus	7	CR
(Lamnidae family)	6	
Alopias sp.	6	
(Odontaspididae family)	2	
Hexanchiformes	227	
Hexanchus griseus	217	LC
Heptranchias perlo	5	
Hexanchus sp.	4	DD
Hexanchus nakamurai	1	DD
Squaliformes	48	
Oxynotus centrina	15	CR
Centrophorus granulosus	10	CR
Dalatias licha	10	VU
Squalus blainvillei	4	DD
Echinorhinus brucus	2	EN
Squalus acanthias	2	EN
(Squalus sp)	2	
(Centrophorus sp)	1	
Etmopterus spinax	1	DD
Somniosus rostratus	1	DD
Carcharhiniformes	333	
Prionace glauca	198	CR
(Carcharhinus sp)	42	
Mustelus mustelus	19	VU
Carcharhinus plumbeus	16	EN
Carcharhinus obscurus	12	DD
(Mustelus sp)	10	
Scyliorhinus canicula	8	LC
Galeorhinus galeus	6	VU
Scyliorhinus stellaris	5	NT
Carcharhinus brachyurus	4	DD
Sphyrna zygaena	3	CR
Galeus melastomus	2	LC
Mustelus punctulatus	2	VU
(Sphyrna sp)	2	
Carcharhinus altimus	1	DD
Carcharhinus brevipinna	1	NA
Carcharhinus falciformis	1	NA
Sphyrna lewini	1	NA
Squatiniformes	20	
Squatina squatina	14	CR
Squatina aculeata	3	CR
Squatina oculata	2	CR
(Squatina sp)	1	

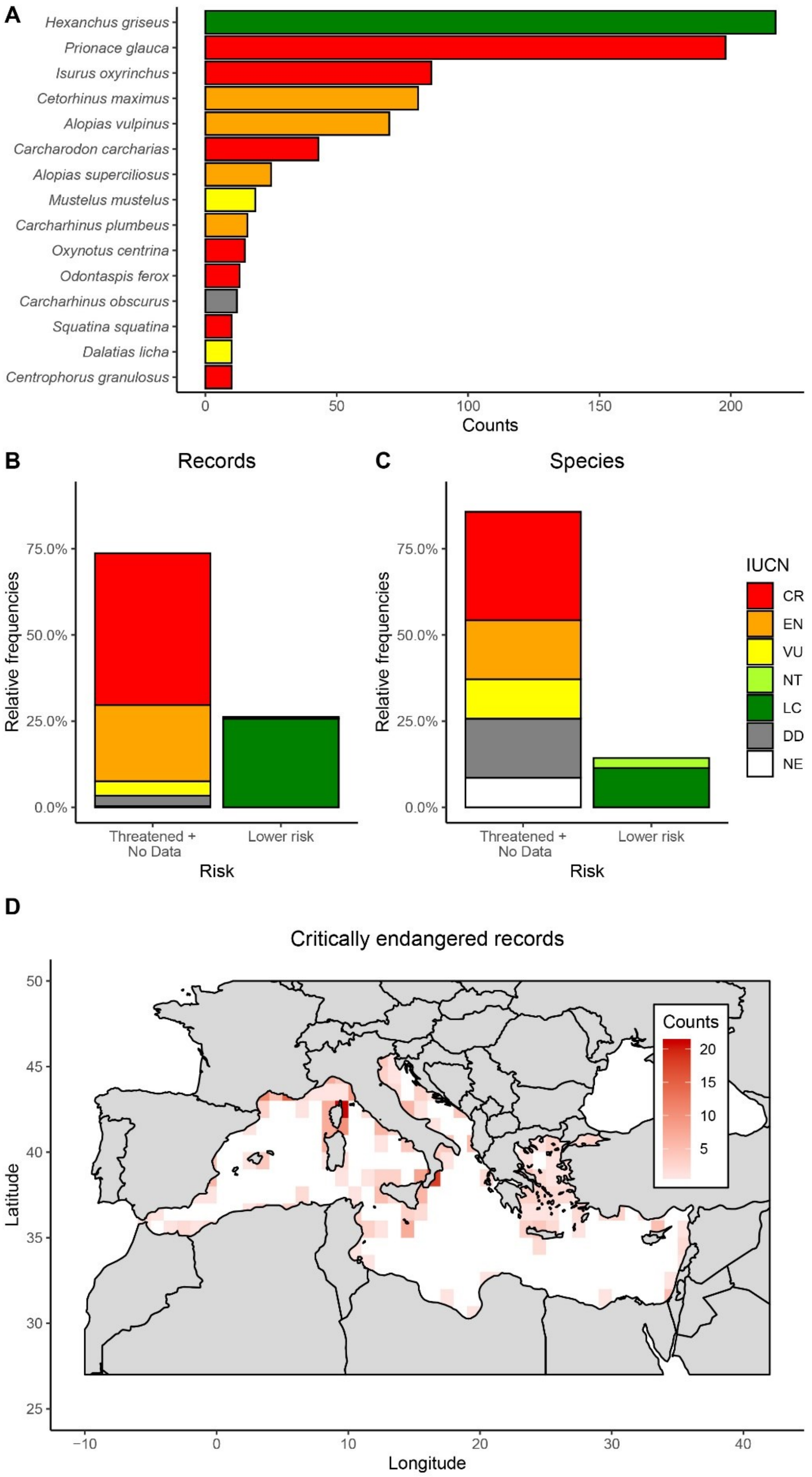

Figure 1. Fifteen most reported species with their conservation status in the area following IUCN (A). Relative frequency of records (B) and species (C) related to the IUCN regional assessment. Raster analysis of the distribution of the critically endangered records (D).

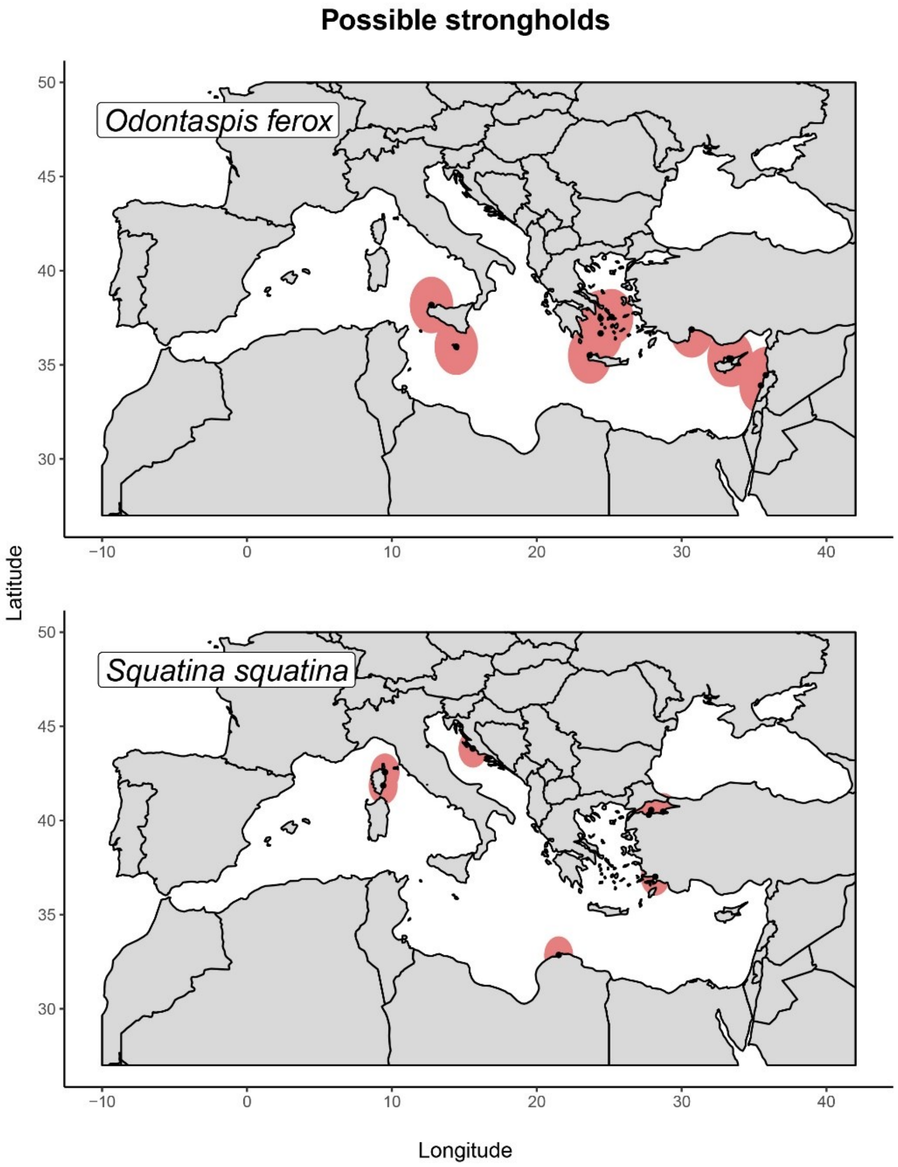

Figure 2. Possible strongholds for small-tooth sand tiger shark (*Odontaspis ferox*), and the angel shark (*Squatina squatina*).

Shapefiles originated from our presence data of rare and endangered species could detect last strongholds. The small-tooth sand tiger shark (*Odontaspis ferox*) has been recorded only in the Eastern part of the Mediterranean Sea, and possible strongholds for the species could be the Sicilian Channel, the central Aegean Sea, Cyprus and Lebanon. Our records of the angel shark (*Squatina squatina*) shows the possibility of strongholds for the species in Corsica, Croatia, Southern Western Turkey and in the Marmara Sea (Figure 2).

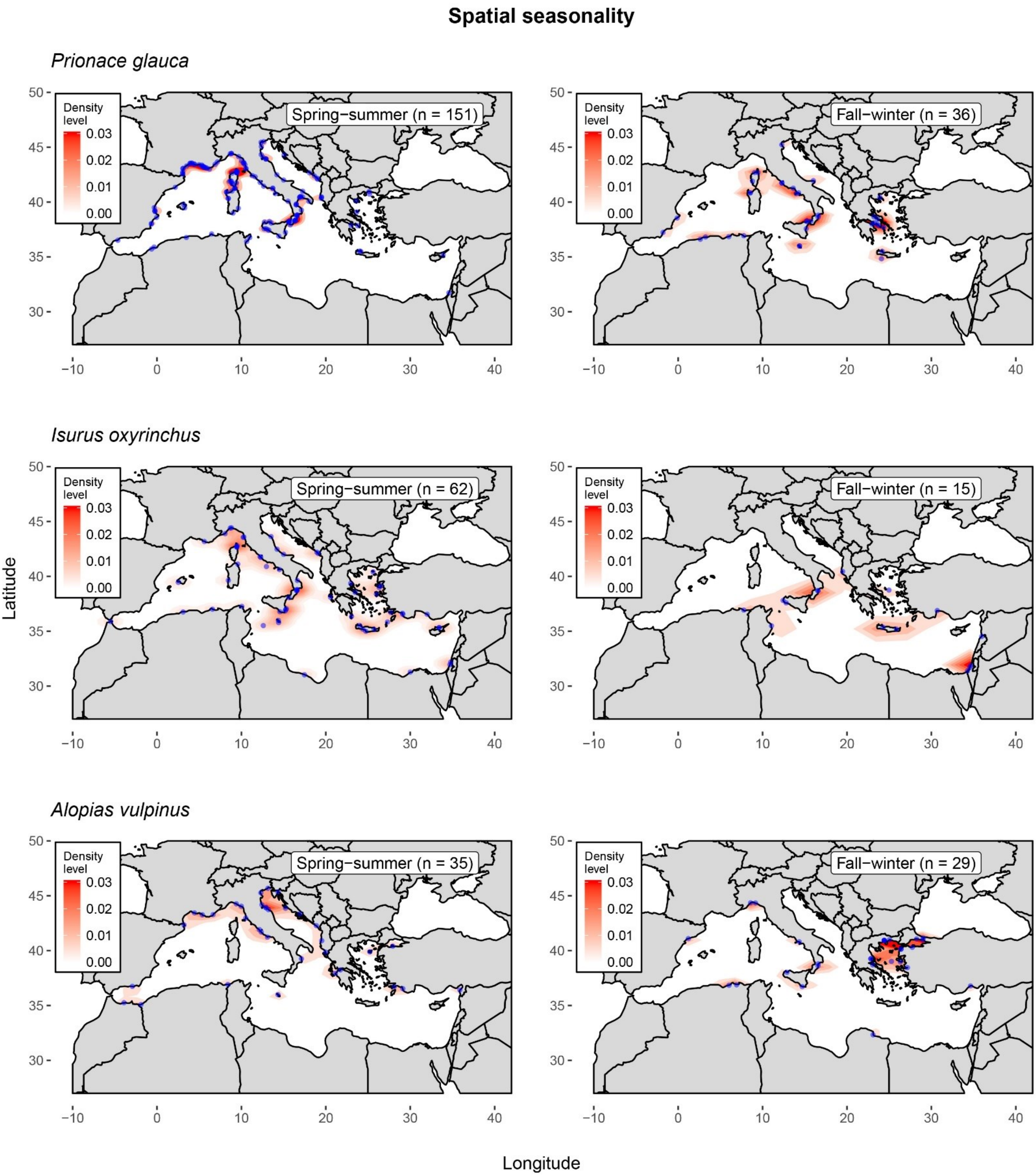

Figure 3. Seasonal patterns of records distribution between spring-summer and fall-winter in three pelagic shark species: blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), and common thresher (*Alopias vulpinus*).

Analyzing the spatial distribution and the density of our sighting records, we identified different spatial patterns for three of the most reported pelagic species throughout the year and, in particular, between the warm (spring-summer) and the cold period (fall-winter). Blue shark, shortfin mako, and thresher shark sighting records clearly shifted southward during the coldest months (Figure 3).

Hotspots for the blues shark sightings in the warm period are represented by the Southern France, Corsica, Tuscany (Italy), and the western Ionian Sea, while in the cold period the majority of records are located in the central Aegean Sea. A similar pattern is showed by the thresher shark, which is mostly sighted in Southern France and in the Northern Adriatic Sea during the warm period, while records seem to be more abundant in the Aegean and the Marmara Sea during the fall-winter seasons. Shortfin mako records are also mostly located in the Ligurian Sea in the warmer period, and totally disappearing from the area during the coolest months.

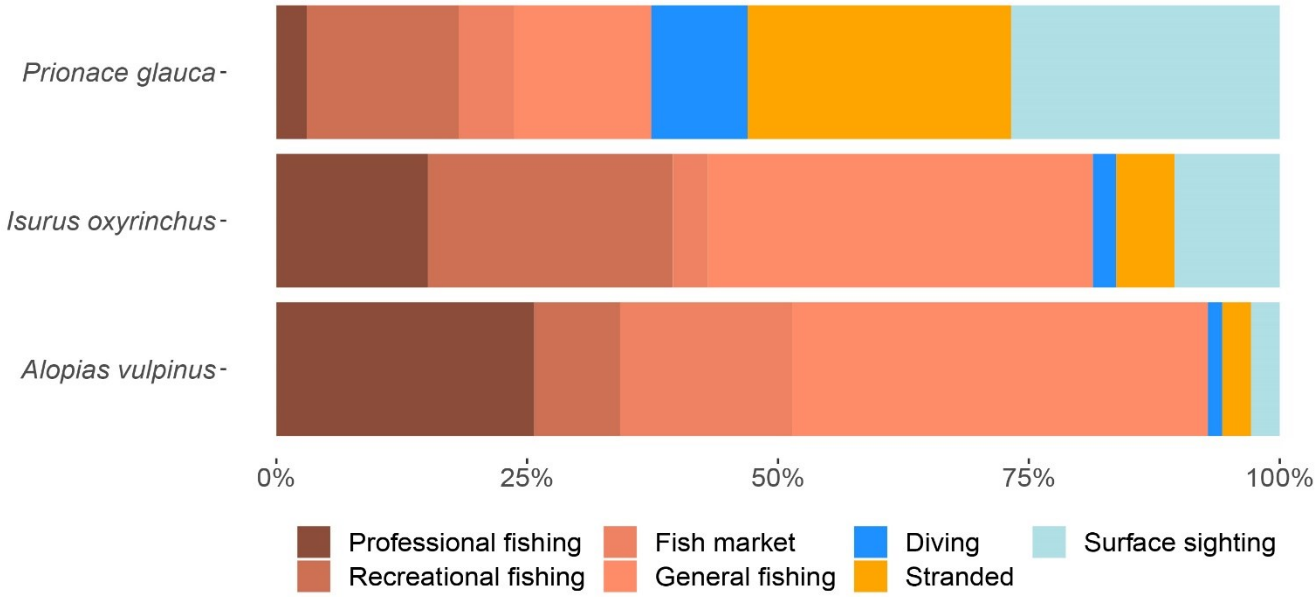

Figure 4. Type of observation among the records of some pelagic sharks' speceis: the blue shark (*Prionace glauca*), the common thresher (*Alopias vulpinus*) and the shortfin mako (*Isurus oxyrinchus*).

Eighty-one percent of the shortfin mako records and 92% of the common thresher records came from observation related to fishing activities (Figure 4). This percentage is lower in the blue shark records (37%), while there is a relevant number of stranded specimens (26%) and record associated with a direct observation of live animals swimming at the surface (27%).

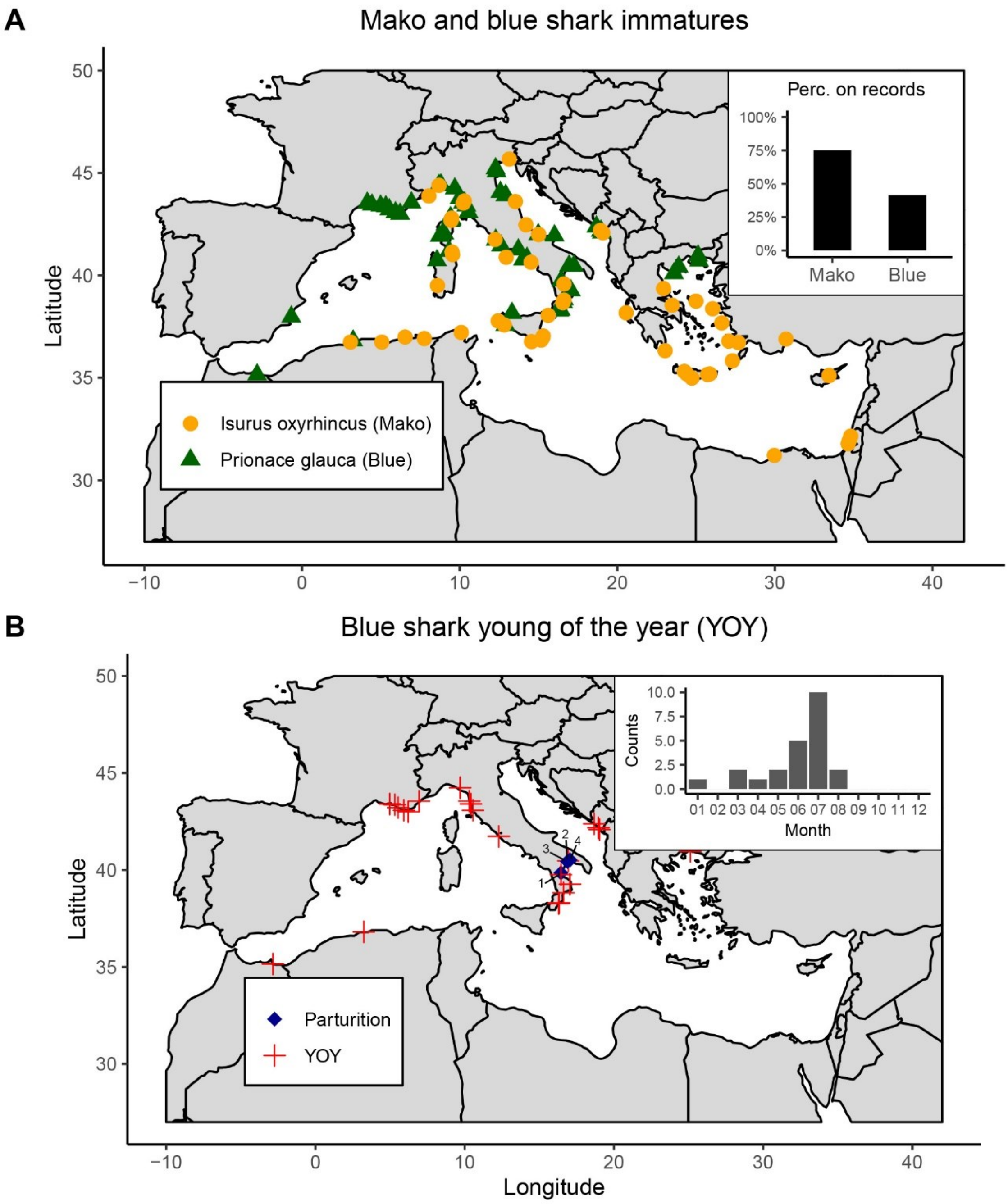

Figure 5. Blue shark (*Prionace glauca*) and shortfin mako (*Isurus oxyrinchus*) immature distribution and percentage on the records from which size class was detectable (A). Spatial and temporal distribution of blue shark young of the year (YOY); blue diamonds mark parturition sites (B), numbers refer to Table 2.

Size classes were analyzed for the shortfin mako and the blue shark (see Materials and Methods for details). The presence of immature specimens of these species in our records is widespred in the study area. However, although shortfin mako immatures are found throughout all the investigated area (with a few exeptions, i.e. Southern France and Spain), the presence of immature blue shark seems to be mainly associated with the Northern part of the Mediterranean, particularly in Southern France, Italy and Northern Aegean Sea (Figure 5A). Interestingly, cosidering only the specimens for which the size was estimated, immatures represented a large portion in both species. Blue shark immatures represent the 41%, and even a higher value (75%) was recorded for the shortfin mako records. Among blue shark records, it was possible to discriminate between immature specimens and newborn less than one-year-old, classified as young-of-the-year (YOY), according with Megalofonou et al. (2009). YOY presence was obseved mainly in spring and summer, with a peak of records in the months of June and July. The spatial distribution of these records shows an interesting aggregation of points in Southern France, the Italian coast of the Northern Tyrrhenian Sea and the Ligurian Sea, and the Northern-Western Ionian Sea (Figure 5B). In addition, four episodes of blue shark parturition were reported in the Northern Ionian Sea (Table 2).

Table 2. Parturitions of blue shark (*Prionace glauca*) recorded. Numbers refer to Figure 5.

N°	Date	Location	Source
1	16th May 2017	Villapiana (CS)	CosenzaApp facebook page
2	12th June 2017	Castellaneta marina (TA)	Nunzio Pasqualicchio facebook page
3	18th April 2019	Ginosa Marina (TA)	Claudio Cart facebook page
4	20th May 2019	Chiatona (TA)	Tommaso Carriero facebok page

In all cases, a stranded female gave birth to several pups. The first episode occurred on 16th May 2017 in Villapiana (CS, Italy), the second one on 12th June 2017 in Castellaneta marina (TA, Italy), the third one on 18th April 2019 in Ginosa Marina (TA, Italy), and the last one on 20th May 2019 in Chiatona (TA, Italy). All episodes were supported by video or photographic documentation, and reported by fecebook users.

4.2.5 Discussion

Our data shows a good coverage of the shark species present in the Mediterranean Sea. Following the last IUCN assessment (Dulvy et al. 2016) only eight species on the 40 assessed for the region were not recorded. Three species were detetected but not yet assessed by IUCN in the area: *Carcharhiunus brevipinna*, *Carcharhinus falciformis* and *Sphyrna lewini*. Taxonomic resolution on our data is high since 92% of the records are identified to the species level. This result is particularly remarkable considering that one of the greatest issues of fisheries dependent data on sharks in the Mediterranean Sea is the low taxonomic resolution (Cashion et al. 2019). Our data collection system based on image sightings can overcome this common issue in Mediterranean sharks' data. Among Mediterranean sharks, species identification starting from pictures seems to be achieved with good results for most of the families and genera. Some issues were found among the genus *Carcharhinus* spp. and families Lamnidae and Odontaspididae. The most challenging group was the genus *Carcharhinus* spp. where for 42 records an identification at species level was not possible, so far. However, new technologies could provide tools to solve this issue. In fact, deep learning processes and convolutional nets are a promising field in automatizing and strengthening the classification processes (LeeCun et al. 2015). Computer-based algorithms could be capable to learn how to classify an image by some peculiar characteristics. It has already been done in other scientific fields (Spanhol et al. 2016) and our project can provide both classified images to feed and improve an image-classifier algorithm on sharks, and images to be classified (Ferretti et al in prep.).

Among our records, 73% belong to threatened species (Figure 1B). Our platform can provide a safe and accessible place to store this kind of data over time and explore spatial and temporal patterns. Data are updated weekly, providing real-time monitoring of the opportunistic occurrence of sharks in the Mediterranean Sea. This allows us to process updated data and to live-monitoring the situation. An analysis of the sighting distribution for critically endangered species shows, for example, how we can identify possible hot spots for sharks conservation (Figure 1D) with a global overview of all the areas. Our data confirm some areas of interest for shark conservation, already suggested by locally-based studies, such as the Gulf of Gabes (Enajjar et al. 2015), the Northern and Central Adriatic Sea (Cugini et al. 2003, Costantini & Affronte 2003, Soldo et al. 2004), the Central Aegean Sea (Damalas & Vassilopoulou 2011) and the Marmara Sea (Kabasakal & Karhan 2015). In other areas such as Southern France, Corse, Northern Tyrrhenian Sea, Malta, and Cyprus the high presence of critically endangered shark species showed by our evidence suggests future deeper investigation.

Among our image-based records, nearly 70% are from threatened species (Figure 1B). Our platform can provide a safe and accessible place to store this kind of data over time and explore spatial and temporal patterns. Data are uploaded to the latest sightings on a weekly based routine, providing real-time monitoring on the opportunistic occurrence of sharks in the Mediterranean Sea. This allows to process updated data and to live-monitoring the situation. An analysis of the distribution of the critically endangered species shows, for example, how we can identify possible hot spots for sharks conservation (Figure 1D) with a global overview of all the area. Our data confirm some areas of interest for shark conservation, already suggested by locally-based studies, such as the Gulf of Gabes (Enajjar et al. 2015), the Northern and Central Adriatic Sea (Cugini et al. 2003, Costantini & Affronte 2003, Soldo et al. 2004), the Central Aegean Sea (Damalas & Vassilopoulou 2011) and the Marmara Sea (Kabasakal & Karhan 2015). In other areas such as Southern France, Corse, Northern Tyrrhenian Sea, Malta, and Cyprus the high presence of critically endangered shark species showed by our evidence suggests future deeper investigation.

Range contraction has been observed in large marine predators when they are overexploited (Moro et al. 2019; Worm & Tittensor 2011). When the spatial distribution of a species goes through reduction and fragmentation, this generates a spatial pattern with relatively small areas where the presence of the species could be still recorded (Mace et al. 2008). These areas are very important since they can act as strongholds from which an endangered species could be preserved and potentially recovered. After exploring our data and focusing on rare and heavily impacted species, we observed that for some of them, records are confined in very restricted areas (Figure 2). Records distributions for the small-tooth sand tiger shark (*Odontaspis ferox*) show a fragmented pattern. This species is a relative of the sand tiger shark (*Carcharias taurus*), which has no confirmed record in our study. Small-tooth sand tiger shark is generally found in deeper waters (200-800 m) than the sand tiger shark, who lives in shallow coastal waters. This might have made the latter more vulnerable to coastal human impact (Fergusson et al. 2002; Fergusson et al. 2008; Bargnesi et al. 2020b). However, deep fishing and trawling can also impact small-tooth sand tiger sharks (Fergusson et al. 2008). Hence, this kind of fisheries in the last decades could have potentially affected the presence and distribution of the species in the Mediterraean Sea. Special attention must be paid to areas where the species is still recorded, with constant monitoring. In particular, it is notable the presence of a dive spot off Beirut (Lebanon) where this species can be still observed in nature with seasonal aggregation (Gallagher & Hammerschlag 2011). Another species that needs a particular attention in the area is the angelshark (*Squatina squatina*), which represented the focal point of several studies in the Eastern Mediterranean Sea (Giovos et al. 2019), Adriatic Sea (Fortibuoni et al. 2016), Turkey (Akyol et al. 2016) and Marmara Sea (Kabasakal & Kabasakal 2014). Our records confirmed the presence of the species in those areas. Constant monitoring is essential for those and other spots still exhibiting the presence of rare and critically endangered species.

For the species well-represented in our database, we had been able to investigate more deeply the spatial and temporal distribution of the records (Figure 3). We found an interesting seasonal shift of records distribution for three big pelagic sharks: blue shark (*Prionace glauca*), shortfin mako (*Isurus* *oxyrhincus*), and thresher shark (*Alopias vulpinus*). Looking at our sighting locations, these speceis seem to prefer the Southern part of the Meditteranean Sea in the cold period (Fall-Winter), moving northward when the temperature of the water starts to rise (Spring-Summer). High mobility has been observed in pelagic sharks (Stevens 1990) and, in order to plan conservation and management actions, it is undoubtedly important to investigate changes in seasonal patterns on a regional scale. Most of the observations of shortfin mako and common thresher derived from fishing activities, and also the relatively high number of stranded blue shark recorded in our database, might be hypothetically related to injured specimens that have been involved in fishing activities. Given that sharks constitute a part of fisheries by-catch (Cavanagh & Gibson 2007, Ferretti et al. 2008), professional and recreational fisheries represent a major threat for sharks in the area. In fact, the presence of pelagic sharks among both professional and recreational fishing catches is confirmed by our records. A recent study has tracked the global footprint of industrial fisheries starting from information collected on the vessels from the automatic identification system (AIS) (Kroodsma et al. 2018). Unfortunately, vessels under 24 m length are not obliged to use the AIS and the effort yield by recreational fishing, normally engaged with relatively small boats, is still not clear in the area of the Mediterranean Sea, though we found evidence of a relatively high incidence on sharks' catches.

Animal movements could be also related to reproductive behavior. For the blue shark, the presence of possible nursery areas has been suggested in the Ligurian Sea, Northern Adriatic Sea and Ionian Sea (Megalofonou et al. 2009). Looking at our YOY blue shark records (Figure 5B), it is reasonable to hypothesize the extension of a possibile nursery from the Ligurian Sea to the Southern France waters. Furthermore, the four parturition events (Table 2), collected along the Ionioan coasts and documented by videos, confirm the hypothesis of Megalofonou et al (2009). YOYs present in our study has been observed from March to August, with a peak in June-July (Figure 5B). A high percentage of immature was also observed both in blue shark and shortfin mako (Figure 5A). The high number of immature shortfin mako observed in our study (75%) suggests that the Mediterranean Sea plays a role during an important life stage of the species. Population dynamics and interconnections within the Atlantic Ocean for those two species are still not recognized and well understood. Genetical analyses revealed a possible grade of connectivity between the Mediterranean and the North-eastern Atlantic population of blue shark and a migratory behavior for the species in the Mediterranean Sea (Leone et al. 2017). The shortfin mako has been recorded both in the North-eastern Atlantic Sea (Koheler et al. 2002) and several sectors of the Mediterranean Sea (Tudela et al. 2005, Megalofonou 2005, Ferretti et al. 2008), but populations connectivity throughout the Gibraltar Strait is not documented, so far.

Although data standardization for opportunistic records is still challenging due to the absence of information about the observation effort carried out to collect them (McPhearson & Myers 2009), our data shows an interesting potential to provide useful information to support sharks' conservation in the area. Further studies will be focused on investigating and testing possible proxies of the observation effort in order to estimate standardized distribution and abundance trends for Mediterranean shark species (Moro et al. 2019).

4.2.6 Conclusions

Our study shows how opportunistic data, collected through the exploration of the new possibilities offered by the nowadays technological development, can represent an important source of information for rare and endangered shark species. In the Mediterranean Sea, these kinds of data can really contribute to improving our knowledge of the presence and distribution of sharks species, providing useful information for both conservation plans and actions. The Mediterranean Sea is an area with a large human pressure on shark populations (Ferretti et al. 2008), resulting in one of the world's highest percentage of locally endangered shark species (Cashion et al. 2019). As a real-time monitoring process, the development of sharkPulse could really fill the data gap on threatened species, so often reaffirmed in several regional and global assessments on shark species (Dulvy et al. 2014). It can also provide an open access data platform to scientists and conservation managers. Collaboration among other local and regional initiatives focusing on sharks' opportunistic data collection is very important in this kind of initiative. To create a network of shark-related citizen science programs in the Mediterranean area is a pivotal goal in order to collect robust and useful data to process. Hence, a solid, free and open access system is required as the base of this kind of process, and sharkPulse reaches all the necessary features.

4.2.7 References

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4.3 “HOW DEEP IS YOUR LOVE FOR SHARKS?”: A CASE OF STUDY ON ATTITUDES AND BEHAVIOR TOWARDS SHARK CONSERVATION AND PARTICIPATORY RESEARCH IN A GROUP OF OCEAN USERS (SCUBA DIVERS)

4.3.1 Abstract

Sharks are one not only one of the most endangered but also one of the most sought groups of animals in marine-based nature tourism, especially scuba diving. Shark diving is an important part of the scuba diving market, with a relevant number of people traveling every year to have an underwater encounter with charismatic species. Southern Africa offers a wide number of opportunities to meet several shark species in the wild and some of the most famous shark diving spots in the world are located in the area. Shark diving could be a trigger to develop local sustainable economies and support shark conservation. Participatory research – Citizen Science, CS – is a great opportunity for scientists to collect data on rare and endangered species, and CS programs could help increasing the ocean literacy of ocean users, such as scuba divers, while also raising awareness on the global conservation status of elasmobranchs. Furthermore, CS programs can provide diving centers with new marketing strategies, giving a higher value to the shark diving experience. Using qualitative research, we assessed scuba divers’ willingness to see sharks, willingness to pay to see sharks, willingness to act for shark conservation, and actual shark conservation efforts, with focus on CS.

Face-to-face interviews were conducted at two famous shark diving locations of Southern Africa: Ponta Do Ouro (Southern Mozambique – 48 interviews) and Aliwal Shoal, south of Durban (South Africa – 38 interviews). In parallel, an online survey was conducted. Scuba divers are prone to travel and spend money in order to see sharks, they are aware of the global conservation status and threats that sharks are facing, and they are interested in becoming involved in CS and conservation programs. However, we observed that real participation in a CS shark-related program at the time of the study was low. A fundamental aspect of gaining participation seems to be the presence of a person who guides divers through the CS program and an easy and smart process of data sharing, supported by new technologies. It would also be fundamental to create a direct link with scientists to validate collected data since our study underlined some difficulty in taxonomic identification among scuba divers. The results of this study suggest that it may be important for the diving industry to create new professional figures who can introduce and guide guests through educational and conservation-oriented experiences. These experiences can add value to shark diving and support the sustainable

growth of this sector for the benefit of local economies while collecting valid data to support shark research and conservation.

4.3.2 Introduction

Diving with sharks represents one of the main components of shark-based tourism and also of scuba diving tourism (Dobson, 2006; Dearden et al. 2008). Indeed, sharks are a highly sought marine wildlife species among divers (Topelko & Dearden 2005). This preference makes the shark diving tourism industry a competitive one and a critical source of revenue for many coastal communities (Dicken & Hosking 2009; Gallagher & Hammerschlag 2011; Cisneros-Montemayor et al. 2013; Huveneers et al. 2017). Importantly, shark diving tourism can contribute to sharks' protection in different ways. Economic incentives derived from shark diving have justified and supported the establishment of shark sanctuaries and marine protected areas (MPAs) (Vianna et al. 2018), as well as community-based management of these areas (Brunnschweiler 2010), and the creation of alternative livelihoods for people who would otherwise fish sharks (Eriksson et al. 2019.). The shark diving experience can have positive effects on knowledge, attitude and behaviour of tourists towards sharks, increasing ecocentric views and promoting agency in shark conservation through participatory research, funding, and advocacy (Whatmough et al. 2011; Mieras et al. 2017; Apps et al. 2018; Sutcliffe & Barnes 2018; Ward-Paige et al. 2018). On the other hand, shark tourism has shown a potential negative impact on health, behaviour and ecology of the animals. Management through code of conducts and educational programs involving stakeholders and local communities are the best candidates to overcome this problem and create sustainable economies (Trave et al. 2017), and Citizen Science (CS) could be a good option for developing educational programs.

Citizen Science (CS) has its origin deep in the past. Involving people who are not scientists in collecting data for scientific research (i.e. Citizen Science, CS) is a practice that has its origin with the science itself: several early naturalists were non-scientists and the earliest known CS project dates back to 1900 (Silvertown, 2009). In the modern scientific era, a great number of CS projects involve millions of individuals (Bonney et al. 2014). New technologies are playing a major role in creating a link between people and science, and in the future, their role in CS projects will be even more fundamental (Newman et al. 2012; Kobori et al. 2016). Marine CS seems to be less developed and widespread than terrestrial CS, but ocean users such as scuba divers have a great potential to provide good quality data given their interest in acquiring knowledge and preserving the marine environment (Lucrezi et al. 2018). Shark-related CS has been less developed compared with CS revolving around other groups of animals (Bargnesi et al. 2020). However, some interesting programs exist. One of the most studied shark species through CS is the whale shark (*Rhincodon thypus*). Divers have been involved in collecting data on the species which have allowed describing migratory patterns, connectivity, distribution, population dynamics and habitat use (Davies et al. 2013; Andrejaczek et al. 2016; Araujo et al. 2017; McCoy et al. 2018). A focused project involving scuba divers has also been done at the Canary Islands on the angelshark (*Squatina squatina*), a critically endangered species, and allowed describing demographics and distribution of the species in the area (Meyers et al. 2017). Scuba guides have shown to provide good quality data on the presence of grey reef sharks (*Carcharhinus amblyrhynchos*) in Palau, comparable with telemetry data (Vianna et al. 2014). A global project had involved scuba divers in evaluating shark sanctuaries (Waird-Paige & Worms 2017) and in Thailand, data provided by scuba divers have allowed describing sharks' local temporal and spatial distributions (Waird-Paige et al. 2018). The methods used to collect data have been various and have included compiling reports on dive sites (Jaine et al. 2012; Vianna et al. 2014), online surveys (Waird-Paige & Worms 2017) and providing pictures for individual photo-identification (Andrejaczek et al. 2016; Araujo et al. 2017). Scuba divers represent a great opportunity for shark CS, and our study confirms their positive inclination to scientific and conservation projects. However, some issues have to be considered, such as the need to create a personal link between scuba divers and scientists, or to guarantee the presence of at least a representative of the CS program proposed, and the need to validate data by a network of experts. Professional, scientific figures (i.e. formed divemasters) created and included in the diving industries could both boost and feed CS shark-related projects and add educational and professional value to the diving experience. This study aims to collect data on the attitudes towards shark and shark conservation among scuba divers, and investigate the best strategies to develop shark-related CS programs with scuba divers in order to collect good data, and improve programs of education and conservation.

4.3.3 Materials and Methods

This study is part of the EU-funded project on sustainable scuba diving, Green Bubbles RISE (www.greenbubbles.eu). For this study, a mixed method of data collection was deployed. The combined use of different research approaches can be valuable thanks to their complementarity, whereby different types of intelligence can be generated regarding a study subject and expand its understanding (Creswell, 2009; Ritchie, 2003). Data collection included face-to-face semi-structured interviews with shark divers at the study areas, and an online questionnaire survey targeting shark divers. Both methods of research served a contextual, explanatory, evaluative, and generative function, by establishing the average profile of shark divers, the reasons behind shark diving, and the knowledge, attitudes, and behavior of shark divers concerning shark conservation and participatory research.

Face-to-face interviews

The two locations (Figure 1) selected for the face-to-face interviews are Ponta do Ouro, the largest base of scuba diving operations in the Ponta do Ouro Partial Marine Reserve (PPMR), Southern Mozambique; and Umkomaas, the largest base of scuba diving operations in the Aliwal Shoal MPA, KwaZulu-Natal, South Africa. The PPMR and the Aliwal Shoal MPA are popular destinations for scuba diving with sharks, not just locally but globally (Dicken & Hosking 2010; Gallagher & Hammerschlag 2011; Daly et al. 2015).

The study areas present three important differences in the way shark diving takes place. First, the PPMR forbids any form of baiting to attract sharks. Baited shark dives are legalized and regulated through permits in the controlled zone of the Aliwal Shoal MPA, which also has a restricted zone where no baiting is allowed. Baited shark dives in Aliwal Shoal are offered at the rate of approximately USD 100 per dive, as opposed to USD 40 for a non-baited shark dive in the same MPA and in the PPMR. Second, shark diving in the PPMR tends to take place at greater depth (approximately 40 m) compared with the Aliwal Shoal MPA (approximately 14 m for baited shark dives and 27 m for non-baited dives). Last, while a variety of shark species can be encountered at both study sites, divers in the PPMR mainly

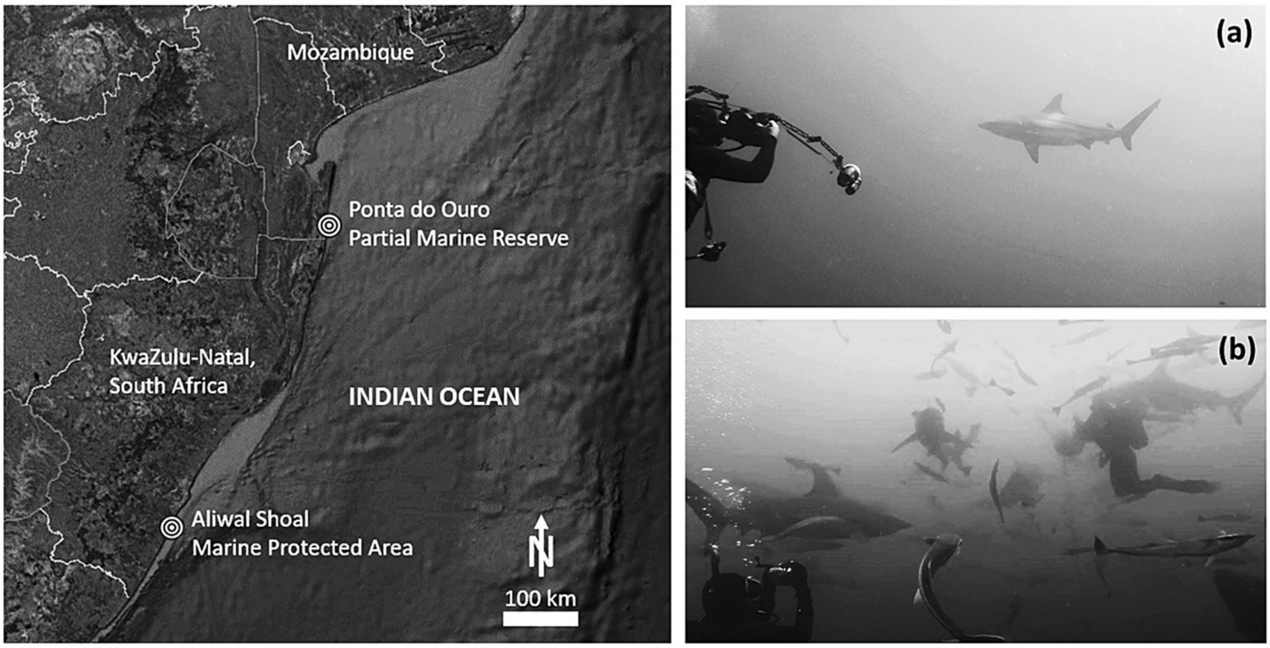

Figure 1. Map of the study areas including Ponta do Ouro Partial Marine Reserve (PPMR) and Aliwal Shoal Marine Protected Area (MPA), with a detail of the non-baited shark diving offered in the former (a), and the latter (b).

dive for species including the hammerhead shark, the bull shark, and the oceanic black tip shark, and those in the Aliwal Shoal MPA mainly dive for tiger sharks and sand tiger sharks. These differences would probably have implications for the findings of this study.

Face-to-face semi-structured interviews (Supporting Information Figure S1) were conducted with scuba divers who had previously dived with sharks at the study areas. The interview was structured into four main sections: demography and scuba diving profile; diving preferences, previous experience diving with sharks, and willingness to dive with sharks; and attitudes towards shark conservation and Citizen Science (CS). Participation in CS was tested by asking divers active submission of sharks' photo-sightings to the sharkPulse project (http://sharkpulse.org).

Data were collected in October 2018, November 2018, and March 2019 in Ponta do Ouro, and in January 2019, March 2019, and April 2019 in Umkomaas. During this time, sampling took place once every three days, yielding between one and two successful interviews per sampling day. On each sampling day, two scuba divers who had completed a shark dive were randomly approached at dive centers by trained fieldworkers, two per study location, and invited to participate in the interview. Agreeing participants were informed of the nature and purpose of the research, that the interview would be audio recorded, that they could leave the research at any point, and that their identity would be kept anonymous. The sampling effort yielded a total of 48 interviews (out of 50 approached divers) for Ponta do Ouro and 38 interviews (out of 40 approached divers) for Umkomaas. The difference in the number of interviews was attributed to issues including the logistics of diving operations, weather, and time available to the scuba divers who were approached. People who did not participate in the research explained it was due to lack of time.

Questionnaire survey

A structured questionnaire survey was developed in order to collect data through the web. The structure of the questionnaire was similar to that of the face-to-face interview, with the exception that most questions were transformed into queries containing single choice items, binary items, multiple choices items, and open-ended items (Supporting Information Figure S2). Virtual snowball sampling is considered valuable in researching hard to get populations remotely (Baltar & Brunet 2012), and its feasibility in reaching shark divers was tested here. The questionnaire was created in Google Forms and promoted online in the social media, specifically Facebook and Twitter, using the main platforms of the Green Bubbles project including the official project page, pages and groups of the participating entities, and personal pages of the researchers involved. In the Facebook posts, people were invited to share the link to the survey with fellow shark divers and in other groups and pages.

Promotion of the online questionnaire began in December 2017 and ended in May 2019, with a total of 333 divers participating.

Data analysis

Audio recordings from the face-to-face interviews were first transcribed verbatim in Microsoft Excel. Where needed, answers to the questions (as well as answers to some of the open-ended questions in the online survey) were coded into either binary, ordinal or continuous variables, so that the data from interviews and the online questionnaire could be merged and analysed together. The matrix containing data from the face-to-face interviews and the online survey was moved to the software Statsoft Statistica (Version 13.3, 2017) for statistical analysis. Answers to the various questions were first reported using descriptive statistics, frequency tables and breakdown statistics. The actual transcripts from the face-to-face interviews, as well as text from open-ended questions in the online survey, were used to complement quantitative answers. These data were analysed manually by the authors using content analysis and inductive coding, whereby meaningful analytical units of content (codes) and keywords are extracted by careful and repeated reading of the text. Graphics and plots were created by using R (version 3.6.1) and several related packages (“ggplot2”, “ggpubr”, “scales”).

4.3.4 Results

A total of 419 scuba divers participated in this study. The demographic profile of the participants is described in Table 1.

The representation of males was greater than that of females (60%) in the face-to-face interviews but similar (50%) in the online survey. Most of the participants were between 30 and 40 years of age. There was a strong proportion of South African participants, especially in the case of Umkomaas (84%). Divers who were not from South Africa originated mainly from Europe, the USA, and sub-Saharan countries. Between 24% and 38% of the participants had attained a university degree. In the case of Ponta do Ouro, many divers had also attained postgraduate qualifications, while the divers in Umkomaas had mainly attained a high school diploma. The origin of participants in the online survey was most widespread, and the main part (70%) came from Europe, USA, and Australia.

The details about the diving experience of the participants are listed in Table 1. Over a third of the participants had a profession in the scuba diving industry, mainly as instructors and divemasters. Over half of the divers interviewed at Umkomaas (63%) worked in the diving industry. On the other hand, in the online survey, only 7% of the participants worked in the diving industry. In the case of the face-to-face interviews, over 60% of the participants possessed qualifications equivalent to either a PADI Divemaster or Instructor level. Most of the remaining divers possessed the equivalent of a PADI

Advanced Open Water Diver qualification. The participants had been diving for an average of ten years, had logged an average of 1,100 lifetime dives, and were logging an average of 100 dives annually.

Table 1. Demographic and scuba diving profile (N = 419).

Variable	Ponta do Ouro - PPMR (n = 48)	Umkomaas – Aliwal Shoal MPA (n = 38)	Online survey (n = 333)
Gender	Male: 58% Female: 42%	Male: 68% Female: 32%	Male: 49% Female: 51%
Age	Mean: 38.6 Min-max: 23-71 SD: 12.18 SE: 1.8	Mean: 32.4 Min-max: 18-65 SD: 10.53 SE: 1.73	Mean: 39.5 Min-max: 17-75 SD: 13.25 SE: 0.7
Country	South Africa: 33% Other: 67% (Europe, USA, Sub-Saharan)	South Africa: 84% Other: 16% (Europe, USA, Canada)	Europe: 37.5 % Other: 35% (USA, Australia)
Highest level of education	No school: 0% High school: 19% Diploma: 8% Degree: 38% Postgraduate: 27% 8% missing data	No school: 3% High school: 50% Diploma: 13% Degree: 24% Postgraduate: 0% 10% missing data	No Data
Profession	Diving: 25% (instructor, divemaster) Other: 75%	Diving: 63% (instructor, divemaster) Other: 26% 11% missing data	Diving: 7% (instructor, divemaster) Other: 85% 8% missing data
Highest diving certification	Open water: 6% Advanced: 31% Divemaster: 27% Instructor: 36%	Open water: 5% Advanced: 24% Divemaster: 37% Instructor: 32% 2% missing data	Open water: 18% Advanced: 42% Divemaster: 19% Instructor: 19% 2% missing data
Years diving	Mean: 12.75 Min-max: 1-44 SD: 10.1 SE: 1.46	Mean: 10.1 Min-max: 0.25-36 SD: 8.84 SE: 1.47	No Data
Lifetime dives	Mean: 771 Min-max: 10-6000 SD: 1281 SE: 189	Mean: 1414 Min-max: 7-12500 SD: 2518 SE: 432	No Data
Annual dives	Mean: 82 Min-max: 2-390 SD: 119 SE: 19	Mean: 159 Min-max: 4-400 SD: 142 SE: 27	No Data

Table 2. Diving preferences, previous experience diving with sharks, willingness to dive with sharks, and attitudes towards shark conservation (N = 419).

Variable	Ponta do Ouro - PPMR (n = 48)	Umkomaas – Aliwal Shoal MPA (n = 38)	Online survey (n = 333)
What kind of scuba diving do you enjoy the most?	Shark diving: 35% Other: 63% (reef, megafauna, deep, wreck, macro) 2% missing data	Shark diving: 32% Other: 68% (reef, wreck, deep, warm water)	Shark diving: 14% Other: 86% (reef, macro, wreck, night)
Which is your favourite species to see when diving?	Shark: 69% (hammerhead shark, bull shark, tiger shark, whale shark) Other: 27% 4% missing data	Shark: 61% (bull shark, tiger shark) Other: 39%	Shark: 65% (whale shark) Other: 34% 1% missing data
Have you ever traveled for the sole purpose of diving to see sharks?	Yes: 83% (Ponta do Ouro, South Africa, Maldives, Red Sea) No: 15%	Yes: 61% (Aliwal Shoal, South Africa, Mozambique, Red Sea) No: 39%	Yes: 46% (Maldives, Bahamas, Red Sea, South Africa, Mexico) No: 13% Other: 41% (seen sharks by chance, occasionally)
Would you like to dive with sharks in the future?	Yes: 96% (hammerhead shark, natural encounters) No / unsure: 0% 4% missing data	Yes: 92% (hammerhead shark, tiger shark, natural encounters) No / unsure: 8%	No Data
Would you be willing to spend (or have you spent) more money than an average dive to be able to see sharks?	Yes: 73% (baited shark dives) Depends: 21% (guaranteed sightings, favourite species) No: 2% 4% missing data	Yes: 55% Depends: 16% (favorite species) No: 18% 11% missing data	No Data
Would you fly internationally to dive with sharks?	Yes: 75% (Mexico, Australia) Depends: 8% No: 11% 6% missing data	Yes: 76% (Philippines, Indonesia, Galapagos) Depends: 8% No: 11% 5% missing data	No Data
Do you believe sharks are threatened at all?	Yes: 92% No: 4% 4% missing data	Yes: 84% No: 16%	Yes: 93% No: 2% Not sure: 5%
Whether sharks are threatened or not, are you concerned for their protection?	Yes: 88% No: 4% 8% missing data	Yes: 66% No: 3% 31% missing data	Yes: 95% No: 3% Not sure: 2%
What problems and threats do you think sharks are facing today, if any?	Overfishing, finning, pollution, public ignorance	Overfishing, finning, shark nets and drumlines	No Data

Although divers at Umkomaas had dived for the least number of years, they reported the greatest average number of lifetime dives and annual dives, probably due to the majority of them working in the diving industry.

In the online survey, 42% of divers possessed a PADI Advanced Open Water Diver qualification or equivalent, and a similar proportion (40 %) possessed a PADI divemaster or Instructor level or equivalent.

Table 2 includes information on diving preferences, previous experiences diving with sharks, and willingness to dive with sharks among the participants. These people enjoyed different kinds of diving, including reef, deep, wreck, macro, night, warm water, and megafauna.

Shark diving was the favourite of about 30% of the scuba divers interviewed, and of 14 % of the divers intercepted through the questionnaire. Sharks were among the preferred species to see underwater by over half of all the scuba divers reached in this study (55-69%). In particular, tiger sharks, hammerhead sharks, bull sharks, and whale sharks were nominated. A large proportion (49-83%) of the scuba divers had previously traveled for the sole purpose of diving with sharks. The destinations mentioned included the PPMR and Aliwal Shoal MPA, other locations in South Africa and Mozambique, the Maldives, the Bahamas, the Red Sea, and Mexico. Reasons to dive with sharks collected in the face-to-face interviews are summarised in a word cloud (Figure 2).

Figure 2. A Word Cloud (© 2019 Enideo) of the main reasons to dive with sharks as provided by the participants in the face-to-face interviews.

From an analysis of the data, it is evident that shark diving was motivated by elements including the fascination for sharks as beautiful and elegant apex predators; and the willingness to shift negative public opinion on sharks. Secondary elements included the desire for thrilling and adrenaline-filled experiences. Nearly all of the participants in the face-to-face interviews (88-96%) stated that they would like to dive with sharks in the future, mostly in their natural habitat and without the use of cages and bait. The majority of them (55-73%) also claimed that they either were willing to spend or had spent more money than the cost of an average scuba dive in order to see sharks, particularly mentioning the baited shark dive in South Africa, which is almost three times the cost of an average dive. Some divers would also spend more money in order to see their favourite species, and if they had the guarantee of a shark sighting. Approximately 70% of the divers were prepared to fly internationally to dive with sharks. Among all the scuba divers intercepted in this study, there were very positive attitudes towards sharks and their conservation, as displayed in Table 2. Nearly all (over 80%) believed that sharks are threatened animals, and the majority (66-90%) were concerned for their protection. The divers identified key threats to sharks, including overfishing, finning, pollution, bycatch, habitat destruction, and general public ignorance about sharks.

The relationship between divers and Citizen Science (CS) is shown in Figure 3.

Divers believed that in general, science can play a role in shark conservation (97%) and that people can help scientists in their work (86%). Most of the divers in Ponta Do Ouro did not know what CS is (84%), while some of them had participated in CS programs (36%). In Umkomaas, 40% of the scuba divers knew about CS and 45% had participated in CS programs. The number of divers who had participated in CS programs was relatively low (14-16%). They mostly mentioned participation in projects related to sharing images of a given species (e.g. manta, leopard shark) for individual identification by scientists through body patterns; some divers mentioned a general submission of pictures of the species sighted in their dives in eco-informatic projects and platforms. Both in Ponta Do Ouro and Umkomaas, divers showed high willingness to participate in shark-related CS programs (80-81%).

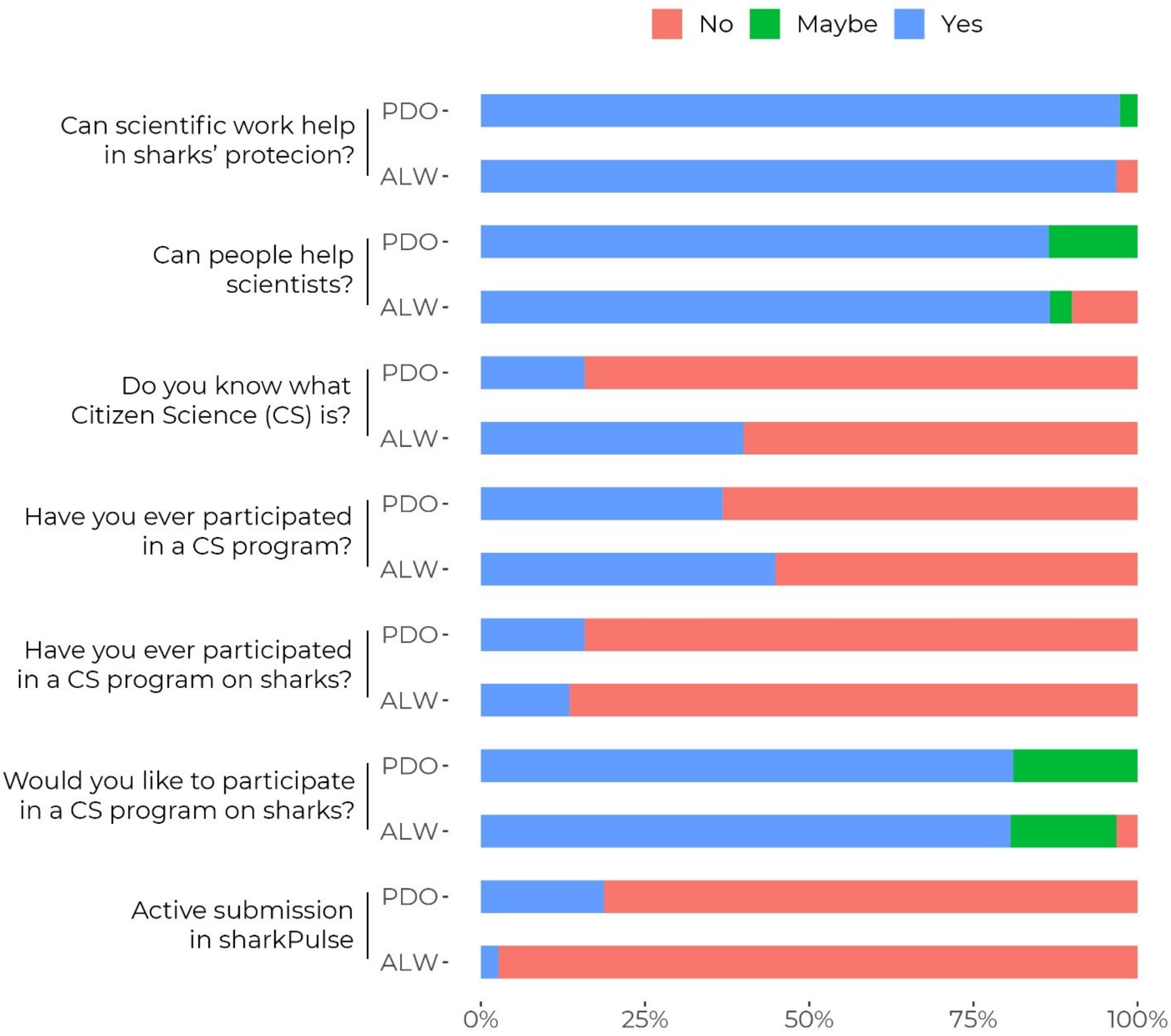

Figure 3. Attitudes of scuba divers in Ponta do Ouro and Umkomaas through science (CS), and shark-related CS, and observed participation in a shark-related CS project.

However, observed active submission of self-reported sightings through images to sharkPulse was low in Ponta Do Ouro (19%) and very low in Umkomaas (3%).

Among divers that answered to the online survey (n = 333) a high number (n = 196) declared to have participated in scientific research (Figure 4A), mostly as volunteers (n = 110) and some as workers (n = 86). Among divers who declared to have never participated in scientific research (n = 123), almost everyone also declared to be interested in participating in future (n = 115). Several scuba divers declared to have participated in marine research (n = 129) and as a scuba diver (n = 122).

The number of scuba divers who had participated in scientific research on sharks (Figure 4B) was lower, with some of them having done projects in collaboration with research institutes (n = 60), and a few with dive centers (n = 23).

Different types of programs were mentioned: identification of species during a dive (n = 37), tagging programs (n = 32), sharing media online (n = 30), and using a mobile app (n = 9).

A high percentage of scuba divers (58%) found that new technologies can play a role in data sharing with scientists (Figure 4C) both through web platforms (37 %) and mobile apps (21 %). Among scuba divers who had participated in CS shark-related programs (Figure 4D), most of them claimed that they will be participating again in the future (80 %).

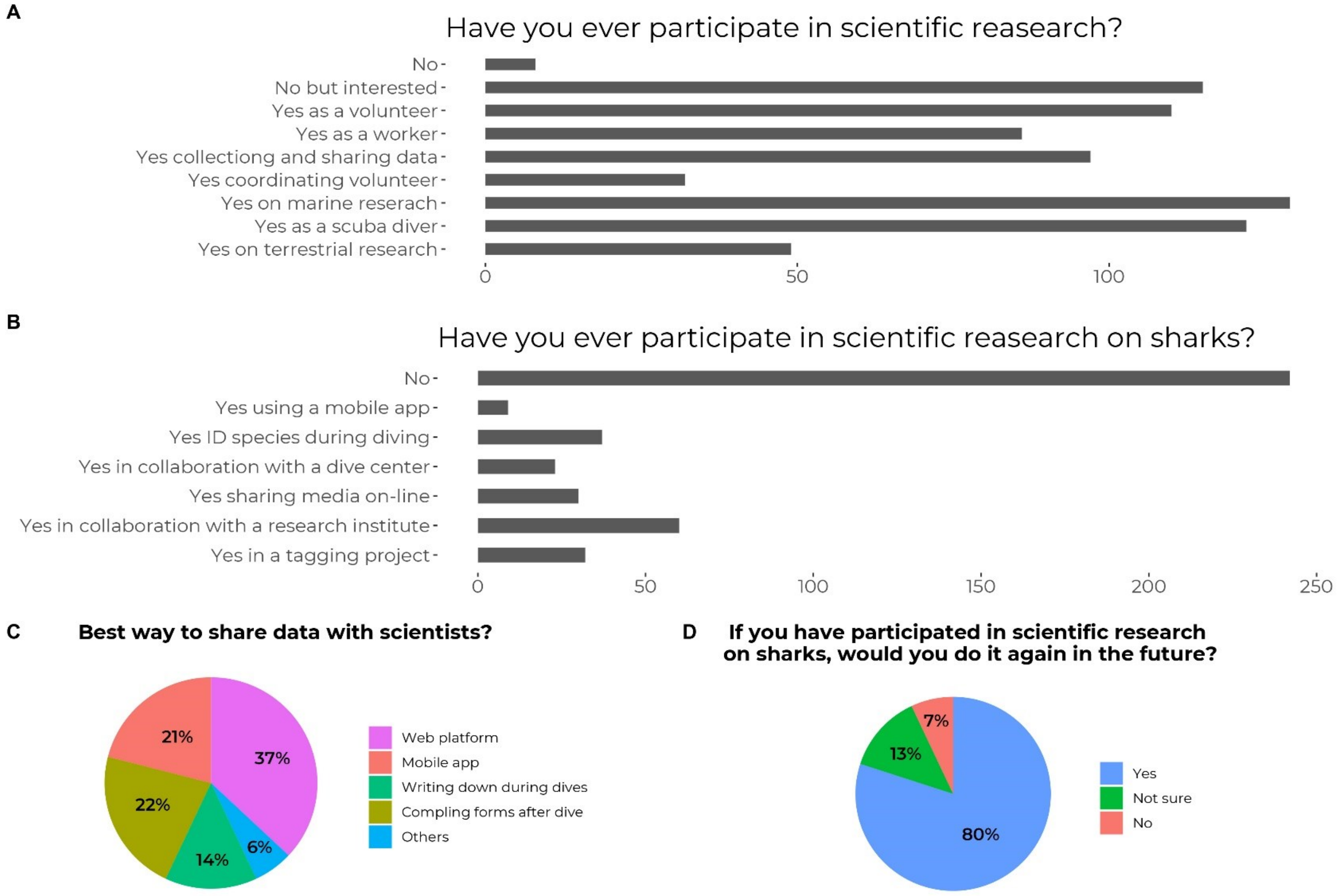

Figure 4. Participation and attitudes in scientific researches and shark-related scientific researches among scuba divers in the online survey.

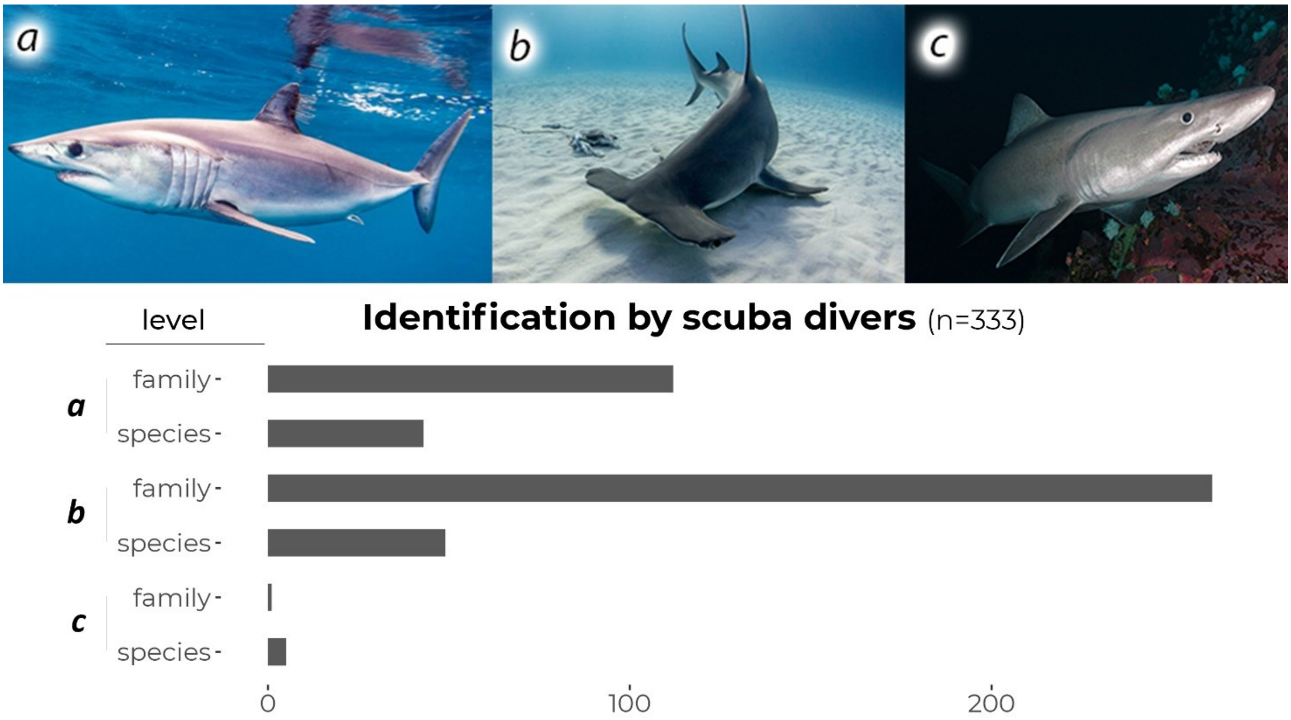

Figure 5. Taxonomical test on the capability of shark identification among scuba divers. Species a: shortfin mako (*Isurus oxyrhinchus*); species b: great hammerhead (*Sphyrna mokarran*); species c: small-tooth sand tiger shark (*Odontaspis ferox*).

At the end of the online survey, scuba divers were asked to identify the picture of three species of three different families of shark, with different levels of difficulty in the identification (Figure 5). The picture showed a shortfin mako (*Isurus oxyrhinchus*), a great hammerhead (*Sphyrna mokarran*), and a small-tooth sand tiger shark (*Odontaspis ferox*). All species are known to have been observed by divers in different places, the rarest one being the small-tooth sand tiger but reported for example, from Lebanon (Gallagher & Hammerschlag 2011). The identification level was not bad at the family level for the shortfin mako (n = 112) and very good for the great hammerhead (n = 261), but in both cases, identification to species level was reached by a low number of scuba divers (n = 43 and 49, respectively). The most common misidentifications of the shortfin mako were with the great white shark (*Carcharodon carcharias*) and the blue shark (*Prionace glauca*). The identification of small-tooth sand tiger shark was very difficult by scuba divers: only 5 of them recognized the species and one provided the right family name. This is because the species is very similar to the sand tiger shark (*Carcharias taurus*), and that generated many misidentifications.

4.3.5 Discussion

The results of this study confirm how scuba divers can be used for the benefit of shark research and conservation. The results, however, also highlight how a CS shark-related program ought to involve both scientists and dive operators. Eco-programs on sharks involving scuba divers can also potentially provide economic benefits to the diving industry, and, on the other hand, support local educational and conservation programs, with the aim to reduce and minimize the potential negative impact that tourism can have on the environment (Trave et al. 2017), and promote responsibility behaviour in scuba divers (Lucrezi et al. 2019).

The participants in this study were mostly male and in their late thirties, which corresponds with the majority of research findings related to the demography of scuba divers and shark divers (Apps et al. 2015; Torres et al. 2017), also at the study locations (Dicken & Hosking 2009; Daly et al. 2015). While education level and origin of the participants were representative of the diving industry for the case of Ponta do Ouro (Daly et al. 2015), the sampling in Umkomaas yielded a biased representation of shark divers, different from that by Dicken and Hosking (2009), according to whom clients are mainly from overseas and not working in the industry. The interviewees here were mostly professionals from South Africa with a high school diploma. This representation may be a result of seasonality or changing markets. At any rate, it created an opportunity to draw differences between recreational divers and professionals. The level of experience of the participants was high, with at least ten years of diving, advanced scuba diving qualification or greater, 700 lifetime dives or higher, and at least 70 annual dives logged. This profile reflected the experience normally required from shark divers due to the challenging environmental conditions (e.g. depth, currents) in which shark diving can take place (Smith et al. 2014; Torres et al. 2017).

The participants in this study tended to be very committed to shark diving. The majority preferred sharks to any other marine species, had travelled for the sole purpose of shark diving, wanted to continue to dive with sharks in the future, and were willing to spend more money and travel internationally to dive with sharks. In line with this commitment, the participants shared positive attitudes towards sharks, both cognitive (knowledge of the threatened status of sharks and human impacts on sharks) and affective (concern for sharks' protection). These findings are recurrent in the literature on shark diving tourism, where divers tend to be dedicated or specialist wildlife tourists possessing some knowledge of sharks, showing pro-environmental attitudes towards sharks, and willing to invest in shark diving (Smith et al. 2009; Torres et al. 2017; Sutcliffe & Barnes 2018). Motivations to dive with sharks also reflected positive attitudes towards sharks and their conservation. Divers were primarily motivated by a fascination for sharks, but also by the desire to draw public attention to problems faced by sharks and to the urgency of shark conservation. Seeking thrilling experiences played a more secondary role in motivating divers. This suggests that commitment to shark diving may have an effect on motivations, whereby the cognitive dissonance between initial fear / perceived threat and the general graceful nature of sharks is overcome, and divers become motivated by more ecocentric reasons, including a responsibility to act in favour of shark conservation (Sutcliffe & Barnes 2018).

Using the collaboration of non-scientists to collect data (Citizen Science, CS) is a procedure that has been used since the origin of the science itself (Silvertown 2009). Most of the CS projects currently in progress are focused on terrestrial species and ecosystems, but scuba divers are an interesting group to focus on to develop marine CS. Scuba divers are usually well disposed to CS programs since they show interest in the knowledge and conservation of the marine environment (Lucrezi et al. 2018). Our sample confirms such observation and tendency. Even if the word "Citizen Science" was not a part of the vocabulary of scuba divers interviewed in Ponta Do Ouro, they showed interest in it and some had previously participated in CS programs. Scuba divers in Umkomaas seemed more aware about the existence of CS, and nearly half of them had participated in some projects. The participation in shark-related CS projects remained, however, low at both locations. Scuba divers who had been involved in research projects on sharks mostly mentioned photo-identification on a single species as tools used by the program, which is a common method already used in other parts of the world (Andrejaczek et al. 2016; Araujo et al. 2017). Scuba divers showed a high interest in participating in CS shark-related programs in future, as it has been previously observed (Lucrezi et al. 2018). However, the active submission of sightings in sharkPulse as a proposed CS shark-related project was low, especially in Umkomaas. One of the issues found was that the participation was proposed not in a framework that involved dive centers and guides, but casually with the scuba divers being reached between one dive and another. External issues such the lack of data connections or the not immediate availability of images (still in the camera storage) also affected the participation in submitting data (i.e. pictures of sharks). The relatively higher participation observed in Ponta Do Ouro may be related to the fact that here, scuba divers were invited to participate in the project by scientists directly involved in the creation and development of the CS shark-related program proposed. Scuba divers suggested that a good CS project must be fun, smart and easy, in cooperation with diving centers and tour operators, where the project must be part of the diving package, also with the help of posters and public presentations.

The online survey confirms the willingness to act in CS programs among scuba divers. Although it must be mentioned that snowball sampling may have inadvertently targeted divers already possessing a pro-shark attitude, given the sustainable objectives of the Green Bubbles project. The sample of scuba divers (n = 333) was slightly different from the one reached by the face-to-face interviews, since near two-thirds of them had already been part of a scientific program, and one fourth actually worked in some scientific research. Also in this case, participation in shark-related CS was low and included identification and reporting of species during a dive and sharing media. Nine scuba divers declared to have used a mobile app to share data. A high percentage of them (58%) declared new technologies as the best way to share data, which confirms the global trend in which web platforms and apps are becoming increasingly central in CS programs (Newman et al. 2012; Sullivan et al. 2014). Among those who had been involved in scientific research on sharks, 80% were satisfied and would participate again, which suggests that shark-related CS projects may benefit from a loyalty relationship with users involved. An issue found was that the participants in the online survey showed a low taxonomic capability of identification. This is a common issue in CS processes that has been overcome by data validation protocols involving both users (with different level of experience) and scientists at different levels in verifying doubtful records (Bonter & Cooper 2012). Developing these kinds of protocol must be a part of the creation of any CS program. Some research has also been carried out with the objective to obtain automatic image classification and verification through machine learning processes (Van Horn et al. 2018), and this underlines the role that progresses of new technologies would have in the future of CS programs. Despite technologies and their future role, CS will always be a matter of people, and our findings confirm the importance of considering all the figures involved at different levels in a CS process. In particular, for researches involving scuba divers, focal figures are the dive operators, the guides, and the scientists. All these figures must be linked with each other and with the scuba divers involved in the project, in a coordinated and shared framework.

4.3.6 Conclusions

Scuba divers are a category of ocean users with great potential for marine Citizen Science (CS), as observed before (Lucrezi et al. 2018). Sharks are one of the most wanted animals to encounter and observe by scuba divers (Topelko & Dearden 2005; Dobson 2006; Dearden et al. 2008;) and this attraction holds a great potential for shark-related CS programs revolving around scuba divers. Due to the charm that sharks have on scuba divers, shark-related CS programs could be an opportunity for the diving industry to develop sustainable economies oriented on conservation and educational programs. This process may benefit from professional figures that may act as a link between diving centers, scuba divers and scientists (Lucrezi et al. 2018). These figures can create an organic framework involving customers and creating a loyalty relationship. Scuba divers are aware of shark conservation status and are prone to contribute to scientific projects, thus their contribution could be of great scientific value.

4.3.7 References

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4.3.8 Supporting information

DIVERS AND SHARKS

INTERVIEWS 2018

Note for researcher, interviews should not exceed 20 minutes duration, unless the participant wishes to speak for longer. For every question, incite a response with a narrative (not simple yes/no responses).

DATE:

DEMOGRAPHY:

- Gender
- Age
- Country of origin and residence
- Highest level of education
- Main occupation

SCUBA DIVING PROFILE:

- Highest level of scuba diving certification
- How many years have you dived?
- How many lifetime dives have you logged?
- On average how many times a year do you dive?
- What kind of scuba diving do you enjoy the most?
- What are your favorite species to see when diving?

SHARK SIGHT/DIVING:

- Have you ever, intentionally or unintentionally, seen a shark and/or dived with sharks? If yes, describe in detail this experience (intentional or unintentional; location; context -- aquarium, in natural habitat, while surfing, while diving, cage diving – species if known; if there was any interaction and the type; feelings and emotions). (*Note for the researcher, if there are people who dived with sharks, you will need to ask them also the questions in the next section "specific questions for shark divers"*)
- Do you think that keeping and seeing sharks in their natural habitat is better than keeping them and seeing them in captivity, for example in an aquarium, and why?
- Whether you have or have not dived with sharks, would you like to do that in the future? And what type of shark diving would you like to experience (e.g. aquarium, natural habitat, large species, small species, deep diving, shallow diving, reef diving, cage diving, specific species)?

SPECIFIC QUESTIONS FOR SHARK DIVERS:

- Have you ever travelled for the purpose of diving to see sharks and if so, please give details (e.g. shark cage diving in Cape Town; hammerhead shark diving in Ponta do Ouro).
- What is attractive to you about shark diving?
- Would you be willing to spend more money (one and half times or higher) than an average dive to be able to see sharks?
- Would you fly internationally to dive with sharks?
- Would you pay to join shark diving research expeditions overseas, and work with scientists to study sharks?
- Have you ever exceeded, or would you, the depths and bottom time limits prescribed by your diving certification in order to see sharks?
- Can you describe a shark diving code of conduct, or at least some rules (dos and don'ts) of shark diving?

- Have you ever broken any shark diving rules given to you (during a pre-dive briefing)?
- Have shark diving operators you made use of worked in an ethically responsible manner and if not, what unethical behaviors have they engaged in?
- Have you ever had any safety issues while diving to see sharks and if so, please describe the nature of these issues.
- Do you believe that shark diving is generally safe and if not, what forms of shark diving are unsafe and why?

SHARK CONSERVATION:

- Do you think sharks are threatened at all?
- Whether sharks are threatened or not, are you concerned for their protection?
- What problems and threats do you think sharks are facing today, if any?
- Do you think that tourism helps or damages sharks and their protection, and how?
- What do you think should be done to protect shark species which are threatened?
- Who is responsible for the protection of sharks?
- Do you think that structures where sharks are kept in captivity, such as aquariums, are positive for the protection of sharks and if so, how? If not, why?

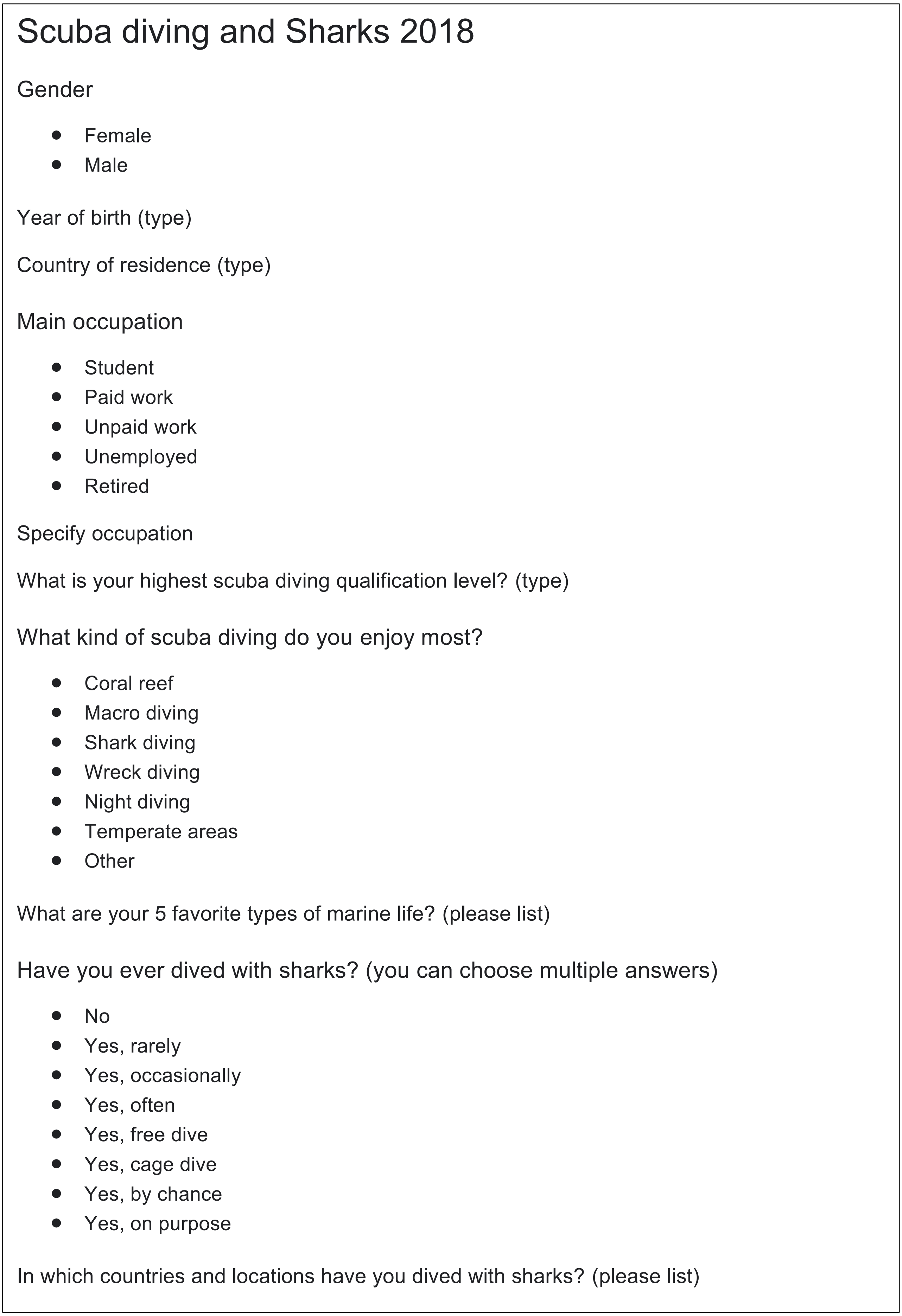
SHARK SCIENCE AND CITIZEN SCIENCE:

- Do you believe that science (scientific research) can help to protect sharks around the world and if so, how?
- Do you think that people who are not scientists (regular citizens) can help scientists to understand and protect sharks, and if so, how? Please describe all possible ways.
- Do you know what participatory research, also called Citizen Science, is? (*Note for researcher, it is very likely that people do not know what CS is and that several types of CS are actually CS, for instance, crowdsourcing and opportunistic CS. You will need to clarify with them and ensure they know what CS includes before moving on to the next two questions. You must do so only after they have answered to this specific questions, so as to not alter the response. So, at this point you will need to provide the definition of CS and its types.*)
- Have you ever participated in scientific research (Citizen Science) and if so, briefly describe your experience.
- Have you ever participated in scientific research (Citizen Science) on sharks and if so, describe your experience in full (name and type of project, species under study, where, when, how long, reasons for participating, method, feedback, satisfaction with participating).
- What do you think are the best ways and methods to make non-scientists easily participate in Citizen Science?
- Whether you have or have not participated in Citizen Science, would you do it in the future and how would you like it to happen? What would make it easy for you to participate?

PARTICIPATION IN SHARKPULSE:

For researcher to say: We have a project on shark Citizen Science we would like to propose to you today. It is an easy project, where you are simply required to share on FB photos of sharks that you may be in possession of. What we do is use the photographs to map the distribution and abundance of shark species in different parts around the world, to monitor sharks and communicate with decision-makers the data, so that conservation plans can be made.

Figure S1. Structure of the face-to-face interview.



Scuba diving and Sharks 2018

Gender

- Female
- Male

Year of birth (type)

Country of residence (type)

Main occupation

- Student
- Paid work
- Unpaid work
- Unemployed
- Retired

Specify occupation

What is your highest scuba diving qualification level? (type)

What kind of scuba diving do you enjoy most?

- Coral reef
- Macro diving
- Shark diving
- Wreck diving
- Night diving
- Temperate areas
- Other

What are your 5 favorite types of marine life? (please list)

Have you ever dived with sharks? (you can choose multiple answers)

- No
- Yes, rarely
- Yes, occasionally
- Yes, often
- Yes, free dive
- Yes, cage dive
- Yes, by chance
- Yes, on purpose

In which countries and locations have you dived with sharks? (please list)

Are you concerned for the well being and protection of sharks?

- No
- Yes
- Not sure

Do you think shark populations and species are:

- Threatened
- Non-threatened
- Not sure

Do you think shark populations and species are:

- Declining
- Increasing
- Overall remaining stable
- Not sure

Please look at these shark species, can you name them?

Species a: (type)

Species b: (type)

Species c: (type)

Do you believe observations by non-scientists can contribute to sharks conservation and research?

- No
- Yes
- Not sure

Do you think that scuba diving tourism can help shark conservation?

- No
- Yes
- Not sure

Have you ever participated in scientific research? (you can choose multiple answers)

- No, I am not interested
- No, but I am interested
- Yes, as a volunteer
- Yes, as a worker
- Yes, for marine research
- Yes, for terrestrial or other research
- Yes, coordinating volunteers
- Yes, collecting and sharing data
- Yes, collecting data and doing other things (analysis etc)
- Yes, as a scuba diver

Have you ever participated in scientific research on sharks? (you can choose multiple answers)

- No
- Yes, I had to share photos/media online (e.g. on social networks)
- Yes, in a tagging project
- Yes, I had to identify species while diving
- Yes, I had to use an app to share data
- Yes, in collaboration with a dive centre
- Yes, in collaboration with research institutes and/or universities

What is in your opinion the best way to share shark data with scientists?

- By sharing information written down during dives
- By compiling forms already prepared for volunteers
- By sharing photos/media on a web platform
- By using apps
- Other

If you have participated in scientific research on sharks, would you do it again in the future?

- No
- Yes
- Not sure

If you have participated in scientific research on sharks, are you satisfied with your experience? (briefly describe)

Figure S2. Structure of the web survey.

5. CONCLUSIONS AND FINAL REMARKS

- For the study of the presence and distribution of sharks in the Mediterranean Sea an approach that includes historical ecology must be considered since scientific knowledge, especially on big sharks, in the area is strongly affected by shifting baseline syndrome.

- Even if the Mediterranean Sea has a little connection through Gibraltar Strait with the worlds' oceanic system, Mediterranean shark species show connectivity at different grades with the other worlds' population. Genetics can explain colonization and dispersion dynamics beyond the species found in the area and outline possible stock borders.

- The long history of exploitation of the Mediterranean Sea has severely reduced populations of big sharks, that are now very rare and elusive. New monitoring strategies need to take place to provide data for conservation assessments and plans.

- Citizen science is a great opportunity for shark science in the Mediterranean Sea. Opportunistic data collected show an interesting potential in describing the presence of strongholds for rare and endangered species. Temporal and spatial dynamics of species distribution can be also detected together with areas of pupping and presence of juveniles. Most of the sightings are related to fishing activities, underlining their impact on shark populations in the area.

- Ocean users are the main target for shark citizen science. Among them, scuba divers show a high propensity for shark encounter through shark diving and interest to collaborate with scientists providing them data and information. To obtain a valid and relevant amount of data is important an integrated process that involves all the diving industry from dive centers, though dive guides, to divers.

- Professional figures formed with a solid scientific background and constantly linked with scientist leading a citizen science project could both improve data collection and give to the diving industry the opportunity to develop sustainable economies oriented on conservation and educational programs.

6. PUBLICATIONS AND PRESENTATIONS

Publications

- Bargnesi F, Gridelli S, Cerrano C, Ferretti F. 2020. Reconstructing the history and ecology of the sand tiger shark (*Carcharias taurus*) in the Mediterranean Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems.* In press. DOI: 10.1002/aqc.3301
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