Head Office: Università degli Studi di Padova

Department of Biology

Ph.D. COURSE IN: Bioscience

CURRICULUM: Evolution, Ecology and Conservation

SERIES XXXIV

MOVEMENT ECOLOGY AND ESSENTIAL HABITAT DELINEATION

IN MEDITERRANEAN SHARKS

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General Abstract

Over the last century, an overall depletion of the marine resources has been documented in many oceans and in particular in the Mediterranean Sea. This depletion has been driven by many anthropogenic activities among which fishing activities have one of the strongest direct impacts. The elasmobranch species are among the most important apex- and meso- predators in the food web, contributing to the balance and functioning of the marine ecosystems. The Mediterranean Sea hosts a wide diversity of elasmobranchs that live in all marine ecosystems. Typically, the elasmobranch life cycle consists in a slower growth rate compared to other marine organisms. This also implies that the time to reach sexual maturity at which an organism can reproduce for the first time takes much longer than other marine species. Within their life cycle, elasmobranch species often require specific habitats to complete their reproduction or foraging needs and no-random choice leads to a specific propriety in the use of space as for instance philopatry, site fidelity and natal homing. Scientific research has reported how essential habitats, used in different stages of their life cycle and across generations, can be subjected to high fishing pressure, especially the coastal areas of several subbasins like the Adriatic Sea where one of the highest fishing pressures is found. As the direct consequence of long period of overfishing, many elasmobranch species have been threatened by extinction. The current scenario in the Mediterranean Sea calls for appropriate management and conservation strategies directed to the commercially-relevant and endangered elasmobranch species. The knowledge of the use of space of elasmobranch throughout the year and among life stages could greatly contribute to the definition of a spatially explicit management which has resulted to be successful in other oceans to sustainably manage the elasmobranch fishery, thus halting or reversing the declining scenario. With the multidisciplinary approach applied to accomplish this PhD thesis, the achieved results have shed some light to define critical periods and locations in which commercially relevant elasmobranchs like the smooth hounds (*Mustelus* spp.) or endangered large coastal shark like the sandbar shark (*Carcharhinus plumbeus*) complete critical steps of their life cycle like birthing, mating and growing using nursery area for foraging purposes. Such multidisciplinary approach could be paramount to provide missing information of elasmobranch species in

the Mediterranean Sea and support the establishment of tailored management towards elasmobranch conservation.

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Chapter 1: General Introduction

1.1 Introduction

Sharks, rays, skates and chimeras belong to the class chondrichthyan that evolved at least 400 million years ago according to the first fossil records (Grogan et al., 2012). Several species of modern chondrichthyans show adaptations as meso- or apex-predators (e.g., electrochemical reception, mimicry, and swimming ability). Together with other vertebrates, these marine fishes are considered k-selected species meaning high longevity, long gestation period, in viviparous species, and late sexually maturity, if compared to other marine species, in particular teleost fish. Their reproductive strategy is characterized by internal fertilization and by either egg-laying, i.e. oviparity, or live-bearing, with some species presenting aplacental and other placental viviparity. Especially in viviparous species, females may be receptive only in a specific time of the year, usually after parturition. Therefore, mating occurs in a limited time. In several species mating systems have been documented to be particularly coercive, with male copulatory organs (claspers) usually armed of spines and hooks, and the occurrence of multiple paternity of litters (Fitzpatrick et al., 2012). In some chondrichthyan species, females present a resting phase after the reproduction, likely related to the high reproductive costs (Carrier, Musick, & Heithaus, 2012).

1.2 The use of space

Among chondrichthyans, the available scientific literature covers several aspects of their biology in elasmobranchs (including sharks, skates and rays). During their life cycle, different use of space has been documented, with different areas where mating and delivery occur or where the extended time of gestation,

or a resting phase between two pregnancies, are spent (Chapman et al., 2015). According to Chapman et al. (2015), the use of space in elasmobranchs not only is drastically different compared for instance to bony fish, but also the role of essential habitats is much more important for key periods such as delivery or egg deposition (Figure 1).
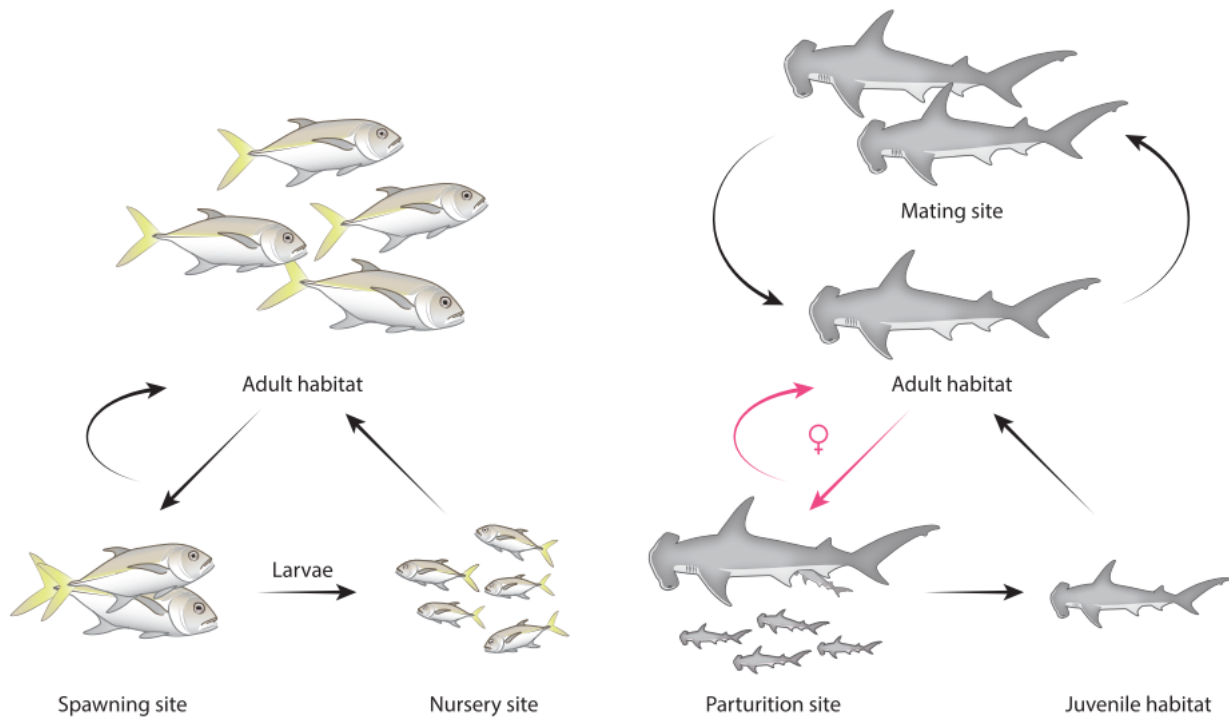
Figure 1: Schematic representation of the use of space and migration of bony fish (left) and coastal sharks (right). Black arrows indicate movements made by both males and females while the red ones only by females. Figure taken from Chapman et al. (2015)

 In addition, sexual and size segregations come into the context of intra-specific use of space. The latter aspect may stem from the mature female avoidance of coercive mating but also from maturing fameless in different use of thermal niches for gamete maturation or pregnancy (Jacoby et al., 2012; Chapman et al., 2015). Other fundamental aspects related to movement proprieties that have emerged in elasmobranch species, are the site fidelity or return to the same birthing place at juvenile stages for foraging purpose, or at mature one for either delivery or mating purposes. In particular, in the present work the definition of philopatry, as "the preferential return of reproducing individuals to their natal sites or regions, resulting in the multigenerational use of these sites", site fidelity as "the return of an individual to a location where it

previously resided after having left it for some defined period of time" are intended according to Chapman et al. (2015). Evidence for such site fidelity have been reported in many studies in which a specific association has emerged between a specific area and an individual or a group of individuals (Chapman et al., 2015). It has been hypothesized that optimal environmental conditions are found in such locations either for foraging, delivery or mating, leading to the definition of essential habitats (Hueter et al., 2004; Chapman et al., 2015). Environmental conditions directly influence the movement in response to species physiological needs (Schlaff et al., 2014). So, residency, site fidelity and philopatry of a species can be determined not only by location-specific environmental conditions that shape the essential habitat, but also location-specific seasonal variation (Schlaff et al., 2014). For instance, the concept of nursery area is strictly connected to the multi-year use of any given locations (Kinney & Simpfendorfer, 2009). According to Heupel et al. (2007), a nursery is located where (*i*) juvenile sharks are more commonly encountered in the area than in other areas, (*ii*) sharks have a tendency to remain or return for extended periods, and (*iii*) there is a repeatedly use of the area across years.

The movement ecology represents an important component even in several behavioural aspects that also play a key role in the species ecology such as the aggregation behaviour which is here intended as a conspecific and high-density aggregation during a specific time of the year (Colin, et al*.,* 2003; Sadovy de Mitcheson & Colin, 2011). The occurrence of such events has been documented in many chondrichthyan species and represents a key moment throughout the life cycle for either a reproductive or defensive scope (Sadovy de Mitcheson & Colin, 2011).

Sharks, rays and skates that display key behaviours such as the use of space in species-specific essential habitats at different life-cycle stages and in key-moments of their life span (e.g., parturition, foraging and mating) and aggregation phenomenon in heavily exploited area may face an additional threat that may further impact the population dynamics (Heuter et al., 2004; Chapman et al., 2015)., besides the main vulnerability aspect due to the life history traits

1.3 Fishery and Conservation Status

In the modern times, it is evident that elasmobranchs have been heavily impacted by anthropogenic activities. The accidental or target catch by professional and recreational fishing, coupled with the life history traits of elasmobranchs compared to other marine species, has led to an evident vulnerability to overfishing, with a consequent high risk or extinction (Dulvy et al., 2021; Pacoureau et al., 2021; Walls & Dulvy, 2021). The number of elasmobranch species compared to other terrestrial or bird species is much larger in term of extinction risk in the oceans and seas where the presence of human activities has been established for centuries (Walls & Dulvy, 2021). Many studies have documented and demonstrated how the elasmobranch decline is to be mainly attributed to extended periods of overfishing at global level by target or accidental catch (Dulvy et al., 2021). Another aspect that globally has determined a heavy exploitation is related to the high commercial value of fins, especially in the Asian tradition (Cardeñosa et al., 2020). Regarding the accidental catch, also called bycatch, most of the fishing gears tend to catch elasmobranch species given their larger size compared to the target species. In fact, the bycatch rate of many elasmobranch species has been well documented in the last years (Oliver et al., 2015; Bonanomi et al., 2018). Even if the commercial value of elasmobranch species is generally relatively low compared to the revenue that professional fishers obtain from other commercial species, nonetheless the decline of several more valuable commercial species is believed to have driven the increase in elasmobranch fishery (Dulvy et al., 2014; Carpentieri et al., 2021). For highly migratory species such as sharks, the high level of fishery exploitation in coastal areas, that are repeatedly used across the years for biological reasons (e.g, nursery, foraging or reproduction), has been documented to be a clear threat to the conservation status of such species (Maguire et al., 2006).

1.4 Mediterranean biodiversity of elasmobranch species

The Mediterranean Sea is widely recognised as hotspot of biodiversity for its richness and abundance of endemic marine species even if its surface is relative smaller compared to other oceans (Coll et al., 2012). A high biodiversity of chondrichthyan species is found in the Mediterranean Sea (Coll et al., 2012; Serena et al., 2020). A total of 88 species, belonging to 30 families of chondrichthyans, have been recently listed in the

Mediterranean and Black Sea. However, only 48% of these species are constantly recorded during surveys, while several other are quite rare or even questionable. The list includes also ten species considered vagrant

Table 1: Current diversity of chondrichthyans the Mediterranean Sea (modified from Serena et al., 2020) and their Conservation status based on IUCN assessment (Dulvy et al., 2016). LC : Least Concern; VU: Vulnerable; NT: Near Threatened; EN: Endangered; CR: Critically Endangered; DD: Data Deficient; NA: Not Available

SHARKS						BATOIDS					
Order	Family / Species		Order	Family / Species		Order	Family / Species		Order	Family / Species	
Carcharhiniformes	**Pentanchidae**		Hexanchidae	**Hexanchidae**		Myliobatiformes	**Dasyatidae**		Rajiformes	**Rajidae**	
	Galeus atlanticus	NT		*Heptranchias perlo*	DD		*Bathytoshia lata*	VU		Dipturus batis	CR
	Galeus melastomus	LC		*Hexanchus griseus*	LC		*Dasyatis marmorata*	DD		Dipturus nidarosiensis	NA
	Scyliorhinidae			*Hexanchus nakamurai*	DD		*Dasyatis pastinaca*	VU		Dipturus oxyrinchus	NT
	Scyliorhinus canicula	LC		**Cetorhinidae**			*Dasyatis tortonesei*	NA		Leucoraja circularis	CR
	Scyliorhinus stellaris	NT		*Cetorhinus maximus*	EN		*Himantura leoparda*	NA		Leucoraja fullonica	CR
	Triakidae		Lamniformes	**Lamniformes**			*Himantura uarnak*	NA		Leucoraja melitensis	CR
	Mustelus asterias	VU		*Alopias superciliosus*	EN		*Pteroplatytrygon violacea*	LC		Leucoraja naevus	NT
	Mustelus mustelus	VU		*Alopias vulpinus*	EN		*Taeniurops grabatus*	DD		Raja asterias	NT
	Mustelus punctulatus	VU		**Lamnidae**			**Gymnuridae**			Raja brachyura	NT
	Galeorhinus galeus	VU		*Isurus oxyrinchus*	CR		*Gymnura altavela*	CR		Raja clavata	NT
	Carcharhinidae			*Isurus paucus*	DD		**Aetobatidae**			Raja miraletus	LC
	Carcharhinus altimus	DD		*Lamna nasus*	CR		*Aetomylaeus bovinus*	CR		Raja montagui	LC
	Carcharhinus brachyurus	DD		*Carcharodon carcharias*	CR		**Myliobatidae**			Raja polystigma	LC
	Carcharhinus brevipinna	NA		**Odontaspididae**			*Myliobatis aquila*	VU		Raja radula	EN
	Carcharhinus falciformis	NA		*Carcharias taurus*	CR		**Rhinopteridae**			Raja undulata	NT
	Carcharhinus limbatus	DD		*Odontaspis ferox*	CR		*Rhinoptera marginata*	DD		Rostroraja alba	EN
	Carcharhinus melanopterus	NA	Squaliformes	**Centrophoridae**			**Mobulidae**		Torpediniformes	**Torpedinidae**	
	Carcharhinus obscurus	DD		*Centrophorus cf. uyato*	CR		*Mobula mobular*	EN		Tetronarce nobiliana	LC
	Carcharhinus plumbeus	EN		**Somniosidae**		Rhinopristiformes	**Rhinobatidae**			Torpedo marmorata	LC
	Galeocerdo cuvier	NA		*Centroscymnus coelolepis*	LC		*Rhinobatos rhinobatos*	EN		Torpedo sinuspersici	NA
	Prionace glauca	CR		*Somniosus rostratus*	DD		**Glaucostegidae**			Torpedo torpedo	LC
	Rhizoprionodon acutus	NA		**Dalatiidae**			*Glaucostegus cemiculus*	EN		**CHIMAERAS**	
	Sphyrnidae			*Dalatias licha*	VU		*Glaucostegus halavi*	NA		**Chimaeridae**	
	Sphyrna lewini	NA		**Echinorhinidae**			**Pristidae**			Chimaera monstrosa	NT
	Sphyrna mokarran	NA		*Echinorhinus brucus*	EN		Pristis pectinata	CR		Hydrolagus mirabilis	NA
	Sphyrna tudes	NA		**Etmopteridae**			Pristis pristis	CR			
	Sphyrna zygaena	CR		*Etmopterus spinax*	LC						
Squatiniformes	**Squatinidae**			**Oxynotidae**							
	Squatina aculeata	CR		*Oxynotus centrina*	CR						
	Squatina oculata	CR		**Squalidae**							
	Squatina squatina	CR		*Squalus acanthias*	EN						
				Squalus blainville	DD						
				Squalus megalops	DD						

The elasmobranch species in Mediterranean Sea have been in an alarming conservation scenario in the last decades and, cumulatively, the situation is much more dramatic compared to the northern-eastern Atlantic Ocean species (Walls & Dulvy, 2021). The last regional assessment from the IUCN included more than 50 % of evaluated chondrichthyan species in the threatened categories (Dulvy et al., 2016). In addition, the excessive fishing effort could generally deplete many marine populations with more commercial value level causing possible and irreversible changes in trophic webs, regime shift or ecological disequilibrium (Myers et al., 2007; Colloca et al., 2015). The Adriatic Sea is one of the Mediterranean subbasin with the hight fishing effort of bottom trawling representing the main anthropogenic impact. (Russo et al., 2019)
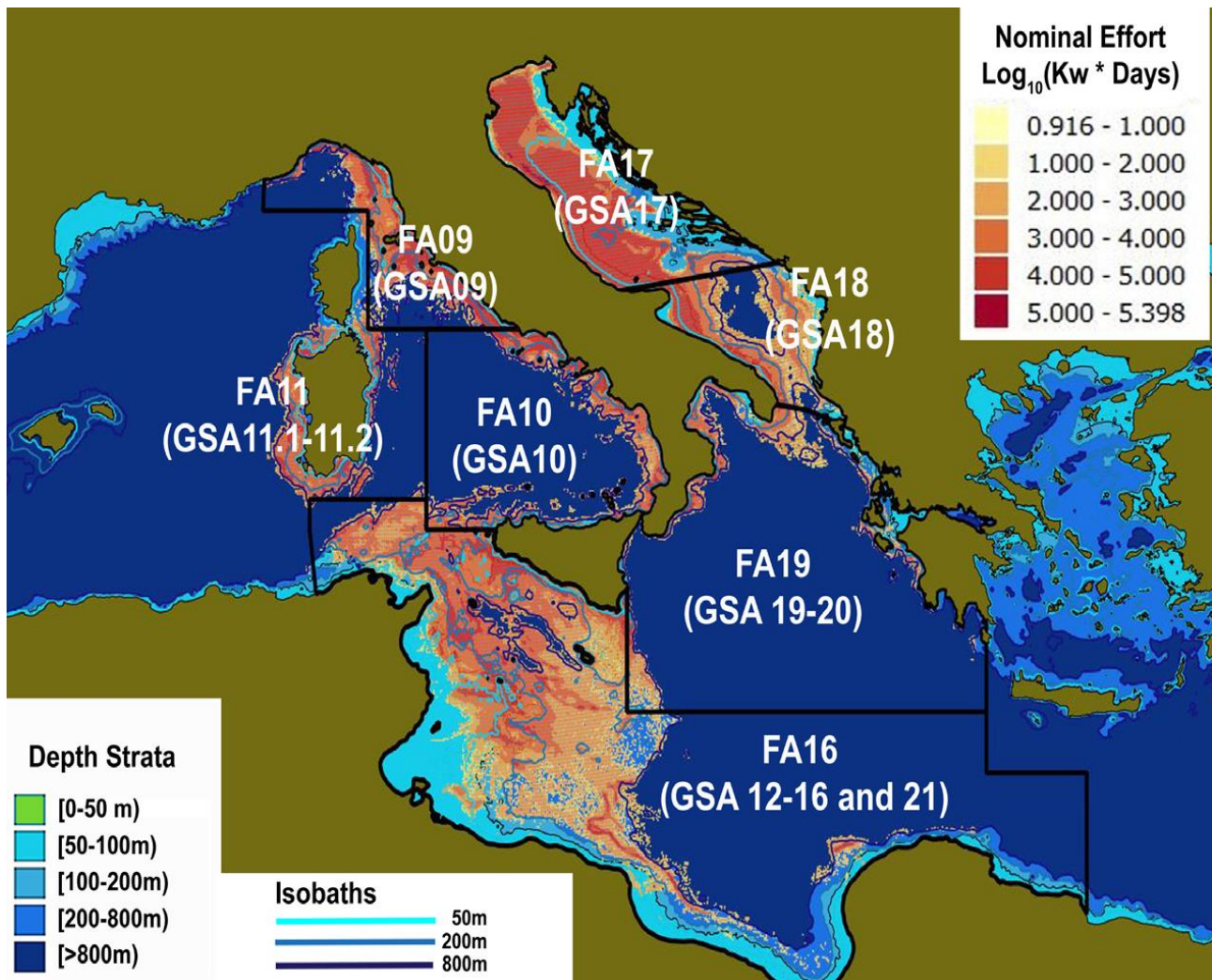
Figure 2: Spatial distribution of bottom trawling fishing effot (edited from Russo et al.,

Several papers highlighted a strong decline or even disappearance in some areas of several elasmobranch species in Mediterranean Sea where large predatory sharks, such as the shortfin mako (*Isurus oxyrinchus*), the porbeagle (*Lamna nasus)*, the blue shark (*Prionace glauca)* the common thresher shark (*Alopias vulpinus)* and the hammerhead sharks (*Sphyrna* spp.), showed declines of more than 90% in the last century (Ferretti et al., 2008). Meso-predatory elasmobranchs showed signs of decline in several Mediterranean areas as well, such as the Adriatic Sea (Fortibuoni et al., 2010; Ferretti et al., 2013; Barausse et al., 2014), Strait if Sicily (Colloca et al., 2020), different Italian waters (Dell'Apa et al., 2012), Tyrrhenian Sea (Ligas et al., 2013), Aegean and Eastern Ionian Seas (Damalas & Vassilopoulou, 2011; Peristeraki et al., 2020). Some species almost disappeared from some areas (Fortibuoni et al., 2016). Currently, a persistence of different abundance and diversity between the northern and the southern parts of the Mediterranean Sea has been suggested due to the different development of fishery activities (Serena et al., 2020).

In the Mediterranean Sea, a growing interest in studying elasmobranch distribution, fishery and biological traits is mainly attributed to fill gaps in knowledge, as documented by the number of papers including review papers (Follesa et al., 2019; Serena et al., 2020) on the biological traits of the most common species (see for instance Geraci et al., 2021; Mulas et al., 2021). In addition, for some species data referring to different sites highlighted intra-specific variability in biological traits noy only between the Mediterranean Sea and the Atlantic Ocean, but also within the Mediterranean (see for instance, Finotto et al., 2015; Bargione et al., 2019).

Some coastal areas have been identified as recurrent egg deposition, parturition or aggregating sites for some commercial species (Colloca et al., 2020), as for instance the Gulf of Gabés for several species (Saidi et al., 201, 2019; Enajjar et al., 2015), some sandy bottoms around Sardinia for *Raja asterias* (Porcu et al., 2017), specific sites for *Dasyatis* spp. and *Glaucostegus cemiculus* along Israel coast (Chaikin et al., 2020), the Gaza strip for *Mobula mobular* (Abudaya et al., 2018). However, despite the urgent need to develop management strategies based on solid scientific data, the use of space of elasmobranchs, in relation also to life cycle and behaviour, is still poorly investigated.

1.5 Elasmobranch management

Despite the continuous call for actions for elasmobranch conservation, as for instance the adoption of the International Action plan for the Conservation and Management of Sharks (IPOA Sharks) by FAO in 1999, the EU-POA Sharks at European level in 2009, the inclusion of some species in CITES appendices or Barcelona convention, and local regulations, the management of chondrichthyan species is still poorly developed. When some regulations are present, they generally include landing and retention restrictions.

In the Mediterranean Sea, very few specific managements for Chondrichthyans have been defined, especially for those species that have commercial relevance. So far, no management measures have been put in force that are based on the use of space of elasmobranch species, with no fishing restriction in parturition or nursery grounds or the aggregation areas where high fishing pressure is present. The 24 bordering countries and the different levels of socio-economic and political development between the northern and southern countries make the Mediterranean basin particularly challenging in management at regional level. The environmental difference in Mediterranean subbasins contributes ultimately to one of the most complex scenarios for nature conservation and fishery management.

To develop effective management strategies, different types of data are needed: reliable landing and catch data, life history traits, use of space, etc. In this regard, the availability of these essential components for fishery management can be limited for some elasmobranch species (Cashion et al., 2019). In particular, landing data are sometimes incomplete and elasmobranch species tend to be reported in aggregated data, such as "sharks", "skates and rays". Even when more species-specific data are included in landing statistics, actual catch data are often missing, and species identification may not be accurate (Dell'Alpa et al., 2012).

In fishery management, various types of temporal and spatial restriction have been implemented aiming at the protection of nursery grounds of many commercial species. In the example concerning the Italian coasts the most important one is the spatial closure of trawling within 3 nautical miles and this spatial restriction changes according to the fishing boat length (EC No. 1967/2006). In other oceans, the spatial management measures in parturition sites and nursery areas have been analysed and discussed for some coastal and

demersal shark species (Kinney & Simpfendorfer, 2009). In the case of the tope shark (*Galeorhinus galeus*) fishery in the Australian waters, the only strict protection of nursery areas by restricted fishing access did not reverse the documented decline since fishing mortality, outside the nursery, remained high and persistent throughout the year. For the gummy shark (*Mustelus antarticus)*, the successful management measures consisted of mild protection to fishery within nursery areas and restricted selectivity of fishing gear such as gillnet that kept the breeding population relatively unfished (Punt 2000; Prince, 2002). In this latter case the information of life history traits and movements contributed significantly to tailor the successful management plan (Kinney & Simpfendorfer, 2009).

For coastal species such as *Carcharhinus* spp., Brewster-Geisz and Miller (2000) applied a population dynamics model to evaluate the effect of fishing mortality at different life stages on the sandbar shark (*Carcharhinus plumbeus*). The results from such model indicate that a significant contribution to the population recovery was given by decreasing the fishing mortality on individuals that were close to the first length of maturity and not only young of the year and juveniles. Therefore, the stand-alone and strict protection of nursery and birthing site of this long-lived shark was not conceivable.

Recently, marine protected areas (MPAs) for elasmobranchs have been evaluated to assess their importance in a spatial strategy for conservation. In highly mobile and tropical species, effectiveness of MPAs is still debated (White et al., 2017; Jacoby et al., 2020). Results indicate that the residency of these species is often beyond MPA boundaries, and this determines that the MPA effectiveness depends on the implementation of law enforcement and patrolling activity. For demersal elasmobranchs, the MPA design has been proposed and evaluated by ecological modelling in the Mediterranean Sea for 5 demersal species, but the use of other approaches, seasonal and life-stage specific data to evaluate its effectiveness are conceivable (Giménez et al., 2020).

1.6 Objectives and thesis structure

Since the scientific research on movement ecology and essential habitat delineation can focus at different time scale and spatial extent according to the methodology (Cadri et al., 2014). This PhD project aims at evaluating the use of space of some elasmobranch species in the Mediterranean Sea by reconstructing movement patterns and frequently used areas across the seasons, identifying key areas that are used in specific life cycle phases (e.g., birthing areas) or for aggregations.

This thesis includes 4 papers:

- The first paper was aimed at using Local Ecological Knowledge in reconstructing movement patterns and aggregation occurrence of elasmobranchs in several Mediterranean Sea areas.
- The second paper reported the first multi-year birthing site of the sandbar shark (*Carcharhinus plumbeus*) and mapped its birthing sites in the Mediterranean basin.
- The third paper investigated the population connectivity and sex biased dispersal of two smooth hound species, *Mustelus mustelus* and *M. punctulatus*, in two Mediterranean areas.
- The fourth paper was focused on the seasonal use of space of *Mustelus* spp. in the northern-central Adriatic Sea
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Bibliography

Abudaya, M., Ulman, A., Salah, J., Fernando, D., Wor, C., & di Sciara, G. N. (2018). Speak of the devil ray (Mobula mobular) fishery in Gaza. *Reviews in Fish Biology and Fisheries*, *28*(1), 229-239.

Barausse, A., Correale, V., Curkovic, A., Finotto, L., Riginella, E., Visentin, E., & Mazzoldi, C. (2014). The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. ICES Journal of Marine Science, 71(7), 1593-1603.

Bargione, G., Donato, F., La Mesa, M., Mazzoldi, C., Riginella, E., Vasapollo, C., ... & Lucchetti, A. (2019). Life-history traits of the spiny dogfish *Squalus acanthias* in the Adriatic Sea. *Scientific reports*, *9*(1), 1-10.

Bonanomi, S., Pulcinella, J., Fortuna, C. M., Moro, F., & Sala, A. (2018). Elasmobranch bycatch in the Italian Adriatic pelagic trawl fishery. *PloS one*, *13*(1), e0191647

Brewster-Geisz, K. K., & Miller, T. J. (2000). Management of the sandbar shark, *Carcharhinus plumbeu*s: implications of a stage-based model. *Fishery Bulletin*, 98 (2), 236-249.

Cadrin, S. X., Karr, L. A., & Mariani, S. (2014). Stock identification methods: an overview. *Stock identification methods*,2nd 1-5.

Carrier, J. C., Musick, J. A., & Heithaus, M. R. (Eds.). (2012). *Biology of sharks and their relatives*. CRC press.

Cashion, M. S., Bailly, N., & Pauly, D. (2019). Official catch data underrepresent shark and ray taxa caught in Mediterranean and Black Sea fisheries. *Marine Policy*, *105*, 1-9.

Cardeñosa, D., Fields, A.T., Babcock, E.A., Shea, S.K.H., Feldheim, K.A., & Chapman, D.D., (2020). Species composition of the largest shark fin retail-market in mainland China. Sci. Rep. 10, 1–10.

Carpentieri, P., Nastasi, A., Sessa, M. & Srour, A., eds. 2021. Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries – A review. Studies and Reviews No. 101 (General Fisheries Commission for the Mediterranean). Rome, FAO. https://doi.org/10.4060/cb5405en

Chaikin, S., Belmaker, J., Barash, A. (2020). Coastal breeding aggregations of threatened stingrays and guitarfish in the Levant. *Aquatic Conservation – Marine and Freshwater Ecosystems*, 30: 1160-1171.

Chapman, D. D., Feldheim, K. A., Papastamatiou, Y. P., & Hueter, R. E. (2015). There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. *Annual review of marine science*, *7*, 547-570.

Colin, P.L., Sadovy, Y.J., Domeier, M.L., 2003. Manual for the Study and Conservation of Reef Fish Spawning Aggregations. Society for the Conservation of Reef Fish Aggregations Special Publication No. 1 (Version 1.0), 1-98+iii pp.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W. W., Christensen, V., ... & Pauly, D. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. *Global Ecology and Biogeography*, *21*(4), 465-480.

Colloca, F., Garofalo, G., Bitetto, I., Facchini, M. T., Grati, F., Martiradonna, A., ... & Spedicato, M. T. (2015). The seascape of demersal fish nursery areas in the North Mediterranean Sea, a first step towards the implementation of spatial planning for trawl fisheries. *PloS one*, *10*(3), e0119590.

Colloca, F., Carrozzi, V., Simonetti, A., & Di Lorenzo, M. (2020). Using local ecological knowledge of fishers to reconstruct abundance trends of elasmobranch populations in the Strait of Sicily. *Frontiers in Marine Science*, *7*, 508.

Council Regulation (EC) No 1967/2006 of 21 December 2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, amending Regulation (EEC) No 2847/93 and repealing Regulation (EC) No 1626/94. [Viewed on 07/01/2022] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1967-20190814

Damalas, D., & Vassilopoulou, V. (2011). Chondrichthyan by-catch and discards in the demersal trawl fishery of the central Aegean Sea (Eastern Mediterranean). *Fisheries Research*, *108*(1), 142-152.

Dell'Apa, A., Kimmel, D. G., & Clò, S. (2012). Trends of fish and elasmobranch landings in Italy: associated management implications. *ICES Journal of Marine Science*, *69*(6), 1045-1052.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., ... & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *elife*, *3*, e00590.

Dulvy, N.K., Allen, D.J., Ralph, G. M., Walls, R.H.L., 2016. The conservation status of Sharks, Rays and Chimaeras in the Mediterranean Sea [Brochure]. IUCN, Malaga, Spain, 14 pp.

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., ... & Simpfendorfer, C. A. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, *31*(21), 4773-4787.

Enajjar, S., Saïdi, B., & Bradaï, M. N. (2015). The Gulf of Gabes (Central Mediterranean Sea): a nursery area for sharks and batoids (Chondrichthyes: Elasmobranchii). *Cahier de Biologie Marine*, 56: 143-150.

Ferretti, F., Osio, G.C., Jenkins, C. J., Rosenberg, A.A., Lotze, H.K., 2013. Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact.*Scientific Reports*, 3, 1057.

Finotto, L., Gristina, M., Garofalo, G., Riginella, E., Mazzoldi C., 2015. Contrasting life history and reproductive traits in two populations of *Scyliorhinus canicula*. *Marine Biology*, 162, 1175-1186.

Fitzpatrick, J. L., Kempster, R. M., Daly-Engel, T. S., Collin, S. P., & Evans, J. P. (2012). Assessing the potential for post-copulatory sexual selection in elasmobranchs. *Journal of Fish Biology,* 80: 1141.1158 (2012).

Follesa, M. C., Marongiu, M. F., Zupa, W., Bellodi, A., Cau, A., Cannas, R., ... & Carbonara, P. (2019). Spatial variability of Chondrichthyes in the northern Mediterranean. *Scientia Marina*, *83*(S1), 81-100.

Fortibuoni, T., Libralato, S., Raicevich, S., Giovanardi, O., & Solidoro, C. (2010). Coding early naturalists' accounts into long-term fish community changes in the Adriatic Sea (1800–2000). *PloS one*, *5*(11), e15502.

Fortibuoni T., Borme D., Franceschini G., Giovanardi O.,Raicevich S., 2016. Common, rare or extirpated? Shifting baselines for common angelshark, *Squatina squatina* (Elasmobranchii: Squatinidae), in the Northern Adriatic Sea (Mediterranean Sea). *Hydrobiologia*, 772, 247-259.

Giménez, J., Cardador, L., Mazor, T., Kark, S., Bellido, J. M., Coll, M., & Navarro, J. (2020). Marine protected areas for demersal elasmobranchs in highly exploited Mediterranean ecosystems. *Marine Environmental Research*, *160*, 105033.

Geraci, M. L., Ragonese, S., Scannella, D., Falsone, F., Gancitano, V., Mifsud, J., ... & Vitale, S. (2021). Batoid Abundances, Spatial Distribution, and Life History Traits in the Strait of Sicily (Central Mediterranean Sea): Bridging a Knowledge Gap through Three Decades of Survey. *Animals*, *11*(8), 2189.

Grogan, E. D., Lund, R., & Greenfest-Allen, E. (2012). The origin and relationships of early chondrichthyans. In Carrier, C. J, Musick, A. J. and Heithaus, R. M: *Biology of sharks and their relatives*. *Second Edition*, 3-29. CRC Press. Taylor&Francis Group, 633 pp

Heupel, M. R., Carlson, J. K., & Simpfendorfer, C. A. (2007). Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series*, *337*, 287-297.

Hueter, R. E., Heupel, M. R., Heist, E. J., & Keeney, D. B. (2004). Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, 35, 239–247.

Jacoby, D.M.P., Croft, D.P., & Sims, D.W. (2012). Social behaviour in sharks and rays: analysis, patterns and implications for conservation. *Fish and Fisheries*, 13: 399-417

Jacoby, D. M., Ferretti, F., Freeman, R., Carlisle, A. B., Chapple, T. K., Curnick, D. J., ... & Block, B. A. (2020). Shark movement strategies influence poaching risk and can guide enforcement decisions in a large, remote marine protected area. *Journal of Applied Ecology*, *57*(9), 1782-1792.

Kinney, M. J., & Simpfendorfer, C. A. (2009). Reassessing the value of nursery areas to shark conservation and management. *Conservation letters*, *2*(2), 53-60.

Ligas, A., Osio, G. C., Sartor, P., Sbrana, M., & De Ranieri, S. (2013). Long-term trajectory of some elasmobranch species off the Tuscany coasts (NW Mediterranean) from 50 years of catch data. *Scientia Marina*, *77*(1), 119-127.

Maguire J.J., Sissenwine M., Csirke J., & Grainger R. (2006). The state of the world highly migratory, straddling and other high seas fish stocks, and associated species. FAO Fisheries Technical Paper. No. 495. Rome: FAO. 2006. 84p.

Mulas, A., Bellodi, A., Carbonara, P., Cau, A., Marongiu, M. F., Pesci, P., ... & Follesa, M. C. (2021). Bio-ecological features update on eleven rare cartilaginous fish in the Central-Western Mediterranean Sea as a contribution for their conservation. *Life*, *11*(9), 871.

Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S. P., & Peterson, C. H. (2007). Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science*, *315*(5820), 1846-1850.

Oliver, S., Braccini, M., Newman, S. J., & Harvey, E. S. (2015). Global patterns in the bycatch of sharks and rays. *Marine Policy*, *54*, 86-97.

Pacoureau, N., Rigby, C. L., Kyne, P. M., Sherley, R. B., Winker, H., Carlson, J. K., ... & Dulvy, N. K. (2021). Half a century of global decline in oceanic sharks and rays. *Nature*, *589*(7843), 567-571. *doi:10.1038/s41586-020-03173-9*

Peristeraki, P., Tserpes, G., Kavadas, S., Kallianiotis, A., & Stergiou, K. I. (2020). The effect of bottom trawl fishery on biomass variations of demersal chondrichthyes in the eastern Mediterranean. *Fisheries Research*, *221*, 105367.

Porcu, C., Marongiu, M. F., Bellodi, A., Cannas, R., Cau, A., Melis, R., ... & Follesa, M. C. (2017). Morphological descriptions of the eggcases of skates (*Rajidae*) from the central-western Mediterranean, with notes on their distribution. *Helgoland Marine Research*, *71*(1), 1-14.

Prince, J.D. (2002) Gauntlet fisheries for elasmobranchs – the secret of sustainable shark fisheries. *Journal of Northwest Atlantic Fishery Science*, *35*, 407-416.

Punt, A.E., Walker T.I., Taylor B.L., Pribac F. (2000) Standardization of catch and effort data in a spatially-structured shark fishery. Fish Res 45, 129–145.

Russo, T., Carpentieri, P., D'Andrea, L., De Angelis, P., Fiorentino, F., Franceschini, S., ... & Cataudella, S. (2019). Trends in effort and yield of trawl fisheries: a case study from the Mediterranean Sea. *Frontiers in Marine Science*, *6*, 153.

Sadovy de Mitcheson, Y., Colin, P. L. (Eds.), (2011). *Reef fish spawning aggregations: biology, research and management*. Fish and Fisheries Series, Vol. 35. Springer Science & Business Media, 621 pp.

Saidi, B., Enajjar, S., & Bradai, M.N., (2016). Elasmobranch captures in shrimps trammel net fishery off the Gulf of Gabés (Southern Tunisia, Mediterranean Sea). *Journal of Applied Ichthyology*, 32: 421-426

Saidi, B., Enajjar, S., Karaa, S., Echwikhi, K., Jribi, I., & Bradai, M.N. (2019). Shark pelagic longline fishery in the Gulf of Gabes: Inter-decadal inspection reveals management needs. *Mediterranean Marine Science*, 20: 532-541.

Schlaff, A. M., Heupel, M. R., & Simpfendorfer, C. A. (2014). Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. *Reviews in Fish Biology and Fisheries*, *24*(4), 1089-1103.

Serena, F., Abella, A. J., Bargnesi, F., Barone, M., Colloca, F., Ferretti, F., ... & Moro, S. (2020). Species diversity, taxonomy and distribution of Chondrichthyes in the Mediterranean and Black Sea. *The European Zoological Journal*, *87*(1), 497-536.

Walls, R. H., & Dulvy, N. K. (2021). Tracking the rising extinction risk of sharks and rays in the Northeast Atlantic Ocean and Mediterranean Sea. *Scientific reports*, *11*(1), 1-15.

White, T. D., Carlisle, A. B., Kroodsma, D. A., Block, B. A., Casagrandi, R., De Leo, G. A., ... & McCauley, D. J. (2017). Assessing the effectiveness of a large marine protected area for reef shark conservation. *Biological Conservation*, *207*, 64-71.

Chapter 2: The use of fishers' Local Ecological Knowledge to reconstruct fish behavioural traits and fishers' perception of the conservation relevance of elasmobranchs in the Mediterranean Sea

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Abstract

The Mediterranean Sea has a long-lasting history of fishery exploitation that, together with other anthropogenic impacts, has led to declines in several marine organisms. In particular, elasmobranch populations have been severely impacted, with drastic decreases in abundance and species diversity. Based on their experience, fishers can provide information on marine species occurrence, abundance and behavioural traits on a long-term scale, therefore contributing to research on the poorly studied biological aspects of elusive or rare elasmobranch species. In this study, for the first time, the Local Ecological Knowledge (LEK) of fishers was applied to study the behavioural traits of sharks, rays and skates in 12 FAO-GFCM geographical sub-areas (GSAs) of the Mediterranean Sea. This study found both new insight and proved the reliability of LEK-based catch seasonality, reflecting seasonal movements, by comparing LEK-based findings and available literature on five elasmobranch taxa *(Mustelus* spp*., Squalus acanthias, Raja* spp*., Myliobatis aquila* and *Scyliorhinus stellaris)* in the Adriatic Sea and 7 taxa (*Mustelus* spp*., Raja* spp*., Prionace glauca, Scyliorhinus canicula, Torpedo* spp*., Pteroplatytrygon violacea* and *Isurus oxyrinchus*) in the remaining Mediterranean GSAs. In addition, LEK provided new insights into a novel comprehensive representation of species aggregations (*Mustelus* spp*., S. acanthias, M. aquila* and *S. canicula*) in the sampled GSAs and supplied the first descriptions of the size, number of individuals and sex composition of the aggregations. When the limits and shortcomings of LEK-based research are considered, this methodology can be a complementary and cost-effective tool used to study elasmobranchs in either a data-poor scenario or a scenario in which a baseline is missing. LEK can also be useful for the evaluation and inclusion of fishers' perceptions of bottom-up management and to provide important evidence for conservation plans.

Keywords

Fishery; space use; aggregations; migration; management; shark; ray

2.1 Introduction

The study of different aspects of the biology of elasmobranchs, a class of cartilaginous fish including sharks, rays and skates, has always been challenging. Their generally low abundances compared to other marine taxa, such as most teleosts, and their elusive nature makes several elasmobranch species difficult to observe and study in the field (Chin & Pecl, 2019; Bargnesi et al., 2020). Therefore, these difficulties have led to a paucity of published data on the behaviour and ecology of elasmobranchs, such as the use of space by the animals at different life stages or sexes as well as the movements, mating and feeding behaviours of the animals. Since these species constitute the target or accidental catch of a wide range of fisheries, such as longliners, trawlers and gillnets, landing data often constitute the most accessible source of information in developed countries (Casey & Myers, 1998; Morgan & Burges, 2005; Serena, 2021). Fishery-independent methods also represent powerful tools to study these species, including scientific surveys (see, for instance, Sguotti et al., 2016), satellite tracking (Hammerschlag et al. 2011), and, more recently, environmental DNA analyses (Bakker et al., 2017) and the citizen science approach (Chin & Pecl, 2019; Bargnesi et al., 2020).

Sharks, rays, and skates are particularly exposed to human activities such as habitat degradation, fishery exploitation and pollution (Myers and Worm, 2003; Barría et al., 2015). One-quarter of chondrichthyans (including chimaeras) are estimated to be threatened by extinction at the global scale (Dulvy et al., 2014). The risk of extinction increases in small ocean basins, such as the Mediterranean Sea; according to the last IUCN regional assessment, in the Mediterranean Sea, among the 73 assessed species (in a total of 88 species registered in the Mediterranean Sea; Serena et al., 2020), 20 are listed as "Critically Endangered" and 11 as "Endangered" (Dulvy et al., 2016). Gaps in the knowledge of some elasmobranch species have not been filled despite research advancements. There are 13 data-deficient species living in the Mediterranean Sea (Dulvy et al., 2016).

The vulnerability of elasmobranchs to fisheries and other anthropogenic activities is tightly related to their life history and behavioural traits. Slow growth rates, late maturity at large sizes, long pregnancies, the deposition of eggs on the seabed and generally large body sizes are recognised as factors that make these

species vulnerable and poorly resilient to overexploitation (Ricklefs, 1979; Dulvy & Reynolds, 2002; Field et al., 2009). Additionally, behaviours such as long migrations, sexual segregations, aggregations for reproduction and site fidelity, and the need for specific, often coastal, areas as nurseries are known to contribute to the risks associated with elasmobranch survival and reproduction and thus to their decline (Maguire et al., 2006; Jacoby et al., 2012; Braccini et al., 2016; Byrne et al., 2017; Dulvy et al., 2017).

The gaps in knowledge encompass several aspects of elasmobranch biology, from their current geographical distribution to the details of their life-history traits and from their trophic ecology to their use of space and behavioural traits (Huepel et al., 2019). Additionally, the incorporation of specific information on these aspects is essential for developing appropriate and effective management strategies (Jacoby et al., 2012; Chapman et al. 2015; Braccini et al., 2016). While several studies in the Atlantic and Pacific Oceans have been performed on the social behaviours, movements and migration of elasmobranchs (see, for review, Jacoby et al., 2012; Braccini et al., 2016), very little information is currently available in the Mediterranean Sea (Abudaya et al., 2018; Barash et al., 2018; Chaikin et al., 2020).

The movements, use of space, and behavioural traits such as the occurrence of sexual segregations, social interactions, and aggregations of elasmobranchs may be investigated using different tools and approaches, such as the application of satellite and radio tracking, genetic analyses, and fishery data (Hammerschlag et al. 2011; Chapman et al., 2015). These approaches may also be combined (Kessel et al., 2014).

An emerging approach to studying wildlife is represented by the collection of information from nonscientitist stakeholders. In particular, so-called Local Ecological Knowledge (LEK), i.e., the knowledge that people in direct contact with wildlife may have on species/ecosystems, often as the result of extensive observation, is increasingly recognised as an important source of information (Huntington, 2000; Anadón et al., 2009; Albuquerque et al., 2021). In the marine environment, LEK usually involves fishers and has been demonstrated to provide relevant information, mainly on species abundances and distributions and their changes over time (e.g., Azzurro et al., 2011, 2019; Maynou et al., 2011; Fortibuoni et al., 2016; Bastari et al., 2017; Peñaherrera-Palma et al., 2018; Taylor et al., 2018; Colloca et al., 2020), as well as on seascape

management (Berkström et al., 2019). For some species, LEK may also provide information on the habitat, diet, reproductive season and even behaviours, such as the occurrence of aggregations (Colin et al., 2003; Moreno et al., 2007; Gerhardinger et al., 2009; Begossi, 2008; Begossi et al., 2019).

Fishing practices are often tightly linked to the knowledge of the behavioural traits of a species, and fishers may adjust fishery activities according to species migrations and uses of space (Moreno et al., 2007). Moreover, fishery exploitation of spawning aggregations is a well-known phenomenon for species such as groupers (Sadovy de Mitcheson & Colin, 2011; Russel et al., 2014), but shark aggregations are also known and exploited (Bada-Sánchez et al., 2019). Therefore, experienced fishers may highly contribute to the knowledge of these biological aspects. The involvement of fishers in gathering information may also constitute the first step towards a comanagement approach (Begossi, 2008; Berkström et al., 2019), especially if fishers perceive these species as important for ecosystems.

This study aims to benefit from the experience gained by fishers in the Mediterranean Sea to 1) evaluate the potential of LEK in reconstructing the behavioural traits of elasmobranchs, in particular movements and aggregations, and 2) collect fishers' perceptions on the relevance of elasmobranch to fisheries and their conservation importance.

2.2 Materials and methods

2.2.1 Study area

The survey was conducted opportunistically in several locations in the Mediterranean Sea, in 12 (6, 9, 10, 11, 13, 16, 17, 18, 19, 20, 22 and 28) out of the 30 Mediterranean GSAs (Resolution GFCM/33/2009/2; FAO, 1990–2021) (Fig. 1a) and in seven countries (Italy, Croatia, Montenegro, Greece, Spain, Tunisia and Turkey). The number of interviews per site depended on fishers' availability and actual opportunities to interview them. In GSA 11, fishers operate in both GSA 11.1 and 11.2, hereafter referred to as GSA 11. Within GSAs 17 and 18, seven different sites were sampled: Ancona (ANC), Chioggia (CHIO), Marano Lagunare (ML), northern Istria (NI; including Funtane, Novigrad, Poréc, Savudrija, Umag, Vabriga, and Vrsar), southern Istria (SI; including Banjole, Pola, Rovinj, and Rabac), the eastern Adriatic coast (EAC; including Crikvenica, Dubrovnik, KrK, Lošinj, Punat, Primošten, Privlaka, Split, and Zadar) and Montenegro (MON; including Bar, Budva, Ber, Herceg Novi, Tivat, and Ulcinj) (Fig. 1b).

Starting from previously available studies (see, for instance, Azzurro et al., 2011, 2019; Maynou et al., 2011), a semistructured questionnaire was developed and translated into different languages. The questionnaire response collection was carried out by trained marine biologists assisted with species tables to allow accurate taxonomic identification by fishers. To facilitate fisher collaboration, all interviewers were local and had previous experience working with fishers. The interviews were completed between spring 2017 and spring 2019.

Fisher interviews were structured in five sections: (*i*) demographics and technical information (question numbers, QNs, 1 to 3); (*ii*) description of catch abundance and diversity (QNs 4 to 12); (*iii*) knowledge about elasmobranch movement and catch seasonality (QNs 13 to 15); (*iv*) knowledge about elasmobranch aggregations (i.e., a conspecific and high-density aggregation during a specific time of the year; Colin, et al., 2003; Sadovy de Mitcheson & Colin 2011) and their characteristics (QNs 16 to 20); and (*v*) fisher opinion on the elasmobranch role and value in the marine environment (QNs 21 to 23) (Fig. S1).

In the first section, personal information (fisher age and years of fishing experience) and information on the fishery (gear type, number of fishing trips per year, past and present fishing areas) were included. The gear types were categorised into gillnets (GNS), longlines (LLS), bottom otter trawls (OTB), beam trawls (TBB) and others, such as purse seines (PS) and traps (FPO).

The catch data section (QNs 4 to 12) included information on four time periods of twenty years each (1940–1960, 1960–1980, 1980–2000 and 2000-present). The interviewed fishers were asked to indicate the relative abundance of sharks, skates and rays in the four time periods, naming the species in an open question and choosing among five categories (Very abundant - more than 3 times more abundant in comparison to the present; Abundant - twice more abundant than in the present; The same; Less abundant; No assessment). The fishers were then asked to indicate the main species perceived as declining or disappearing and if they witnessed any change in size. These data were collected to provide a key for the interpretation of the general framework of species presence in the different Mediterranean areas according to fisher perception and therefore to help to understand the answers of the fishers to the following questions.

Seasonality (QNs 13 to 15) was investigated by collecting responses on the main seasons of catch and the main migration drivers (e.g., reproduction, foraging or abiotic factors).

In the fourth section, questions on elasmobranch aggregations included their occurrence (QN 16), frequency (QN 17) and features such as number, size, and sex composition over the four abovementioned periods (QN 18). Additionally, the area where and the period of the year when the aggregation takes place were assessed (QNs 19 and 20).

Finally, fishers were asked to express their opinion on the ecological value, commercial significance and conservation importance of sharks, skates, and rays, as well as what measures they would adopt to conserve the species (QNs 21, 22 and 23).

2.2.2 Data processing and analyses

No-answer entries (NA) and null answers (NULL) were identified and discarded because they were too vague (e.g., use of general terms such as shark, ray, and skate instead of specifying a species name) throughout the whole questionnaire. Concerning the second section (description of catch abundance and diversity), the ranges of the frequency of NA and null answers were between 18% and 30%. The third section, regarding the catch season (movement and catch seasonality), had an overall frequency of discarded NA and null answers of 14%. To depict general patterns, a minimum response threshold was set in which only species that were indicated by at least 25% of the interviewees in each GSA were considered (Annex I, Tables S1 and S2). Genera were used instead of species when scientific names were not reported in the answers (e.g., *Raja* spp. and *Mustelus* spp.). The fourth section (elasmobranch aggregations and their features) had different percentages of NULL and NA answers among different periods, with valid answers collected only for the 1960–1980 period. Data on aggregation features were retrieved while considering only the last three time frames (1960–1980, 1980–2000 and 2000-present) and keeping the Adriatic and other GSA entries separate (AnnexI, Tables S3 and S4). The other GSA data were pooled together due to the limited sample size (Annex,I, Table S5). QNs 14, 15, 19 and 20, from the third and fourth sections, were excluded from further analyses because of overall inconsistent and generalist answers. Only a few fishers marked an aggregation area on the provided map; thus, this part was not included.

Data on fisher age, years of fishing activity and annual fish trips were evaluated to check similarity by the Kruskal–Wallis test in R studio (R Studio team 2020).

For the questions related to the second section (catch abundance and diversity), the interviews were analysed according to GSA (GSA 22 and 28 were grouped since fishers from GSA 28 declared to also fish in GSA 22) and period (A: 1940–1960; B: 1960–1980; C: 1980–2000; D: 2000-present). Because the different species of the genera *Mustelus* (Marino et al., 2018) and *Alopias* (Serena et al., 2005) present similar morphological traits, thus possibly favouring misidentification, we chose to pool together in the genus all answers related to the species belonging to these taxa.

Data on the declared most-fished species per period and GSA were first transformed into ratios relative to the total number of interviews that answered the related question. Then, the data were analysed by calculating a Bray-Curtis similarity matrix and were represented through cluster analysis (group average as cluster mode). SIMPER analysis was applied to investigate which main species were responsible for the similarity within each GSA, with a threshold of 10% relative contribution to the similarity. Multivariate analyses were performed using Primer 6 and PERMANOVA plus (Clarke & Gorley, 2006; Anderson et al., 2008).

To analyse which species were declared to have declined (from the answers to questions in the catch abundance and diversity section), the data on the declined species per GSA were transformed into ratios relative to the total number of interviews that answered the related question.

Only in the Adriatic Sea were seasonality data transformed by ratios over the total number of interviews and visualised by QGIS (https://qgis.org) according to the geographic area or city. To investigate whether the fishers' knowledge about seasonality was related to the fishers' experience (years of fishing activity, days of fishing and change in fishing area) and whether seasonality varied according to sub-basins, we applied generalised linear modelling (GLM) (Dobson & Barnett, 2008) by using R (R studio team,2020). The presence of seasonality was considered a binomial (yes/no) and dependent variable. Three Mediterranean areas, the Adriatic Sea (GSAs 17 and 18), Central-Western Mediterranean (GSAs 6, 9, 10, 11, 16, 13) and Eastern Mediterranean (GSAs 19, 20, 22, 28), were considered categorical dependent variables, and years of fishing experience, days of fishing and change in the fishing area (binomial, yes/no) were used as independent variables. Years of experience and fishers' age showed collinearity, so only the first was kept in the analysis. Based on the Akaike information criterion (AIC) values, the best GLM model was chosen.

2.3 Results

In total, 218 questionnaires were collected in the 12 GSAs of the Mediterranean Sea (Fig. 1a). The Adriatic Sea (GSAs 17 and 18) was the sub-basin where the largest number of interviews was gathered (N = 92) (Fig. 1b). In the other areas, the number of interviews varied from 4 to 21 per GSA.
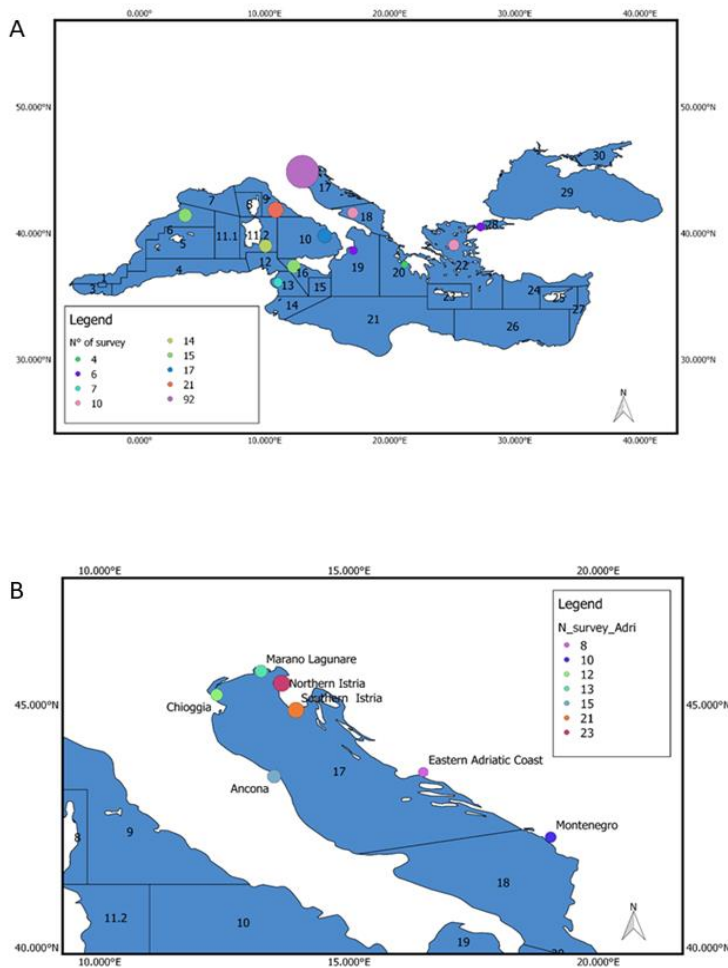
Fig. 1: Sampled GSA of the Mediterranean Sea with a focus on Adriatic sampling points. Coloured bubbles represent the number of interviews in (A) the different Mediterranean GSAs and (B) sampling sites in the Adriatic Sea.

The age of the fishers was not different between GSA (Kruskal–Wallis chi-squared = 7.3876, df = 10, p value = 0.6884), whereas year of fishing activity (Kruskal–Wallis chi-squared = 21.104, df = 11, p value = 0.0323)

and number of fishing trips (Kruskal–Wallis chi-squared = 65.179, df = 11, p value = 9.971e-10) significantly differed among sampled GSA (Fig. 2a, 2b, 2c). Many interviewed fishers (65%) did not change the fishing area from the beginning of their activity compared to the current one, whereas 31% of them operated in other areas within the same GSA, and 9% did not reply. Nearly all fishers had caught sharks in the past (96%), as in the present study (90%). Likewise, rays and skates were frequent catches in the past (95%) and the present (81%). Across GSAs, the fishers' LEK was based on different fishing gear (Annex I, Table S6), among which the TTB was sampled only in GSAs 13, 17 and 28 and the PTM was sampled only in GSAs 6, 17, 18 and 28. LEK based on GNT fishers was not collected in GSA 6 or GSA 28. The LLS and OTB gears were represented in every GSA.
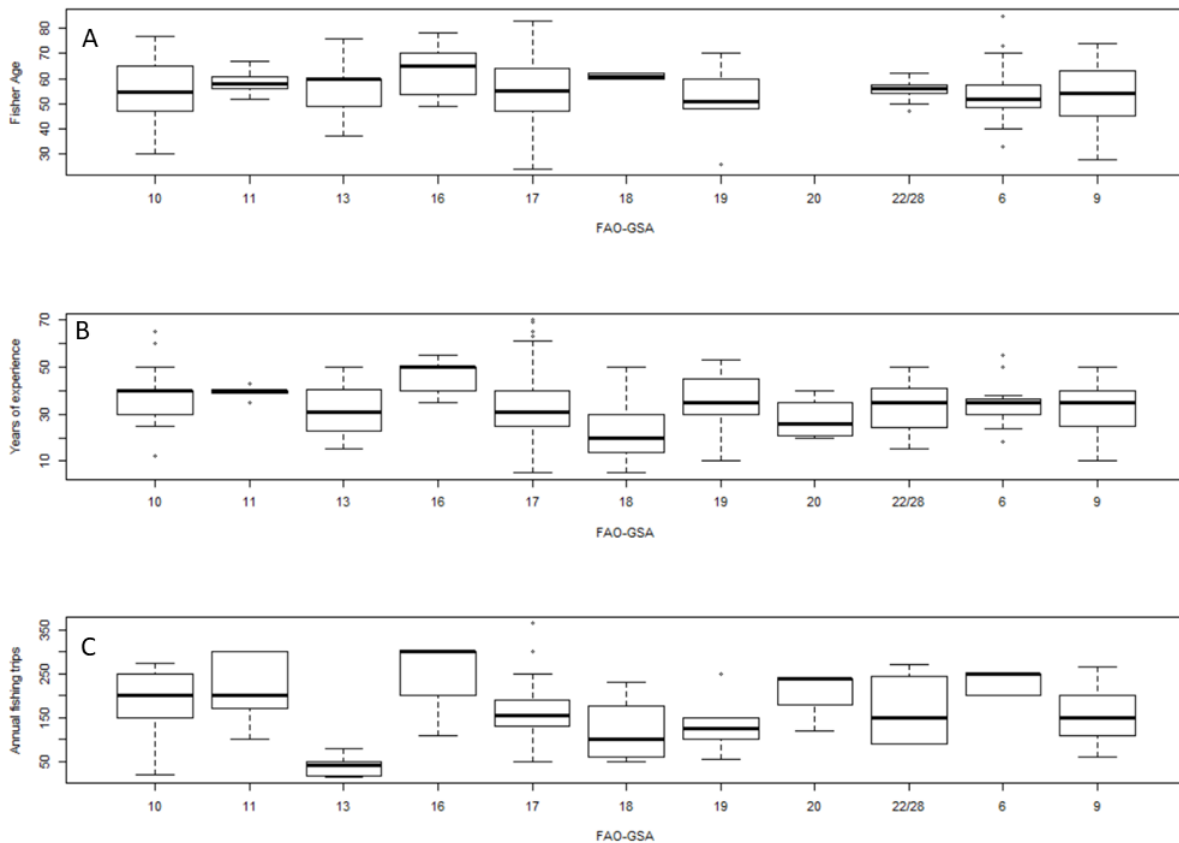
Fig. 2: Boxplots reporting the data on interviewed fishers for each GSA. A) Fisher age. B) Years of fishing experience. C) Annual fishing trips.

No LEK-based information was gathered for the periods 1940–1960 and 1960–1980 for either shark or ray/skate abundance catches. Between 1980 and 2000, shark catches were higher than those at present. In detail, forty-two percent of fishers in all sampled GSAs indicated that catches were either twice (21%) or three times (21%) more abundant than in the present, while 25% perceived that catch abundance remained the same. Only 9% indicated that catches were less abundant between 1980 and 2000 than at present.

For rays and skates, fishers highlighted a sharp decline in catch abundance in the last twenty-year period; 30% of fishers expressed present catches as being less abundant, 34% described them as remaining the same, and only 16% suggested an increase in captures compared to the present. Similar to sharks, in the 1980–2000 period, ray and skate catches were shown to be greater than those in the present. Between 2000 and the present, a decline in catches was highlighted as well (Fig. 3a and 3b).
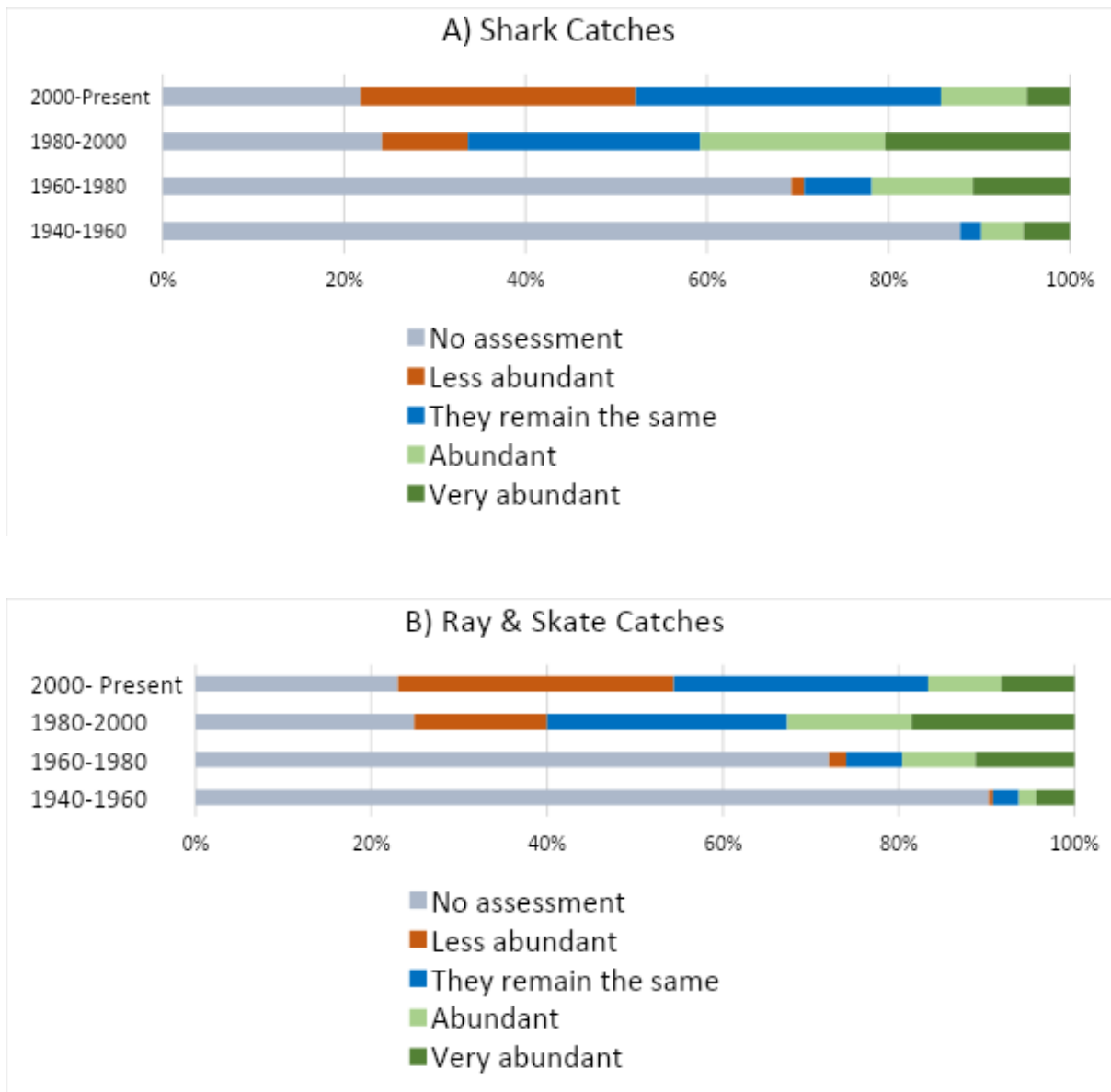
Fig. 3: Catches in the different time periods of Sharks (a) and Skates and Rays (b)

2.3.1 Most-fished species

Considering the species that were declared to be the most fished reported per period and GSA, the cluster analysis grouped the samples mainly according to geographic area, regardless of the period (Fig. 4).
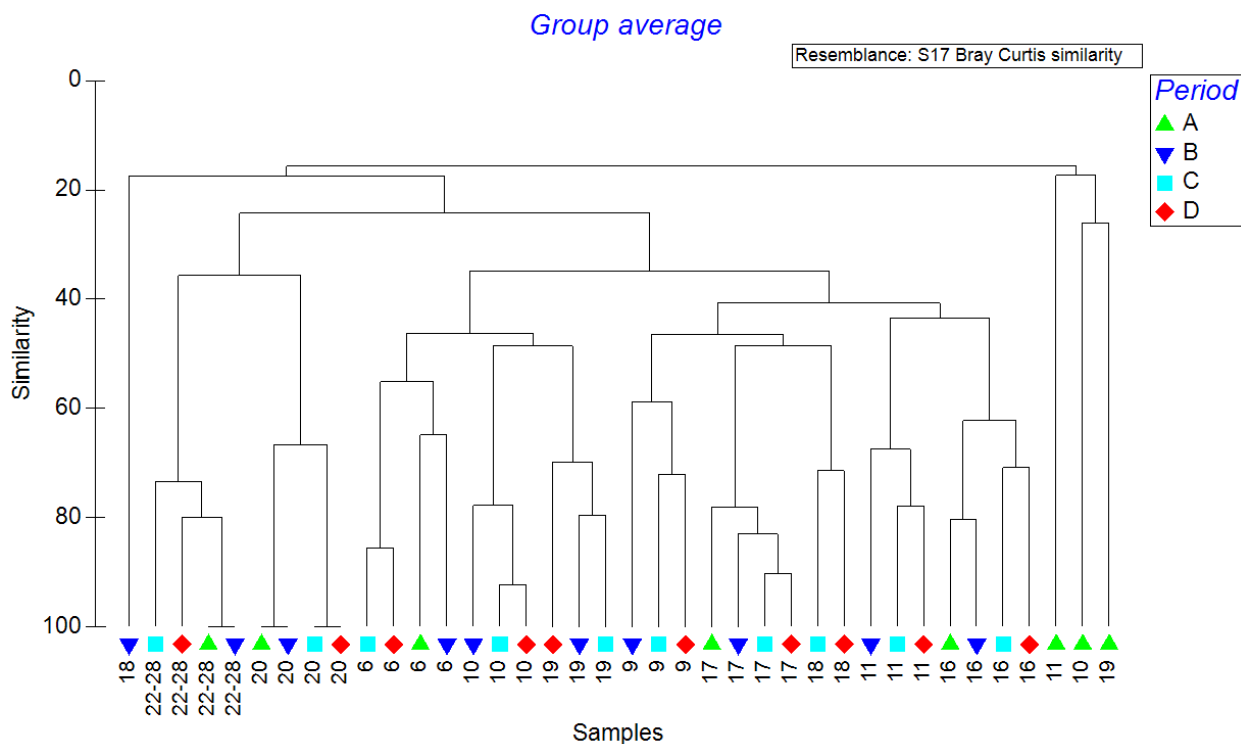
Fig. 4: *Cluster analysis of elasmobranch diversity. GSAs are indicated by numbers and time periods by letters (A: 1940–1960; B: 1960–1980; C: 1980: 2000; D: 2000 – present).*

However, clustering was not completely related to the contiguity of the GSAs; some GSAs were clustered with other distant GSAs (see, for instance, the clustering of GSA 9 with GSAs 17 and 18). Within each GSA, some temporal trends are recognisable, with sample clustering mainly by period. SIMPER analyses identified the main species responsible for the similarity within each GSA, therefore characterising the different GSAs (Table 1).

Table 1: *Species responsible for GSA similarity, listed by relative contribution (SIMPER analyses).*

Species	Average relative presence	Contribution (%)	Species	Average relative presence	Contribution (%)
GSA 6: average similarity 61.67 %			GSA 17: average similarity 81.83 %		
Scyliorhinus canicula	0.67	18.69	*Mustelus* spp.	1.00	17.53
Galeus melastomus	0.55	13.06	*Squalus acanthias*	0.74	12.64
GSA 9: average similarity 63.26 %			*Raja clavata*	0.76	11.16
Raja clavata	0.43	19.29			

Scyliorhinus canicula	0.40	15.50	GSA 19: average similarity 56.93 %		
Raja asterias	0.38	15.50	*Prionace glauca*	0.60	19.73
Pteroplatytrygon violacea	0.28	10.26	*Alopias* spp.	0.38	12.53
GSA 10: average similarity 59.07 %			*Isurus oxyrinchus*	0.43	12.53
Raja clavata	0.91	19.77	*Sphyrna zigaena*	0.34	11.20
Prionace glauca	0.65	12.29	GSA 20: average similarity 77.78 %		
GSA 11: average similarity 43.23 %			*Mustelus* spp.	0.25	29.76
Scyliorhinus canicula	0.60	11.73	*Squalus acanthias*	0.25	29.76
Raja polystigma	0.56	11.30	*Scyliorhinus canicula*	0.25	29.76
Prionace glauca	0.48	10.39	*Rhinobatos rhinobatos*	0.38	10.71
GSA 16: average similarity 66.73 %			GSA 22-28: average similarity 80.07		
Mustelus spp.	0.80	30.10	*Dasyatis pastinaca*	0.54	18.33
Raja miraletus	0.63	26.14	*Alopias* spp.	0.42	12.77
Raja clavata	0.67	19.54	*Scyliorhinus canicula*	0.42	12.77
GSA 18: average similarity 54.04			*Galeus melastomus*	0.42	12.77
Mustelus spp.	0.80	45.73			
Myliobatis aquila	0.65	31.50			

2.3.2 Declined species

Fishers indicated that 40 species declined in their fishing areas. The species that were perceived as declining the most, in more than half of the analysed GSAs (11 GSAs, with GSAs 22 and 28 grouped), were *Alopias* spp. and *Mustelus* spp., with 8 GSAs reporting their decline over time, followed by *S. acanthias, P. glauca*, *Squatina squatina* and *Raja clavata.* GSA 17 was the one with the highest number of declining species (n = 27), followed by GSA 11, with 17 species shown to have declined.

In general, there was no wide consensus among fishers on the declining species; indeed, the percentage of fishers who indicated that the same species had declined was generally below 20% among the GSAs (Fig. 5).
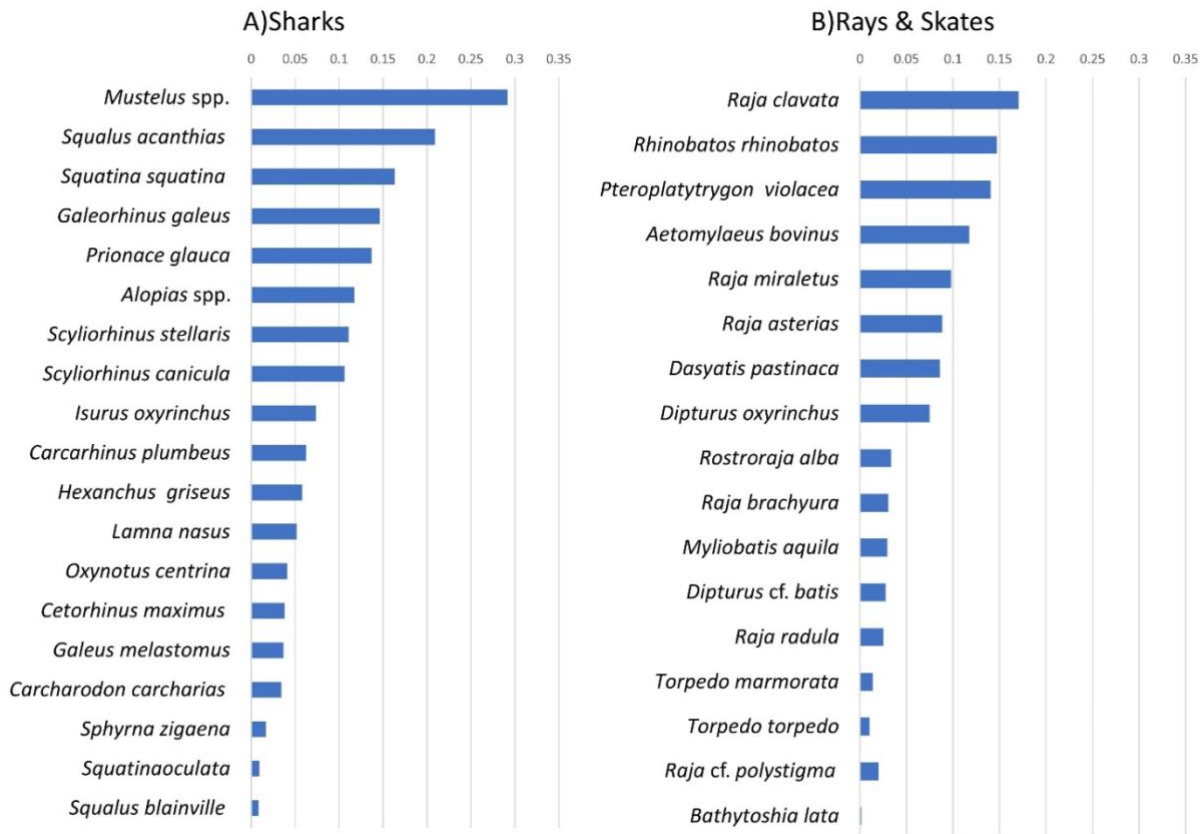
Fig. 5: *Declined sharks (A) and rays and skates (B) according to fishers' perception (ratio calculated over the total answers for each species).*

There are many factors at play, such as the different distributions of elasmobranch species, different fishing pressures, and different gear types. Considering the species most reported by fishers, pelagic sharks such as *P. glauca*, *Alopias* spp. and *I. oxyrinchus* and some demersal species, such as *Mustelus* spp., *S. acanthias*, S. *stellaris* and *S. squatina,* were reported to decline in the Adriatic Sea (GSAs 17 and 18). Similarly, rays and skates such as *R. clavata*, *Raja asterias* and *Raja miraletus* have become less abundant than in the past. In the other sampled GSAs, different species were indicated as relatively abundant. The most-indicated species within GSAs were *Mustelus* spp. in GSAs 9 and 11, *S. acanthias* in GSA 9, *Squatina spp.* in GSA 11, *R. clavata* in GSA 6, *Aetomylaeus bovinus* in GSA 19, *Dipturus oxyrinchus* in GSA 6, and *Rhinobatos rhinobatos* in GSAs 16 and 20 (Table 2). The other survey questions in this section (QNs 6, 7, 10 and 11) were not included in the analyses due to the extensive lack of answers.

Table 2: *Species indicated to be declined in each GSA. The ratio consists of species frequency in the answers over the total.*

GSA	Species	Ratio	GSA	Species	Ratio
GSA6	*Raja clavata*	0.57	GSA 18	*Mustelus* spp.	0.50
	Prionace glauca	0.36		*Raja asterias*	0.40
	Cetorhinus maximus	0.36		*Squalus acanthias*	0.30
				Isurus oxyrinchus	0.30
GSA9	*Mustelus* spp.	0.76	GSA 19	*Aetomylaeus bovinus*	0.67
	Squalus acanthias	0.41		*Pteroptlatytrygon violacea*	0.67
				Dasyatis pastinaca	0.33
				Mustelus spp.	0.33
				Carcarhinus plumbeus	0.33
GSA 10	*Lamna nasus*	0.43	GSA 17	*Squalus acanthias*	0.45
	Pteroplatytrygon violacea	0.33		*Raja clavata*	0.42
	Raja clavata	0.33		*Mustelus* spp.	0.33
	Raja miraletus	0.33		*Prionace glauca*	0.33
	Rostroraja alba	0.33		*Scyliorhinus stellaris*	0.32
	Aetomylaeus bovinus	0.33			
GSA 11	*Mustelus* spp.	0.58	GSA 20	*Rhinobatos rhinobatos*	0.75
	Scyliorhinus canicula	0.33			
GSA 16	*Scyliorhinus stellaris*	0.36	GSA 22-28	*Squalus acanthias*	0.33
	Rhinobatos rhinobatos	0.50		*Prionace glauca*	0.33
				Dasyatis pastinaca	0.33

2.3.3 Seasonality

The seasonality of catches was indicated for 5 elasmobranchs *(Mustelus* spp*., S. acanthias, Raja* spp*., M. aquila* and *S. stellaris)* in the Adriatic Sea and 7 (*Mustelus* spp*., Raja* spp*., P. glauca, S. canicula, Torpedo* spp*., P. violacea* and *I. oxyrinchus*) in the remaining Mediterranean GSAs.

According to the lowest AIC value, the best-fit model (dispersion parameter equal to 0.8) included the Mediterranean subdivisions, years of fishing experience, fishing days and fishing area as variables (Table 3).

Table 3: *Formula of tested GLMs and corresponding AIC value*

Models ID	Formula	AIC value
Model 1	glm (formula = Seasonality ~ Subdivision + Years of experience + Fishing days + Fishing area, family = binomial (link ="logit"))	136
Model 2	glm (formula = Seasonality ~ Subdivision + Years of experience + Fishing days, family = binomial (link ="logit"))	147

Model 3	glm (formula = Seasonality~ Subdivision + Years of experience, family = binomial (link ="logit"))	155

The GLM parameters showed that species seasonality was correlated with the considered subdivisions (Adriatic Sea and Central-Western Mediterranean, both p values < 0.001, and Eastern Mediterranean, p < 0.05), whereas years of experience of the fishers, days of fishing and fishing area change were found not to be significantly correlated with the occurrence of seasonality (Table 4).

Table 4. Model 1: Estimated regression parameters, standard errors, z-values and P-values. Significant values in bold.

Variable	Estimate	Std. Error	z-value	p-value
Adriatic Sea	3.756273	1.012022	3.712	**< 0.001**
Central-Western Mediterranean	-3.182497	0.669038	-4.757	**< 0.001**
Eastern Mediterranean	-1.775696	0.896434	-1.981	**< 0.05**
Years of experience	-0.004263	0.019640	-0.217	> 0.05
Fishing Days	-0.002594	0.003186	-0.814	> 0.05
Fishing Area	0.138850	0.547402	0.254	> 0.05

Adriatic fishers indicated a north-south (GSAs 17) and in-offshore migration (GSA 18) of *Mustelus* spp., as represented by a stronger seasonality of catches in the north (CHIO, NI and ML) in summer than in the SI site. At the central and southern sites of the Adriatic (EAC and MON), strong seasonality also emerged in summer. Winter was broadly a time period with low catches (Fig. 6a).

S. acanthias did not show any clear pattern of seasonality across the Adriatic sites, except for ECA and MON, where catches reached a peak between spring and summer (Fig. 6b). *M. aquila* showed a marked peak at the

northeastern sites (ML and NI) in summer. In contrast, this species presented no seasonality at the ECA site (Fig. 6c). Regarding *Raja* spp., LEK information showed that catches were equally common throughout the year, whereas marked seasonality was reported exclusively in EAC and MON (Fig. 6d). The lack of seasonal movements indicated for *S. stellaris* was not surprising given that this species showed no seasonality in abundance at the ML and NI sites.
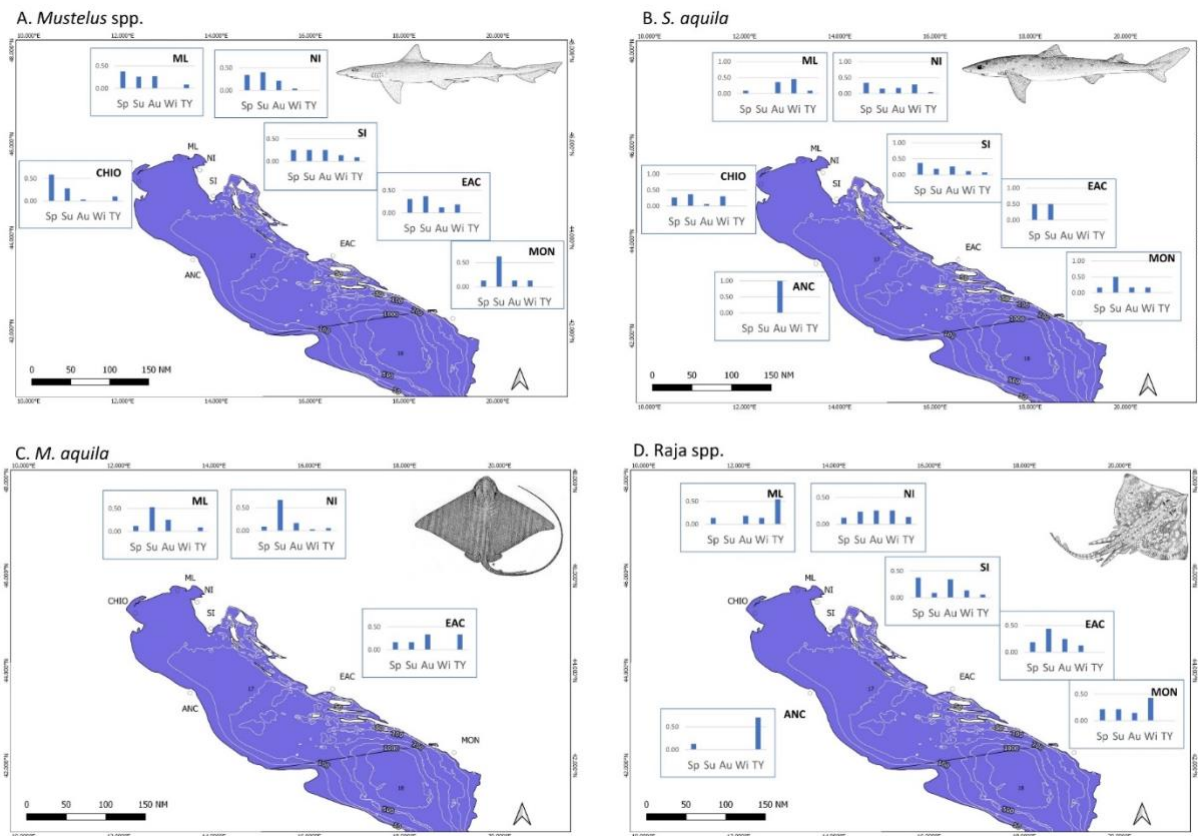
Fig. 6: *Seasonality of Mustelus spp. (M), Squalus acanthias (SA), Myliobatis aquila (MA), Raja spp. (R) as perceived by fishers at the Adriatic sites: Ancona (ANC), Chioggia (CHIO), Marano Lagunare (ML), northern Istria (NI) and southern Istria (SI), the eastern Adriatic coast (EAC) and Montenegro (MON). The ratio of answers over the total in the four seasons (spring (SP), summer (SU), autumn (AU) and winter (WI) and throughout the year (TY)).*

Even though it was not possible to sample several sites in the other Mediterranean GSAs, LEK indicates that some species do follow a seasonal trend in catches, while others appear to have an unclear pattern. For instance, spring and summer seem to be the catch seasons for *Torpedo* spp. (GSAs 11 and 19), *I. oxyrinchus*

(GSA 19), *S. canicula* (GSA 11) and *P. violacea* (GSA 6). Moreover, some aspects of species seasonality are consistent with the data from the Adriatic Sea; for instance, *Raja* spp. shows highly variable seasonality across GSAs 9, 11, 22/28 and is catchable year-round. In contrast, *Mustelus* spp. does not appear to have strong seasonality in GSA 16, likely since LEK-based information may suffer from seasonal shifts in the fishery distribution in that GSA. *P. glauca* catches present an equivocal pattern; no seasonal trend appears in GSAs 19 and 6, while the species is likely to occur in autumn and winter in GSA 9 (Table 5).

Table 5: Seasonality in Mediterranean GSAs (Italy (ITA), Turkey (TUR), Spain (SPA)). GSAs 17 and 18 are not included. Values are reported as a ratio, meaning the frequency of each species in fisher's answers over the total. Throughout the year (TY).

GSA	Country	Species	Spring	Summer	Autumn	Winter	TY	N
9	ITA	*Raja* spp.	0.1	0.2	0.3	0.3	0.1	21
9	ITA	*P. glauca*	0.0	0.0	0.5	0.5	0.0	21
28/29	TUR	*Raja* spp.	0.8	0.0	0.0	0.0	0.3	10
11	ITA	*Raja* spp.	0.5	0.3	0.1	0.0	0.1	14
11	ITA	*S. canicula*	0.3	0.8	0.0	0.0	0.0	14
11	ITA	*Torpedo* spp.	0.5	0.5	0.0	0.0	0.0	14
16	ITA	*Mustelus* spp.	0.1	0.1	0.3	0.2	0.3	15
6	SPA	*P. violacea*	0.1	0.5	0.0	0.2	0.2	15
6	SPA	*P. glauca*	0.0	0.5	0.0	0.3	0.3	15
10	ITA	*I. oxyrinchus*	0.0	0.5	0.5	0.0	0.0	6
19	ITA	*P. glauca*	0.3	0.2	0.2	0.0	0.3	6
19	ITA	*Torpedo* spp.	0.3	0.8	0.0	0.0	0.0	6

2.3.4 Aggregations

Many fishers experienced the occurrence of elasmobranch aggregations in the Mediterranean Sea, either through catches or visual witnesses. In the Adriatic Sea, such events were experienced by 80% of the interviewed fishers, while 13% did not report having experienced them, and 7% did not answer. *Mustelus* spp. (57%), *S. acanthias* (32%), *M. aquila* (26%) and *Raja* spp. (11%) were the most frequent species caught in aggregations in both GSA 17 and 18. Other species, such as *P. glauca* and *Dasiatis pastinaca,* were indicated

to be rare catches in aggregations (Fig. 7). In the other sampled GSAs, a large portion of the interviewed fishers (58%) confirmed having fished on an elasmobranch aggregation. In comparison, 31% of fishers declared to have not had this experience, and 11% did not answer. By combining all sampled GSAs, *Raja* spp. (25%), *S. canicula* (19%) and *Mustelus* spp. (14%) appeared to be the most common species caught in aggregations. Other elasmobranchs, such as *Mobula mobular, P. violacea, Galeus melastomus, P. glauca, D. pastinaca, Squalus blainville, Cetorhinus maximus, Torpedo* spp.*, Etmopterus spinax, Hexanchus griseus, Sphyrna zygaena,* and *S. acanthias,* were occasionally caught in aggregations. Nevertheless, such events appear to be rare (between 1% and 8% of answers) (Fig. 7). The latter species group includes less commercially relevant species, such as *P. violacea*, *D. pastinaca* and *Torpedo* spp., and rare species, according to the results of this survey regarding the most fished elasmobranchs. Overall, some species aggregations were present in more GSAs than other species. For instance, *Raja* spp. and *Mustelus* spp. aggregations appeared in the highest number of GSAs in eight and five GSAs, respectively (Fig. 7).
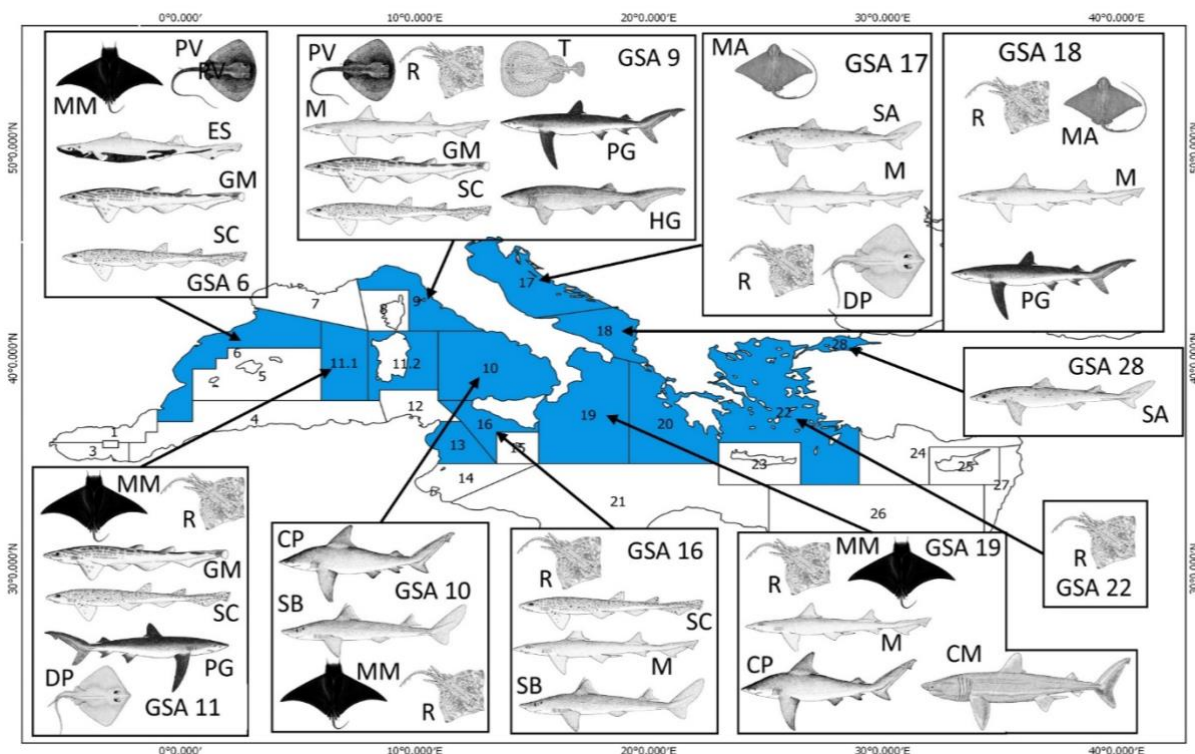
Fig. 7: Data on aggregating species in sampled GSAs in the Mediterranean Sea. The absence of icon means zero aggregating species (GSA 19) or no-available data for the GSA (GSA 13). Species name and used abbreviations: Cetorhinus maximus (CM), Carcharhinus plumbeus (CP), Dasyatis pastinaca (DP), Etmopterus spinax (ES), Galeus melastomus (GM), Hexanchus griseus (HG), Myliobatis aquila

(MA), Mobula mobular (MM), Mustelus spp. (M), Prionace glauca (PG), Pteroplatytrygon violacea (PV), Raja spp. (R), Squalus acanthias (SA), Scyliorhinus canicula (SC), Squalus blainville (SB), Sphyrna zygaena (SZ), Torpedo marmorata (TM).

In both the Adriatic Sea and the other Mediterranean GSAs, LEK indicated declining trends in aggregation occurrence for *Mustelus* spp., *S. acanthias*, *M. aquila* and *S. canicula*. Regarding the frequency of *Raja* spp., a slight trend seems to indicate an increase only in the Adriatic Sea (Fig. 8a and 8b).
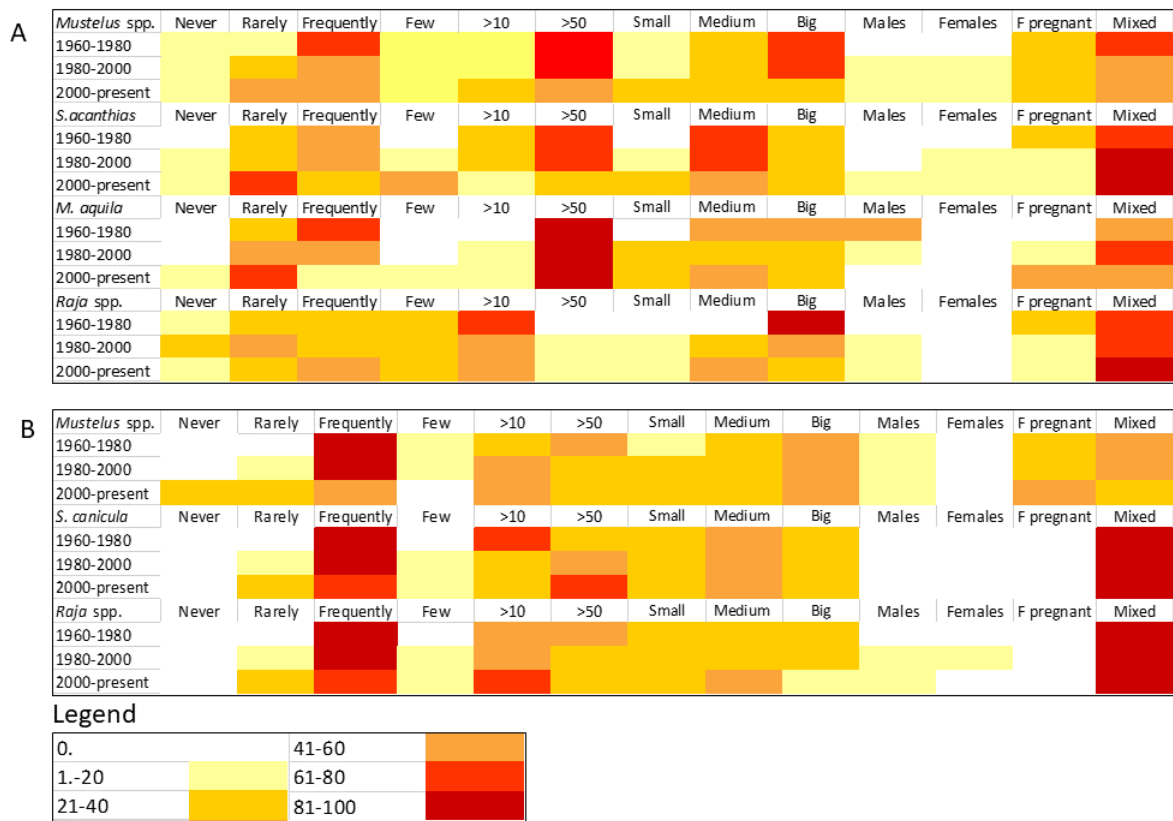
Fig. 8: *Characteristics of the aggregations of the main elasmobranch species indicated by fishers in the Adriatic Sea (11a) and in other Mediterranean GSAs (11b). Legend by colours refers to answer ratio in percentage.*

LEK information allowed the description of some aggregation features, such as individual number, size, and sex composition (Fig. 8a and b). There is a general consistency in the results among the Mediterranean GSAs regarding the aggregation characteristics of *Mustelus* spp. Comparing the three periods represented by the fishers' answers, more than fifty individuals were usually found in the aggregations, there was a prevalent presence of mixed sexes in the aggregations, and pregnant females were commonly encountered. Adriatic LEK on *S. acanthias* showed that the individual composition of aggregations changed over time in number,

decreasing from more than fifty animals to a few per aggregation. The individual size remained the same over time, as did the presence of mixed sexes. The characteristics of *M. aquila* aggregations in the Adriatic Sea were similar across periods; most of the aggregations were formed by more than fifty individuals and were composed of mixed sizes and sexes. *Raja* spp. were frequently found in aggregations that were consistently composed of more than ten individuals and mixed sexes. In all the Mediterranean GSAs, a size reduction was noted for *Raja* spp. aggregations. Only for GSAs 9, 11 and 6 was information on *S. canicula* aggregations collected. The data showed an increase in the number of individuals per aggregation and a constant presence of medium-size individuals. Mixed sexes were common across the investigated periods.

2.3.5 Fishers' perceptions of elasmobranch value

Regarding the value and role of elasmobranchs in ecosystems, it was generally acknowledged by fishers that elasmobranch species are important for the marine environment (77% YES, 8% NO and 15% no answer-NA). Similarly, sharks, rays and skates were also recognised to have relevant economic value for fishery revenue (75% YES, 21% NO and 4% NA). Interestingly, 74% of the interviewed fishers answered that there is a need for conservation actions for elasmobranchs. In comparison, 10% did not agree, and the rest (16%) of the fishers did not answer the question (Fig. 9a). Fishers in favour of elasmobranch protection indicated three actions: (i) spatial-temporal closures (17%), for instance, during the reproduction season; (ii) release of captured small relative-sized individuals (e.g., newborn or juveniles) as good fishing practice (10%); and (iii) catch control, such as regulation surveillance and enforcement, to more broadly reduce illegal, unreported and unregulated fishing (21%). Other measures made up 8% of the answers, whereas 44% of interviewed fishers did not give any indication of specific measures, although they were in favour of conservation measures (Fig. 9b).
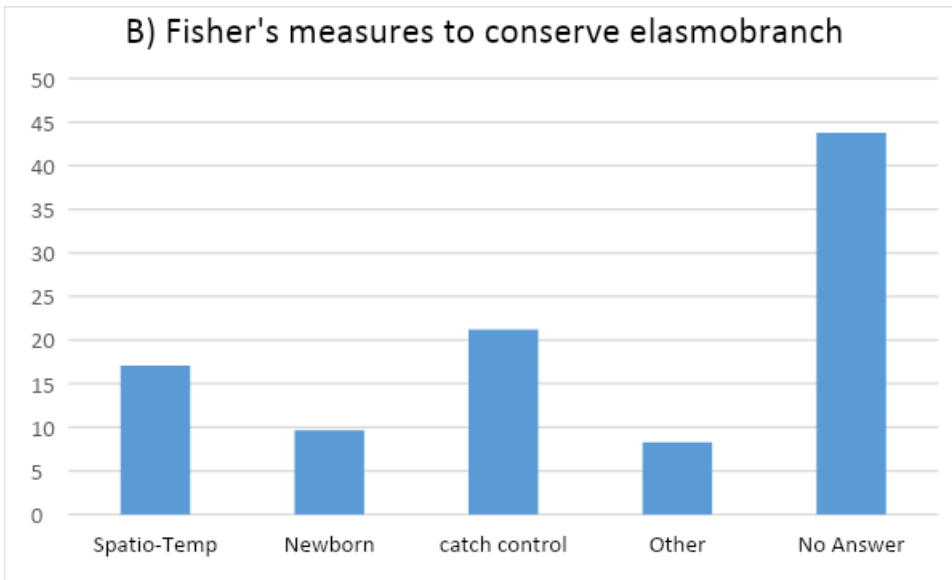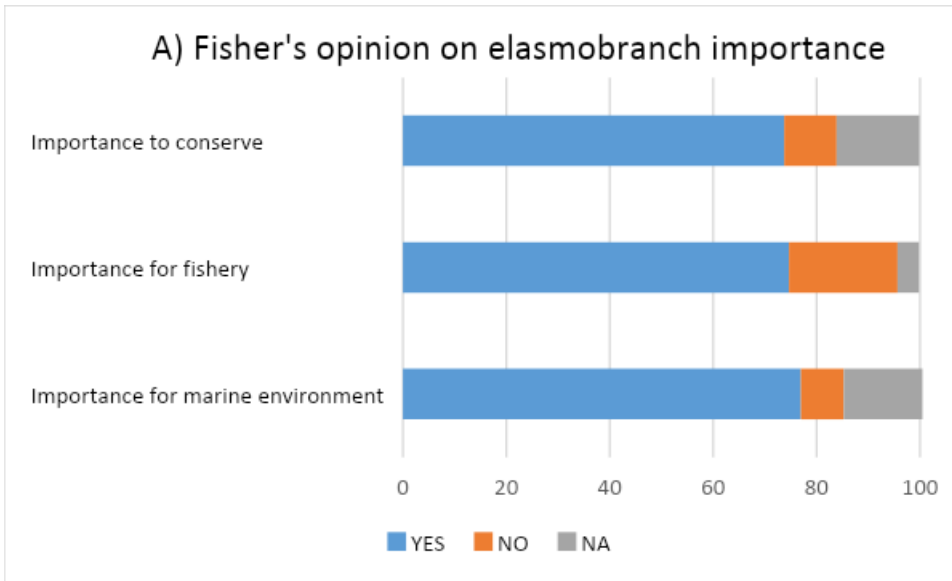
Fig. 9: *Elasmobranch importance in the marine environment, fishery revenue and conservation aspects according to fishers (12a).*

Measures for elasmobranch conservation proposed by fishers: spatial-temporal closure (Spatio-Temp), newborn release (newborn),

catch control (e.g., quotas, law enforcement) and others.

2.4 Discussion

This study has shown that LEK can be useful for collecting behavioural and ecological information on elasmobranch populations. LEK has already proven to be important for studying abundance trends of commercially exploited elasmobranchs in GSA 16 (Colloca et al., 2020) as well as several aspects of other marine species (Azzurro et al., 2011, 2019; Maynou et al., 2011), but this is the first time that the study of elasmobranch behaviour has been applied in Mediterranean GSAs collectively. As a general perspective on LEK-based information, the robustness of the data collected from fishers was supported by their long average fishing experience and by the fact that many fishers did not change fishing areas during their activity, and if changed, the new fishing areas were within the same GSA. As a consequence, the fishers provided long-term data referring to specific areas. Interviewed fishers used different fishing gear and were therefore able to provide information on different species. Their ability to provide a reliable picture is supported by the high correspondence of the species distribution and changes over time reconstructed by fishers' LEK with those evaluated with scientific and fishery surveys in the Mediterranean Sea (Ferretti et al., 2013; Barausse et al., 2014; Colloca et al., 2017; Follesa et al., 2020; Ramirez-Amaro et al., 2020). For instance, according to LEK, *Mustelus* spp., *S. acanthias* and *S. squatina* were widely caught in several GSAs in the past. The decline of these species has already been documented in the Mediterranean Sea (Ferretti et al., 2013; Fortibuoni et al., 2016; Colloca et al., 2017; Gordon et al., 2019). Similarly, pelagic species such as *Alopias* spp.*, Lamna nasus, P. glauca* and *I. oxyrinchus* have recently experienced drastic decreases, and the LEK results indicated similar trends (Ferretti et al., 2008). Conversely, other species have remained at stable levels or are less affected by fishery exploitation, as is the case for *G. melastomus*, *S. canicula* and *R. clavata* in the western Mediterranean Sea (Abella et al., 2017; Ramirez-Amaro et al., 2020) and in the northern Ionian Sea (Serena, 2014; Ricci et al., 2021). If the correspondence between LEK and scientific data is important to evaluate LEK reliability, fishers provided new insights for other species, both commercial, such as *D. oxyrinchus* in GSA 6, and even noncommercial, such as *A. bovinus*, in GSA 19.

2.4.1 Species seasonality

The present study suggests that LEK can be an important source of information about seasonal migrations of species in the Mediterranean Sea. Several species were indicated by fishers as showing seasonality in catches as the likely consequence of seasonal movements. Moreover, some differences in migratory patterns among areas emerged. It could have been expected that fishers' knowledge of fish movement could be related to their experience at sea. Our analyses did not show any influence of fishers' experience on this information, highlighting either that our sample included experienced fishers or that even fishers with a short time of activities can have a clear perception of fish movements. Before discussing the results for the different species, it is also worth noting that the distribution of fishing efforts may influence the perceptions of catch seasonality if fishing grounds change seasonally. While this point was not highlighted by fishers, it could limit the reliability of some information, especially in some areas, such as the largest Mediterranean subbasins (e.g., the central, western and eastern Mediterranean areas), and therefore the comparability between areas. However, this issue appears to be less relevant for other areas, such as the Adriatic Sea, where fishery distribution does not show a wide spatial difference across seasons (Russo et al., 2020). In addition, to overcome this potential bias, the 25% answer threshold was set to establish coherence across the fishers' replies. In general, both static and active fishing gear may be used in different areas to follow fish movements; therefore, the use of different fishing gear is not expected to provide differently biased results.

2.4.2 Species seasonality of demersal species

Migration patterns in the Adriatic Sea have already been suggested for some demersal species (Fortuna et al., 2010; Bonanomi et al., 2018). The application of seasonality in catches as an indicator of seasonal migrations has been proposed for *Mustelus* spp., *S. acanthias* and *M. aquila* (Bonanomi et al., 2018). In the present study, fishers indicated similar seasonality patterns for these species as well.

The LEK from the two sides of the Adriatic Sea provided a more comprehensive picture of the movements of *Mustelus* spp. and *M. aquila* with respect to the available data. For these species, indeed, in the Adriatic Sea, two patterns emerged from LEK: a north-south pattern in the northern Adriatic, as suggested by Bonanomi

et al. (2018), and an off-inshore pattern in the southern Adriatic. Interestingly, *Mustelus* spp. seasonality at the Montenegro site may indicate a different migration pattern compared to those indicated at the northern Adriatic sites, similar to what was found for other species whose movement is influenced by the abiotic characteristics of the Adriatic Sea (Papetti et al., 2013). The greater depth of the southern Adriatic, in comparison to the northern-central Adriatic, may favour winter migration into deep waters with mild temperatures, which are more favourable for shark physiological needs such as metabolism and somatic growth (Schlaff et al., 2014). Moreover, for the *Mustelus* spp. no clear evidence of seasonality emerged in GSA 16. Differences in movement behaviours among areas are not unexpected, considering that the common drivers of such migrations, such as environmental factors, may indeed vary in their seasonality among areas. For instance, compared to the Adriatic Sea, the Strait of Sicily does not have a strong seasonal variation in sea water temperature (Bethoux, 2003).

A comparison of the results between the two coastal areas of the Adriatic Sea showed seasonality but did not reveal any clear pattern (i.e., north-south or west-east movement) in the migrations of *S. acanthias*. In the Atlantic Ocean, this species can have different movement ranges in different study areas (Carlson et al., 2014), and the distribution of *S. acanthias* has been found to be affected by bottom temperatures and prey availability (Sagarese et al., 2014).

In addition to providing new information on species known to perform migrations, LEK also provided new insights for some less-studied species in the Adriatic Sea and the Mediterranean Sea. The interview results suggested that *Raja* spp., *S. stellaris* and *S. canicula* may not undertake migrations in Mediterranean GSAs, while the seasonality that emerged for *P. violacea* is consistent with its migratory behaviours reported in the Atlantic Ocean (Weidner et al., 2014). For what concerns *Torpedo* spp., little is known about its movement, and more research is therefore required.

2.4.3 Species seasonality of pelagic species

This study suggests the existence of seasonality in several GSAs (6, 9, 10, 19) for some pelagic species, such as *P. glauca* and *I. oxyrinchus*. The movement of *P. glauca* has been largely studied in the Atlantic and Pacific

oceans (Kohler et al., 1998; 2002; Mucientes et al., 2009; Stevens et al., 2010; Vandeperre et al., 2014). Long-term migrations of *P. glauca* have been documented by tagging studies, with some individuals moving from the Atlantic Ocean to the Mediterranean Sea and within the Mediterranean (Kohler et al., 1998; 2002). In particular, in the Mediterranean Sea, where mainly immature individuals were tagged, only short movements were observed for the two species (Kohler et al., 2002), supporting the observation that juveniles display residency for at least two years after birth within the same area (Vandeperre et al., 2014). Similarly, *I. oxyrinchus* showed seasonal movements in the Atlantic Ocean (Rogers et al., 2015), while no data on this species are available for the Mediterranean Sea. Considering the migratory behaviour of these two species, it is conceivable that the seasonality found in this study may be due to migrations. Considering the lack of information on this issue, the results of this study encourage future studies on these two species in the Mediterranean Sea to reconstruct their movements as an accessory approach to a monitoring scheme focused on large elasmobranchs (Mancusi et al., 2020).

2.4.4 Aggregation

This study allowed the investigation of elasmobranch aggregations (i.e., conspecific and high-density aggregation during a specific time of the year) in the Mediterranean Sea. In addition to sporadic and opportunistic events published in the literature, the occurrence, frequency, and species-specific characteristics of aggregations have often been overlooked for many elasmobranch species. In this study, LEK provided information on species known to perform aggregations but also some initial insights into the aggregation occurrence of six species (*P. violacea*, *M. aquila*, *G. melastomus*, *S. blainville*, *T. marmorata*, *E. spinax*) and one genus (*Raja* spp.) for which, to our knowledge, no previous data are available.

2.4.5 Aggregation - Demersal species

The occurrence of aggregations of *Mustelus* spp. and *S. acanthias* are known in the Atlantic Ocean (Smale & Compagno, 1997; da Silva et al., 2013; Carlson et al., 2014) and hypothesised also in the Adriatic Sea (GSA 17) (Bonanomi et al., 2018) and the Strait of Sicily (GSA 16) (Colloca et al., 2017). LEK confirmed the occurrence of aggregations in those GSAs and indicated new aggregation areas for *Mustelus* spp. in other

GSAs (9 and 19). For *S. acanthias*, the aggregation areas (GSAs 17 and 28) indicated by LEK correspond to the areas where the species is mostly found (Serena et al., 2009; Follesa et al., 2020). However, to our knowledge, this is the first report of aggregation of *S. acanthias* in the Mediterranean Sea. *S. canicula* aggregations have not been reported in Mediterranean sites (Wearmouth et al., 2012). Nonetheless, LEK suggests the occurrence of such aggregations in 4 GSAs (6, 9, 11, 16), in close correspondence with the main Mediterranean distribution of the species (Follesa et al., 2020). Among Mediterranean batoids, aggregation records have been published only for *D. pastinaca* in the Levantine Sea (GSA 27) (Chaikin et al., 2020), but this species occurrence has been documented in other coastal areas of the central Mediterranean Sea (GSA 16, Tiralongo et al., 2020). This study highlighted two more GSAs (11 and 17) where *D. pastinaca* aggregations occur according to LEK.

For the first time, specific descriptions and temporal occurrences of aggregations were recovered for four species in the Mediterranean Sea. In the Adriatic Sea, considering LEK in only three time frames (1960–1980, 1980–2000 and 2000-present), the catch and sight frequencies of aggregations decreased for *Mustelus* spp. and *S. acanthias,* likely due to fishery-driven decline. The general decline of these species was highlighted by fishers in the interviews and is consistent with the findings of Barausse et al. (2014). The opposite trend noted for *Raja* spp. may reflect an increase in abundance observed in landings (Clodia database, 2020) after a period of documented decline (Jukić-Peladić et al., 2001), but more studies are necessary to confirm this increase. In other GSAs, *Mustelus* spp. Aggregation occurrence and the number of individuals decreased over time, as expected due to recent exploitation-driven decline (Ligas et al., 2013; Colloca et al., 2017). In contrast, *Raja* spp. and *S. canicula* did not show substantial changes in aggregation occurrence; this was expected since no decreasing trend in abundance was observed over time in the western Mediterranean (Ramírez-Amaro et al., 2020; Follesa et al., 2020).

Based on LEK interviews, the reported size and sex composition of individuals in aggregations (large individuals, pregnant females) may support the role of aggregations in reproductive scope (*Mustelus* spp., *S. acanthias* and *Raja* spp.). In these species in other geographic areas, aggregations have been previously

reported to be composed of adults of both sexes (Jacoby et al., 2012). On the other hand, in *M. aquila* and *S. canicula,* the reported occurrence of aggregating individuals of different sizes may support the role of aggregations as a defence against predators (Sadovy de Mitcheson & Colin, 2011).

In commercially exploited species, such as *Mustelus* spp. and *Raja* spp., LEK has shown how well fishers know this behavioural-reproductive driven phenomenon. Therefore, fishers can effectively exploit such aggregations, further impacting species abundance and leading to their decline, as supported by the decline in the aggregating species *Mustelus* spp. (Ligas et al., 2013; Colloca et al., 2017). On the other hand, if fishers report the frequent occurrence of large aggregations for a species, this may indeed be an indicator of a good conservation status, as suggested for *S. canicula* in non-Adriatic GSAs (6, 9, 11, 16) (Abella et al., 2017; Ramírez-Amaro et al., 2020).

2.4.6 Aggregation - Pelagic species

Among pelagic sharks, *P. glauca* has been observed to aggregate in other oceans, such as the aggregation of adult individuals around seamounts (Litvinov, 2007) and of juveniles in coastal areas (Litvinov, 2006; Serena & Silvestri, 2018). In the Adriatic (GSAs 17 and 18) and Ionian Seas (GSA 19), aggregations of *P. glauca* have already been observed (Clò & Bianchi, 1997; Pomi et al., 1997). The present study provides, for the time, indications of *P. glauca* aggregations in GSAs 11 and 9.

Carcharhinus plumbeus has been seen aggregating in Boncuk Cove (Turkey) (Filiz et al., 2019) and forming seasonal aggregations in Israel (Barash et al., 2018) and Lampione Island (Sicily) (Cattano et al., 2021). In addition, GSAs 10 and 19 were recognised as previously unreported aggregating areas of *C. plumbeus* by LEK in the present study.

Aggregating areas of *C. maximus* have been proposed in the Ligurian Sea (Northern Tyrrhenian) and the Balearic region (Mancusi et al., 2005). GSA 19 was indicated as an additional aggregating area where the presence of this species has been indeed reported (Carlucci et al., 2014).

In GSA 19, *S. zygaena* aggregation was indicated by fishers, as already documented (Sperone et., 2012). In addition, aggregation of this species was reported in the central Mediterranean (Lampedusa Island) (Bigelow & Schroeder, 1948).

Among *Mobula* species, the aggregation phenomenon is well known (Ward-Paige et al., 2013). In the Mediterranean Sea, studies on the abundance and habitat associations of *M. mobular* have already suggested the occurrence of aggregations (Notarbartolo di Sciara et al., 2015). Winter aggregations have also been observed in the Levantine Sea (Gaza strip) for mating (Couturier et al., 2013; Abudaya et al., 2018). Overall, these published studies confirmed the LEK findings for this species.

2.4.7 Use of LEK in elasmobranch behavioural research

The use of LEK for the study of the behavioural traits of elasmobranchs has some shortcomings and limitations. First, LEK might tend to suffer cognitive biases, such as judgement deviation, shifting baseline syndrome (Pauly, 1995), changes in fishing effort over time, or difficulties in reconstructing past scenarios, as some elasmobranch species are not the target but accidental catch for fisheries. Second, fishers' attention may be more focused on more commercially relevant species, and biological traits may not be easily observed. For instance, for several species, sex composition information based on LEK may suffer from erroneous attribution of sex. However, in elasmobranchs, sex is easily determined based on external prominent copulatory organs called as claspers, which are extensions of the posterior bases of the pelvic fins (Musick & Ellis, 2005), and indeed, interviewed fishers provided information on sex in aggregations. Among the species reported by fishers, clasper presence may go unnoticed in *S. canicula,* since they are enwrapped in pelvic fins (ICES, 2013). Therefore, sex misidentification for this species may indeed occur and explain a possible inconsistency between the indication of mixed-sex aggregations by LEK and the well-known sexual segregation of this species (Wearmouth et al., 2013; Finotto et al., 2015). Third, after the progress in elasmobranch taxonomy by new genetic tools (see, for instance, Cariani et al., 2017; Marino et al., 2018), interviewers and fishers, depending on the geographical area, might have different taxonomy expertise about the studied species. Fourth, the quality of the LEK results may strongly depend on the level of

established trust between the interviewers and fishers. All these factors should be carefully considered when using LEK (Begossi et al., 2019). To minimise these limits and collect more reliable data, in our study, we chose to perform some analyses only above a certain minimum sample size. In addition, we used available scientific data to corroborate the fishers' information, as shown above. Moreover, the interviewers were scientists working on fishery and/or elasmobranch species. Finally, the structure of a questionnaire survey should always be examined with regard to target questions and sampling locations. The trade-off between the usage of open and specific questions may dramatically affect the objectives of a study in terms of the degree of freedom as well as the quality of the results (Azzurro et al., 2019). In this questionnaire survey, some questions were discarded due to the inconsistency and discontinuity of the replies.

2.4.8 Fishers' perceptions of elasmobranch value

Halting or reversing the dramatic situation of the decline in fishery resources requires a co-management plan based on a bottom-up approach to provide practical and feasible measures (Moller et al., 2004).

This study, in collecting fishers' opinions on shark importance, value, conservation and management, provides key information for understanding the feasibility of fishers' involvement in the management process. The ecological importance of sharks and their relevance for fisheries was highlighted by fishers' answers. In fact, shark and ray meat consumption is still an important category of sea-origin food in Mediterranean countries (FAO 2020), so the demand is still high enough to make this resource commercially valuable. Not only were more than half of the interviewed fishers in favour of conservation measures, but most of them had a pro-active and collaborative attitude to propose their own ideas on shark and ray management plans that go beyond the existing national and international protection and management regulations. Interestingly, in addition to general catch control, fishers indicated some more specific management strategies, such as the temporal closure of specific areas hosting vulnerable stages (e.g., reproductive areas) and the release of newborns. These answers confirm the knowledge of fishers about the biology of these species. Moreover, some fishers voluntarily and regularly release newborns, at least in some areas, such as the northern Adriatic Sea (Barbato and Mazzoldi, personal observation). The protection of

areas used during vulnerable stages of elasmobranch life is recognised to be an effective tool by the scientific world (e.g., Martins et al., 2018). On the other hand, the conservation efficacy of newborn release should be evaluated considering the population dynamics of the species and explored along with other management strategies (Prince, 2002). Although the efficacy of the management actions proposed by fishers might not be optimal, these strategies should be considered and carefully evaluated.

These results highlight that it may be possible to recognise priority measures in collaboration with fishers, both for commercial elasmobranch species, which are more sensitive to exploitation, and for nontarget and commercially less important species.

Acknowledgements

Bibliography

Abella, A., Mancusi, C., Mannini, A., Serena F., 2017a. *Galeus melastomus*. In: *Sintesi delle conoscenze di biologia, ecologia e pesca delle specie ittiche dei mari italiani / Synthesis of the knowledge on biology, ecology and fishery of the halieutic resources of the Italian seas.* Sartor, P., Mannini, A., Carlucci, R., Massaro, E., Queirolo, S., Sabatini, A., Scarcella, G., Simoni, R. (Eds), *Biologia Marina Mediterranea*, 24 (Suppl. 1), 136-143.

Abella, A., Mancusi, C., Serena, F., 2017b. *Scyliorhinus canicula.* In: *Sintesi delle conoscenze di biologia, ecologia e pesca delle specie ittiche dei mari italiani / Synthesis of the knowledge on biology, ecology and fishery of the halieutic resources of the Italian seas.* Sartor, P., Mannini, A., Carlucci, R., Massaro, E., Queirolo, S., Sabatini, A., Scarcella, G., Simoni, R. (Eds), *Biologia Marina Mediterranea*, 24 (Suppl. 1), 157-164.

Abudaya, M., Ulman, A., Salah, J., Fernando, D., Wor, C. *et al.*, 2018. Speak of the devil ray (*Mobula mobular*) fishery in Gaza. *Review in Fish Biology and Fishery*, 28, 229-239.

Albuquerque, U. P., Ludwig, D., Feitosa, I. S., de Moura, J. M. B., Gonçalves, P. H. S., da Silva, R. H. *et al.*, 2021. Integrating traditional ecological knowledge into academic research at local and global scales. *Regional Environmental Change*, *21*(2), 1-11.

Anadón, J. D., Giménez, A., Ballestar, R., Pérez, I., 2009. Evaluation of local ecological knowledge as a method for collecting extensive data on animal abundance. *Conservation Biology*, 23(3), 617-625.

Anderson, M. J., Gorley, R. N., Clarke, K. R., 2008. *PERMANOVA+ for PRIMER: Guide to software and statistical methods*. PRIMER-E, Plymouth, 214 pp.

Azzurro, E., Moschella, P., Maynou, F., 2011. Tracking signals of change in Mediterranean fish diversity based on local ecological knowledge. *PLoS One*, 6(9), e24885.

Azzurro, E., Sbragaglia, V., Cerri, J., Bariche, M., Bolognini, L., *et al.*, 2019. The shifting distribution of Mediterranean fishes: a spatio-temporal assessment based on Local Ecological Knowledge. *Global Change Biology*, 25, 2779-2792.

Bada-Sánchez, E., Pérez-Jiménez, C., Martínez-Cruz, L. E., Méndez-Loeza, I., Sosa-Cordero, E., 2019. Fishery indicators during a predictable aggregation of Atlantic sharpnose sharks *Rhizoprionodon terraenovae* in the southern Gulf of Mexico: An alternative to assess a heterogeneous data-poor fishery. *Fisheries Management and Ecology*, 26, 354-364.

Bakker, J., Wangensteen, O. S., Chapman, D. D., Boussarie, G., Buddo, D. *et al.*, 2017. Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Scientific Reports*, 7(1), 1-11.

Barash, A., Pickholtz, R., Pickholtz, E., Blaustein, L., Rilov, G., 2018. Seasonal aggregations of sharks near coastal power plants in Israel: an emerging phenomenon. *Marine Ecology Progress Series*, 590, 145-154

Barausse, A., Correale, V., Curković, A., Finotto, L., Riginella, E. *et al.*, 2014. The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. *ICES Journal of Marine Science*, 71(7), 1593-1603.

Bargnesi, F., Lucrezi, S., Ferretti, F., 2020. Opportunities from citizen science for shark conservation, with a focus on the Mediterranean Sea. *The European Zoological Journal*, 87(1), 20-34.

Barría, C., Coll, M., Navarro, J., 2015. Unravelling the ecological role and trophic relationships of uncommon and threatened elasmobranchs in the western Mediterranean Sea. *Marine Ecology Progress Series*, 539, 225-240.

Bastari, A., Beccacece, J., Ferretti, F., Micheli, F., Cerrano, C., 2017. Local ecological knowledge indicates temporal trends of benthic invertebrates species of the Adriatic Sea. *Frontiers in Marine Science*, 4, 157.

Begossi, A., 2008. Local knowledge and training towards management. *Environment, Development and Sustainability*, 10(5), 591.

Begossi, A., Salyvonchyk, S., Glamuzina, B., de Souza, S. P., Lopes, P. F. *et al.* 2019. Fishers and groupers (*Epinephelus marginatus* and *E. morio*) in the coast of Brazil: integrating information for conservation. *Journal of Ethnobiology and Ethnomedicine*, 15(1), 53.

Berkström, C., Papadopoulos, M., Jiddawi, N. S., Nordlund, L.M., 2019. Fishers' Local Ecological Knowledge (LEK) on Connectivity and Seascape Management. *Frontiers in Marine Science*, 6, 130.

Bethoux, J. P., 2003. Long Term Data Series on Mediterranean Sea Temperature, Nutrients and Hydrology Changes. p. 301-306. In: *Mediterranean climate*. *Variability and trends.* Bolle, H.-J. (Ed). Springer, Berlin, Heidelberg, 372 pp.

Bigelow, H. B., Schroeder, W. C., 1948. Fishes of the western north Atlantic. Lancelets, cyclostomes and sharks. Memoir. *Sears Foundation for Marine Research,* 1(1), 1-576.

Bonanomi, S., Pulcinella, J., Fortuna, M. C., Moro, F., Sala, A., 2018. Elasmobranch bycatch in the Italian Adriatic pelagic trawl fishery. *PLoS ONE*, 1381, E0191647.

Braccini, M., Aires-da-Silva, A., Taylor, I., 2016. Incorporating movement in the modelling of shark and ray population dynamics: approaches and management implications. *Reviews in Fish Biology and Fisheries*, 26(1), 13-24.

Byrne, M.E., Cortés, E., Vaudo, J.J., Harvey, G.C.M.N. *et al.*, 2017 Satellite telemetry reveals higher fishing mortality rates than previously estimated, suggesting overfishing of an apex marine predator. *Proceedings of the Royal Society B*, 284, 20170658.

Cariani, A., Messinetti, S., Ferrari, A., Arculeo, M., Bonello, J.J. *et al.*, 2017. Improving the Conservation of Mediterranean Chondrichthyans: The ELASMOMED DNA Barcode Reference Library. *PLoS One,* 12(1), e0170244.

Carlson, A. E., Hoffmayer, E. R., Tribuzio, C. A., Sulikowski, J. A., 2014. The use of satellite tags to redefine movement patterns of spiny dogfish (*Squalus acanthias*) along the US east coast: implications for fisheries management. *PLoS One*, 9(7), e103384

Carlucci, R., Battista, D., Capezzuto, F., Serena, F., Sion, L., 2014. Occurrence of the basking shark *Cetorhinus maximus* (Gunnerus, 1765) (Lamniformes: Cetorhinidae) in the central-eastern Mediterranean Sea. *Italian Journal of Zoology*, 81(2), 280-286.

Casey, J. M., Myers, R. A., 1998. Near extinction of a large, widely distributed fish. *Science*, 281, 690–692.

Cattano, C., Turco, G., Di Lorenzo, M., Gristina, M., Visconti, G., Milazzo, M., 2021. Sandbar shark aggregation in the central Mediterranean Sea and potential effects of tourism. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(6), 1420-1428.

Chaikin, S., Belmaker, J., Barash, A., 2020. Coastal breeding aggregations of threatened stingrays and guitarfish in the Levant. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30(6), 1160-1171.

Chapman, D. D., Feldheim, K. A., Papastamatiou, Y. P., Hueter, R. E., 2015. There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. *Annual Review of Marine Science*, 7, 547-570.

Chin, A., Pecl, G., 2019. Citizen science in shark and ray research and conservation. Strengths, opportunities, considerations, and pitfalls. p. 299-317. In: *Shark research. Emerging technologies and applications for the field and laboratory*. Carrier, J. C., Heithaus, M. R., Simpfendorfer, C. A. (Eds). CRC Press, Boca Raton, FL.

Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth, 192 pp.

Clò, S., Bianchi, I., 1997. Osservazioni sul comportamento alimentare della verdesca, *Prionace glauca* (Linnaeus, 1758) (Chondrichthyes: Carcharhinidae). *Quaderni della Civica Stazione Idrobiologica di Milano*, 22, 79-93.

Clodia database, 2020. Database of Fishery Data from Chioggia, Northern Adriatic Sea. http://chioggia.biologia.unipd.it/banche-dati/

Colin, P. L., Sadovy, Y. J., Domeier, M. L., 2003. Manual for the Study and Conservation of Reef Fish Spawning Aggregations. Society for the Conservation of Reef Fish Aggregations Special Publication No. 1 (Version 1.0), 1-98+iii pp.

Colloca, F., Enea, M., Ragonese, S., Di Lorenzo, M., 2017. A century of fishery data documenting the collapse of smooth-hounds (*Mustelus* spp.) in the Mediterranean Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27(6), 1145-1155.

Colloca, F., Carrozzi, V., Simonetti, A., Di Lorenzo, M., 2020. Using local ecological knowledge of fishers to reconstruct abundance trends of elasmobranch populations in the Strait of Sicily. *Frontiers in Marine Science*, 7, 508.

Couturier, L. I., Bennett, M. B., Richardson, A. J., 2013. Mystery of giant rays off the Gaza strip solved. *Oryx*, 47(4), 480-480.

Dobson, A. J., Barnett, A.G., 2008. *An Introduction to Generalized Linear Models.* CRC Press, London, 301 pp.

Dulvy, N. K., Reynolds, J.D., 2002. Predicting extinction vulnerability in skates. *Conservation Biology*, 16, 440–450.

Dulvy, N. K, Fowler, S. L, Musick, J. A., Cavanagh, R. D., Kyne, P. M. *et al.*, 2014. Extinction risk and conservation of the world's sharks and rays. *eLife*, 3, e00590.

Dulvy, N. K., Allen, D. J., Ralph, G. M., Walls, R. H. L., 2016. The conservation status of Sharks, Rays and Chimaeras in the Mediterranean Sea [Brochure]. IUCN, Malaga, Spain, 14 pp.

Dulvy, N. K., Simpfendorfer, C. A., Davidson, L. N., Fordham, S. V., Bräutigam, A. *et al.*, 2017. Challenges and priorities in shark and ray conservation. *Current Biology*, *27*(11), R565-R572.

FAO 1990-2021. FAO Major Fishing Areas. MEDITERRANEAN AND BLACK SEA (Major Fishing Area 37). CWP Data Collection. In: FAO Fisheries Division [online]. Rome. Updated. [Cited 22 July 2021].

FAO, 2018. *The State of World Fisheries and Aquaculture 2020*. *FAO*, Rome, 206 pp.

Ferretti, F., Myers, R. A., Serena, F., Lotze, H. K., 2008. Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology*, 22(4), 952-964

Ferretti, F., Osio, G. C., Jenkins, C. J., Rosenberg, A. A., Lotze, H. K., 2013. Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact. *Scientific Reports*, 3, 1057.

Field, I. C., Meekan, M. G., Buckworth, R. C., Bradshaw, C. J. A., 2009. Susceptibility of sharks, rays and chimaeras to global extinction. *Advances in Marine Biology*, 56, 275-363.

Finotto, L., Gristina, M., Garofalo, G., Riginella, E., Mazzoldi C., 2015. Contrasting life history and reproductive traits in two populations of *Scyliorhinus canicula*. *Marine Biology*, 162, 1175-1186.

Filiz, H., 2019. Year-round aggregation of sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), in Boncuk Cove in the southern Aegean Sea, Turkey (Carcharhiniformes: Carcharhinidae). *Zoology in the Middle East*, 65(1), 35-39.

Follesa, M. C., Marongiu, M. F., Zupa, W., Bellodi, A., Cau, A. *et al.*, 2020. Spatial variability of Chondrichthyes in the northern Mediterranean. *Scientia Marina*, 83(S1), 81-100.

Fortibuoni T., Borme D., Franceschini G., Giovanardi O., Raicevich S., 2016. Common, rare or extirpated? Shifting baselines for common angelshark, *Squatina squatina* (Elasmobranchii: Squatinidae), in the Northern Adriatic Sea (Mediterranean Sea). *Hydrobiologia*, 772, 247-259.

Fortuna, C.M., Vallini, C., De Carlo, F., Filidei, E. Jr, Lucchetti, A. *et al.*, 2010. *Relazione finale del progetto "Valutazione delle catture accidentali di specie protette nel traino pelagico (BYCATCH III)." ISPRA*, Roma, 137 pp.

Gallagher, A. J., Kyne, P. M., Hammerschlag, N., 2012. Ecological risk assessment and its application to elasmobranch conservation and management. *Journal of Fish Biology*, 80(5), 1727-1748.

Gerhardinger, L. C., Hostim-Silva, M., Medeiros, R. P., Matarezi, J., Bertoncini, Á. A. *et al.*, 2009. Fishers' resource mapping and goliath grouper *Epinephelus itajara* (Serranidae) conservation in Brazil. *Neotropical Ichthyology*, *7*(1), 93-102.

Gordon, C.A., Hood, A.R., Al Mabruk, S. A. A., Barker, J., Bartolí, A., Ben Abdelhamid, S., Bradai, M.N. *et al.,* 2019. *Mediterranean Angel Sharks: Regional Action Plan*. The Shark Trust, United Kingdom. 36 pp.

Hammerschlag, N., Gallagher, A. J., Lazarre, D. M., 2011. A review of shark satellite tagging studies. *Journal of Experimental Marine Biology and Ecology*, 398(1-2), 1-8.

Heupel, M. R., Papastamatiou, Y. P., Espinoza, M., Green, M. E., Simpfendorfer, C. A., 2019. Reef shark science - Key questions and future directions. *Frontiers in Marine Science*, 6, 12.

Huntington, H. P., 2000. Using traditional ecological knowledge in science: methods and applications. *Ecological applications*, 10(5), 1270-1274.

ICES 2013. Report of the Workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL2) ICES CM 2012/ACOM:59. 11-14 December 2012, Lisbon, Portugal. 113 pp.

Jacoby, D. M. P., Croft, D. P., Sims, D. W., 2012. Social behaviour in sharks and rays: analysis, patterns and implications for conservation. *Fish and Fisheries* 13, 399-417.

Jukic-Peladic, S., Vrgoc, N., Krstulovic-Sifner, S., Piccinetti, C., Piccinetti-Manfrin, G. *et al.,* 2001. Long-term changes in demersal resources of the Adriatic Sea: comparison between trawl surveys carried out in 1948 and 1998. *Fisheries research*, *53*(1), 95-104.

Kessel, S. T., Chapman, D. D., Franks, B. R., Gedamke, T., Gruber, S. H. *et al.*, 2014. Predictable temperature-regulated residency, movement and migration in a large, highly mobile marine predator (*Negaprion brevirostris*). *Marine Ecology Progress Series, 514*, 175-190.

Kohler, N. E., 1998. NMFS cooperative shark tagging program, 1962-93: An atlas of shark tag and recapture data. *Marine Fisheries Review*, 60(2), 1-87

Kohler, N. E., Turner, P. A., Hoey, J. J., Natanson, L. J., Briggs, R., 2002. Tag and recapture data for three pelagic shark species: blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), and porbeagle (*Lamna nasus*) in the North Atlantic Ocean. *ICCAT Collective Volume of Scientific Papers*, 54(4), 1231-1260.

Ligas, A., Osio, G. C., Sartor, P., Sbrana, M., De Ranieri, S., 2013. Long-term trajectory of some elasmobranch species off the Tuscany coasts (NW Mediterranean) from 50 years of catch data. *Scientia Marina*, 77, 119–127.

Litvinov, F. F., 2006. On the role of dense aggregations of males and juveniles in the functional structure of the range of the blue shark *Prionace glauca*. *Journal of Ichthyology*, 46(8), 613-624.

Litvinov, F. F., 2007. Fish visitors to seamounts: aggregations of large pelagic sharks above seamounts. *Seamounts: Ecology, Fisheries and Conservation. Fisheries and Aquatic Resource Series, Blackwell Scientific*, 202-206.

Maguire, J.-J., Sissenwine, M., Csirke, J., Grainger, R., 2006. The state of the world highly migratory, straddling and other high seas fish stocks, and associated species. FAO Fisheries Technical Paper, No. 495. Rome: FAO. 2006. 77 pp.

Mancusi, C., Clò, S., Affronte, M., Bradaï, M. N., Hemida, F. *et al.*, 2005. On the presence of basking shark (*Cetorhinus maximus*) in the Mediterranean Sea. *Cybium*, 29(4), 399-405.

Mancusi, C., Baino, R., Fortuna, C., De Sola, L. G., Morey, G. *et al.,* 2020. MEDLEM database, a data collection on large Elasmobranchs in the Mediterranean and Black seas. *Mediterranean Marine Science*, 21/2,276-288.

Marino, I. A. M., Finotto, L., Colloca, F., Di Lorenzo, M., Gristina, M. *et al.,* 2018. Resolving the ambiguities in the identification of two smooth-hound sharks (*Mustelus mustelus* and *Mustelus punctulatus*) using genetics and morphology. *Marine Biodiversity*, 48(3), 1551-1562.

Martins, A.P.B, Heupel, M.R., Chin, A., Simpfendorfer, C. A., 2018. Batoid nurseries: definition, use and importance. *Marine Ecology - Progress Series*, 595, 253-267.

Maynou, F., Sbrana, M., Sartor, P., Maravelias, C., Kavadas S. *et al.*, 2011. Estimating trends of population decline in long-lived marine species in the Mediterranean Sea based on fishers' perceptions. *PLoS One*, 6(7), e21818.

Moller, H., Berkes, F., Lyver, P., Kislalioglu, M., 2004. Combining science and traditional ecological knowledge: monitoring populations for co-management. *Ecology and Society*, 9, 2.

Moreno, G., Dagorn, L., Sancho, G., Itano D., 2007. Fish behaviour from fishers' knowledge: the case study of tropical tuna around drifting fish aggregating devices (DFADs). *Canadian Journal of Fishery and Aquatic Science*, 64, 1517-1528.

Morgan, A. C., Burgess, G. H., 2005. Fishery-dependent sampling: total catch, effort and catch composition. In Management Techniques for Elasmobranch Fisheries, p. 182–200. In: *Fisheries Technical Paper 474.* Musick J. A., Bonfil R. (Eds). Food and Agriculture Organization, Rome.

Mucientes, G. R., Queiroz, N., Sousa, L. L., Tarroso, P., Sims, D. W., 2009. Sexual segregation of pelagic sharks and the potential threat from fisheries. *Biology Letters*, 5(2), 156-159.

Musick, J. A., Ellis, J. K., 2005. Reproductive evolution of Chondrichthyans, 45-79. In: *Reproductive biology and phylogeny of Chondrichthyes. Sharks, Batoids and Chimaeras*. Hamlett W. C. (Ed). Science Publishers, INC., Enfield, New Hampshire, 562 pp.

Myers, R.A., Worm, B., 2003. Rapid worldwide depletion of predatory fish communities. *Nature*, 423, 280–283

Notarbartolo di Sciara, G., Lauriano, G., Pierantonio, N., Cañadas, A., Donovan, G. *et al.*, 2015. The devil we don't know: investigating habitat and abundance of endangered giant devil rays in the North-Western Mediterranean Sea. *PloS One*, 10(11), e0141189.

Papetti, C., Di Franco, A., Zane, L., Guidetti, P., De Simone, V. *et al.*, 2013. Single population and common natal origin for Adriatic *Scomber scombrus* stocks: evidence from an integrated approach. *ICES Journal of Marine Science*, 70(2), 387-398.

Pauly, D., 1995. Anecdotes and the shifting baseline syndrome of fisheries. *Trends in Ecology & Evolution*, *10*(10), 430.

Peñaherrera-Palma, C., van Putten, I., Karpievitch, Y. V., Frusher, S., Llerena-Martillo, Y. *et al.*, 2018. Evaluating abundance trends of iconic species using local ecological knowledge. *Biological Conservation*, 225, 197-207.

Pomi C., 1997. Morphometric study of *Prionace glauca* (Linnaeus, 1758) in the Central and Northern Adriatic Sea. *Quaderni della CivicaStazione Idrobiologica di Milano*, 22, 159–161.

Prince, J. D., 2002. Gauntlet fisheries for elasmobranchs–the secret of sustainable shark fisheries. *Journal of Northwest Atlantic Fishery Science*, *35*, 407-416

R Studio Team, 2020. R Studio: integrated development for R. R Studio, PBC, Boston, MA URL https://www.Rstudio.com/.

Ramírez-Amaro, S., Ordines, F., Esteban, A., García, C., Guijarro, B. *et al.*, 2020. The diversity of recent trends for chondrichthyans in the Mediterranean reflects fishing exploitation and a potential evolutionary pressure towards early maturation. *Scientific Reports*, 10(1), 1-18.

Ricci, P., Sion, L., Capezzuto, F., Cipriano, G., D'Onghia, G., Libralato, S., Maiorano, P., Tursi, A., Carlucci R., 2021. Modelling the trophic roles of the demersal Chondrichthyes in the Northern Ionian Sea (Central Mediterranean Sea). *Ecological Modelling*, 444, 109468

Ricklefs, R.E., 1979. *Ecology* (2nd ed.). Chiron Press, New York, 966 pp.

Rogers, P. J., Huveneers, C., Page, B., Goldsworthy, S. D., Coyne, M. *et al.*, 2015. Living on the continental shelf edge: habitat use of juvenile shortfin makos *Isurus oxyrinchus* in the Great Australian Bight, southern Australia. *Fisheries Oceanography*, 24(3), 205-218.

Russell, M. W., Sadovy de Mitcheson, Y., Erisman, B. E., Hamilton, R. J., Luckhurst, B. E. *et al.*, 2014. Status Report – World's Fish Aggregations 2014. Science and Conservation of Fish Aggregations, California, USA. International Coral Reef Initiative, 12 pp.

Russo, E., Monti, M. A., Mangano, M. C., Raffaetà, A., Sarà, G. *et al.*, 2020. Temporal and spatial patterns of trawl fishing activities in the Adriatic Sea (Central Mediterranean Sea, GSA17). *Ocean & Coastal Management*, 192, 105231.

Sadovy de Mitcheson, Y., Colin, P. L. (Eds.), (2011). *Reef fish spawning aggregations: biology, research and management*. Fish and Fisheries Series, Vol. 35. Springer Science & Business Media, 621 pp.

Sagarese, S. R., Frisk, M. G., Cerrato, R. M., Sosebee, K. A., Musick, J. A. *et al.*, 2014. Application of generalized additive models to examine ontogenetic and seasonal distributions of spiny dogfish (*Squalus acanthias*) in the Northeast (US) shelf large marine ecosystem. *Canadian Journal of Fisheries and Aquatic Sciences*, 71(6), 847-877.

Schlaff, A. M., Heupel, M. R., Simpfendorfer, C. A., 2014. Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. *Reviews in Fish Biology and Fisheries*, 24(4), 1089-1103.

Serena F., 2005. *Field identification guide to the sharks and rays of the Mediterranean and Black Sea*. FAO Species Identification Guide for Fisheries Purposes. Rome, 97 p. 11 colour plates + egg cases.

Serena F., 2014. *Lo status degli elasmobranchi dei mari italiani (Elasmostat). I Programma Nazionale triennale della pesca e dell'acquacoltura 2007-2009 (prorogato a tutto il 2012). Progetto di ricerca: "7 – Tematica A3"*. Rapporto finale, 28 febbraio 2014. 321 pp.

Serena F. 2021. Elasmobranchs, 111-197. In: *Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries – A review.* Carpentieri, P., Nastasi, A., Sessa, M., Srour, A. (Eds) General Fisheries Commission for the Mediterranean. Studies and Reviews. No. 101. Rome, FAO, 320 pp. https://doi.org/10.4060/cb5405en.

Serena, F., Papaconstantinou, C., Relini, G., De Sola, L. G., Bertrand, J. A. *et al.*, 2009. Distribution and abundance of spiny dogfish in the Mediterranean Sea based on the Mediterranean International Trawl Survey Program. p. 139-149. In: *Biology and Management of Dogfish Sharks*. Gallucci, V. F., McFarlane, G. A., Bargmann, G. (Eds). American Fisheries Society, Bethesda, Maryland, USA, 435 pp. https://doi.org/10.47886/9781934874073.ch13.

Serena, F., Silvestri, R., 2018. Preliminary observations on juvenile shark catches as by-catch of the Italian fisheries with particular attention to the Tuscany coasts. *Proceedings of XX Congress of Codice Armonico*, 18b / Scientific Sez. Rosignano Marittimo (Li), 158-169

Serena, F., Abella, A. J., Bargnesi, F., Barone, M., Colloca F., Ferretti, F., Fiorentino, F. *et al.*, 2020, Species diversity, taxonomy and distribution of Chondrichthyes in the Mediterranean and Black Sea, *The European Zoological Journal*, 87:1, 497-536,

Sguotti, C., Lynam, C. P., García-Carreras, B., Ellis, J. R., Engelhard G. H., 2016. Distribution of skates and sharks in the North Sea: 112 years of change. *Global Change Biology*, 22 (8), 2729-2743.

da Silva, C., Kerwath, S. E., Attwood, C. G., Thorstad, E. B., Cowley, P. D. *et al.*, 2013. Quantifying the degree of protection afforded by a no-take marine reserve on an exploited shark. *African Journal of Marine Science*, 35(1), 57-66.

Smale, M. J., Compagno, L. J. V., 1997. Life history and diet of two southern African smoothhound sharks, *Mustelus mustelus* (Linnaeus, 1758) and *Mustelus palumbes* Smith, 1957 (Pisces: Triakidae). *South African Journal of Marine Science*, 18(1), 229-248.

Sperone E., Parise G., Leone A., Milazzo C., Santoro G. *et al.*, 2012. Spatiotemporal patterns of distribution of large predatory sharks in Calabria (Central Mediterranean, Southern Italy). *Acta Adriatica*, 53 (1): 13-24

Stevens, J. D., Bradford, R. W., & West, G. J., 2010. Satellite tagging of blue sharks (*Prionace glauca*) and other pelagic sharks off eastern Australia: depth behaviour, temperature experience and movements. *Marine Biology*, 157(3), 575-591.

Taylor, S. M., Braccini, J. M., Bruce, B. D., McAuley, R. B., 2018. Reconstructing Western Australian white shark (*Carcharodon carcharias*) catches based on interviews with fishers. *Marine and Freshwater Research*, 69(3), 366-375.

Tiralongo, F., Messina, G., Lombardo, B. M., 2020. Biologic aspects of juveniles of the common stingray, *Dasyatis pastinaca* (Linnaeus, 1758) (Elasmobranchii, Dasyatidae), from the Central Mediterranean Sea. *Journal of Marine Science and Engineering*, 8(4), 269.

Vandeperre, F., Aires-da-Silva, A., Fontes, J., Santos, M., Santos, R. S. *et al.*, 2014. Movements of blue sharks (*Prionace glauca*) across their life history. *PLoS One*, 9(8), e103538.

Ward-Paige, C. A., Davis, B., Worm, B., 2013. Global population trends and human use patterns of *Manta* and *Mobula* rays. *PloS One*, 8(9), e74835.

Wearmouth, V. J., Southall, E. J., Morritt, D., Sims, D. W., 2013. Identifying reproductive events using archival tags: egg-laying behaviour of the small spotted catshark *Scyliorhinus canicula*. *Journal of Fish Biology*, 82(1), 96-110.

Weidner, T. A., Cotton, C., Kerstetter, D. W., 2014. Habitat utilization and vertical movements of the pelagic stingray *Pteroplatytrygon violacea* (Bonaparte, 1832) in the Western North Atlantic Ocean using short-duration pop-up archival satellite tags" Marine & Environmental Sciences Faculty Proceedings, Presentations, Speeches, Lectures. 227

Chapter 3: Too young to die: mapping nursery area for early juveniles of the endangered sandbar shark (*Carcharhinus plumbeus*) to inform conservation in the Mediterranean Sea

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Abstract

The declining status of elasmobranch populations in the Mediterranean Sea is alarming. Reversing such dramatic trends requires tackling fishing pressure using different methods, including a fine-tuned spatial resolution conservation strategy, incorporating robust scientific evidence on fundamental ecological aspects such as spatial use at different life stages and its overlap with fishery grounds. Using a small-scale fishery, this study unambiguously identified a multiyear nursery area for early juveniles of the sandbar shark (*Carcharhinus plumbeus*), an endangered neritic species, in the northern Adriatic Sea. Then, we combined such novel information with a review on the presence and absence of newborns across Mediterranean subbasins to construct an ecological model predicting suitable nursery areas for early juveniles of the sandbar shark across the Mediterranean Sea. The model shows key areas, for this species recruitment, that critically overlap with fishing activities, where conservation actions should be implemented. This methodology could be extended to other neritic elasmobranchs, whose transboundary migratory nature requires spatially explicit conservation efforts.

Keyword

Adriatic Sea; nursery ; juveniles; conservation; elasmobranch; fishery; Mediterranean Sea.

3.1 Introduction

The decline in elasmobranchs represents a global environmental concern. Fishing pressure has caused a worldwide decrease in the abundance of cartilaginous fishes in past decades (Sguotti et al., 2016; Pacoureau et al., 2021) and centuries (Fortibuoni et al., 2010; Lotze et al., 2011). Elasmobranchs are prone to overfishing, either as targets or bycatches, due to their characteristic life history traits, such as large size, slow growth, late sexual maturity, and low fecundity, which increase their catchability, especially before reproduction, with respect to most bony fish (Dulvy et al., 2017). Typical elasmobranch behaviours such as long migrations, aggregations, philopatry and site fidelity further increase their vulnerability to fishing pressure (Chapman et al., 2015). Despite the pervasive disappearance of these predatory fish worldwide, its ecological consequences, including far-reaching trophic cascades (Myers et al., 2007), are largely unexplored, management actions are rarely enforced (Milazzo et al., 2021), and the urgent search for sustainable conservation solutions remains incomplete (Dulvy et al., 2017; Pacoureau et al., 2021). For neritic sharks, a promising conservation approach is an integrative management strategy that includes the identification and protection of birthing and nursery areas as well as subadult life stages (Kinney & Simpfendorfer, 2009).

The Mediterranean Sea experienced one of the longest-lasting and strongest declines in elasmobranch abundance worldwide (Fortibuoni et al., 2010; Lotze et al., 2011; Barausse et al., 2014), especially for large sharks (Ferretti et al., 2008). Environmental management in the Mediterranean Sea is complex: here biodiversity, disproportionately high for the surface of this sea, faces multiple threats, including fisheries, eutrophication, pollution, transportation, habitat loss and degradation, climate change and alien species (Coll et al., 2012; Lotze et al., 2011). These threats are mostly related to human activities along the coasts, which have been inhabited for millennia, explaining why the Mediterranean elasmobranch decline dates so far back into history (Fortibuoni et al., 2010; Lotze et al., 2011). The institutional framework is also fragmented: the Mediterranean, at the crossroad of three continents, is bordered by 24 sovereign countries, a potential obstacle to the implementation of joint conservation strategies. Gathering robust ecological information to

make informed decisions represents a precondition to achieve conservation goals in the face of this complexity.

Here, we contribute to building a better knowledge base for the conservation of the sandbar shark (*Carcharhinus plumbeus*), a neritic large-body species classified as endangered in the Mediterranean by the International Union for Conservation of Nature (Ferretti et al., 2016).

Biological information on this placental viviparous shark is incomplete in this basin: the reproductive and pupping seasons occur in summer (Saïdi et al., 2005), but only opportunistic and scattered research have been published on the occurrence of early juveniles and nursery area identification (Costantini & Affronte, 2003; Bradai e al., 2005; Başusta etal., 2021). Based on a systematic data collection at fine-spatial scale, we document the existence of an important nursery area for early juveniles of this species in the northern Adriatic Sea, one of the most human-impacted Mediterranean subbasins (Lotze et al., 2011; Barausse et al., 2014). We then review the scientific literature on the presence and absence (PA) of nursery for early juveniles in the Mediterranean Sea and combine this information with our novel information to construct an ecological model predicting the probability of suitable nursery area for early juveniles in the Mediterranean Sea. We have focused on early juvenile occurrence since the birthing sites of the sandbar shark are often found within nursery areas and since older juveniles may have a wider movement range than younger ones (Conrath & Musick, 2010), possibly leading to an overestimation of nursery extension. In the western Atlantic Ocean, juveniles up to 10 years old showed site fidelity and natal homing for foraging purposes after seasonal migration in more favourable conditions (Merson &Pratt, 2001; Conrath & Musick, 2010). Furthermore, secondary nursery sites were reported for *C. plumbeus*, presumably the results of break-off groups from migrating adults (Baremore & Hale 2012). The potential nursery sites for early juveniles inferred from this study may cover primary and secondary nursery areas.

3.2.Materials & Methods

3.2.1. Study Area

The Mediterranean Sea is a semi-enclosed basin in the temperate climate zone displaying high heterogeneity in chemico-physical and biological features. Hydrodynamics are influenced by the cold and inflowing Atlantic waters with lower salinity, while river inputs affect regional primary production patterns. The annual mean sea surface temperature increases from north to south and from west to east. Steep and narrow shelves are found in the southern Mediterranean, except in the Gulf of Gabès, whereas extended shelves are present in the northern subbasins, such as the northern Adriatic Sea (Coll et al., 2012). In the northern Adriatic, the shallow depth coupled with intense river discharges drives high nutrient availability, which, in turn, sustains strong planktonic productivity fuelling nekton and benthic communities, which are heavily exploited by semi-industrial and artisanal fisheries (FAO, 2018).

3.2.2 *Adriatic nursery sites*

To identify possible nursery areas for early juveniles of *C. plumbeus*, we used data from small-scale fisheries (SSF, "fishing vessels of an overall length of less than 12 m and not using towed gear", FAO 2018) in the north-western Adriatic Sea. This fleet segment comprises low-tonnage vessels (1-4 GT) with seasonal turnover of passive fishing gear. Gillnets are deployed from April to January, mainly targeting the common sole (*Solea solea*), with peak effort occurring in August (Grati et al., 2018). SSF data collection was carried out in Cervia (Emilia-Romagna region, Italy, Fig.2) in July-August 2019 and July-August 2020 through (*i*) a daily survey to record the number of fishing trips (i.e., number of fishers who went out to fish) and, for each landed shark, its capture coordinates, total length (TL), sex, and umbilical scar presence (Costantini & Afronte, 2003); and (*ii*) the passive monitoring of fishing effort distribution of gillnets by a GPS tracker (GARMIN® eTrex20) in one of the seven fishers operating in 2019 and one of the five in 2020.

All landed sharks were identified using morphological characters (Ebert et al., 2020). The number of landed sharks was corrected for the high variability of SSF fishing practices (Humphries et al., 2019) by dividing it by the number of fishing trips to calculate the landing per unit effort (LPUE), a relative abundance indicator.

Biases in the sex ratio and differences in the LPUE or TL of the landed sharks were tested with χ^2 and Mann-Whitney tests, respectively. To reconstruct the fishing effort distribution and generate the captured shark distribution through a minimum convex polygon approach, the GPS coordinates of each gillnet start point and end point were superimposed to catch coordinates using QGIS v3.1 (www.qgis.org). Such a distribution was plotted against potential abiotic and biotic predictors averaged over July-August 2019 and 2020: sea surface temperature (SST), net primary production (NPP), turbidity (KD), sea surface salinity (SAL) and current velocity and direction (CUR), taken from the Copernicus Marine Environment Monitoring Service (Annex II, Tab. S2.1).

3.2.3 Nursery areas in the Mediterranean Sea

To collect PA data on the location of nursery of early juveniles of sandbar sharks in the Mediterranean Sea, we performed a review of peer-reviewed and grey literature using the following criteria to construct a birthing PA dataset. The selected studies (Tab. S1 and Fig. S1) should (*i*) report elasmobranch catch or bycatch; (*ii*) be conducted during summer, the birthing season of *C. plumbeus* (Saïdi et al., 2005), in the last 20 years; (*iii*) describe sampling effort with monthly replicates; and (*iv*) be based on fisheries using trammel nets (GTR), gillnets (GNS) or set/drifting longlines (LLS/LLD, with hook sizes N. 2 and 3, which in our experience allow to catch neonates) or scientific surveys such as underwater visual census and baited underwater video. To qualify as a nursery for early juveniles, the occurrence of at least one neonate considered an early juvenile had to be reported with an open fresh or partially open scar given the potential healing capacity of umbilical scar to last 30 days in congeneric species (Chin et al., 2015); while the occurrence of larger juveniles or adults was not considered. Following the selection of studies reporting nursery for early juveniles, the published maps with the study results were georeferenced by QGIS3 to obtain the latitude and longitude coordinates for PA data (Annex II, Fig.S1).

The areas of each georeferenced PA maps from selected studies were divided into regular point grid (0.0277° spacing, ~3 km) and each point was attributed either a value of zero for absence or one for presence. The spatial data presented in this study regarding the Adriatic birthing site were included in the PA dataset.

To characterize the environmental conditions at each PA point, abiotic and biotic factors (SST, SAL, CUR, KD and NPP; Tab. S2.2) were obtained taken from the Copernicus Marine Environment Monitoring Service in raster format which were matched to the PA areas. The function 'extraction' from the 'raster' R package (Hijmans, 2021) was used for matching the PA dataset with environmental conditions within the sampling period by averaging the monthly values for June-August months and for each sampling year of the selected studies (Tab. S2.2). For those areas not covered by the environmental dataset resolution (e.g., too close to the coast or inside narrow bays), values were taken from the nearest available cell. Bottom depth and slope were also extracted for each point to describe the PA sites (Tab. S2.2).

3.2.4 Modelling and validation of Mediterranean nurseries

Given their strongly asymmetric distributions, slope, SAL, KD and NPP were ln-transformed prior to analysis only if distribution would become normal after ln-transformation. Point-density plots were drawn for each variable to explore their distribution in the PA sites. Collinear variables (Pearson's r>0.8) were excluded from subsequent analyses (Zuur et al., 2010). To investigate how the suitability of birthing areas could be shaped by environmental features, a generalized lineal model (GLM), shown in Equation 1, and a generalized additive model (GAM), shown in equation 2, were fitted to the PA data as a binary response variable, using different combinations of abiotic and biotic factors as predictors:

$$\text{Eq. 1} \;\; logit(PA) = \beta_0 + \beta_1 x_1 + \cdots + \beta_p x_p$$

$$\text{Eq. 2} \;\; logit(PA) = \beta_0 + \beta_1 x_1 + f_1(x_2) + \cdots + \beta_p x_p + f_m(x_m)$$

In case of GAM, a smoothing function (f in Eq.2) was applied to SST, SAL and NPP according to the plot-density of PA and on biological sense otherwise a linear relation was kept for CUR, slope and depth. The latter liner parameters were standardized prior to building the model (Zuur et al., 2009). To find the right amount of smoothing and avoid overfitting each smoother was first modelled alone with the PA data and smoothing function inspected for biological sense. GAM was performed on mgvc R package (Wood, 2011)

By the 'dredge' function in the MuMIn R package (Barton, 2020), all possible combinations of predictors were calculated, and AIC was computed for each resulting model to select the best predictor combination (Burnham & Anderson 2002). The best GLM or GAM model, with the lowest AIC (and, in case of models with similar AIC's, the smaller number of predictors according to the principle of parsimony), was selected.

To choose between the two types of regression model, ten random partitions were created using *caret* R package (Kuhn, 2021) resulting in an 80% subset. On each partition, the best GLM and GAM in predictor combinations was applied, and AIC was computed to compare the performance on the same subset.

To validate the accuracy, type I and II errors for the assessment of model performance, the independent variables from the remaining 20% entries (~ 3'128) of the same ten subsets, were used to predict the PA and then to compare it to the observed PA from the same 20% subsets. The resulting accuracy, type I and type II errors were averaged from the ten-validation models and their standard deviation was computed as well.

 The best-fitting model was used to predict the probability of occurrence of birthing sites in the whole Mediterranean Sea. The averaged summer values for abiotic and biotic factors over 2016-2020 were used as inputs for the best model to calculate the birthing probability over a 0.0277°-cell grid across the Mediterranean Sea. This time interval was chosen long enough to average short-term environmental oscillations out, and recent enough to be representative of the typical values of abiotic and biotic factors at present and hence to estimate potential birthing areas which remain valid nowadays. Analyses were run in RStudio (R studio Team 2020).

3.3. Results

3.3.1. Nursery sites in the north-western Adriatic Sea

The total number of sandbar sharks caught by Cervia's SSF was 20 in 2019 and 14 in 2020. All individuals were categorized as newborns (presence of open fresh or partially healed umbilical scars), except for two juveniles, of which there was one in each year (completely healed scar, Fig. 1). The TL distribution did not differ between years (W=99, p=0.109). The sex ratio was balanced in each year (χ^2=0.40, p=0.502 in 2019, χ^2=0.57, p=0.789 in 2020). Because of gear turnover and weather conditions, fishing occurred on 69% (29 over 42) and 68% (37 over 59) of the monitored days in 2019 and 2020, respectively.
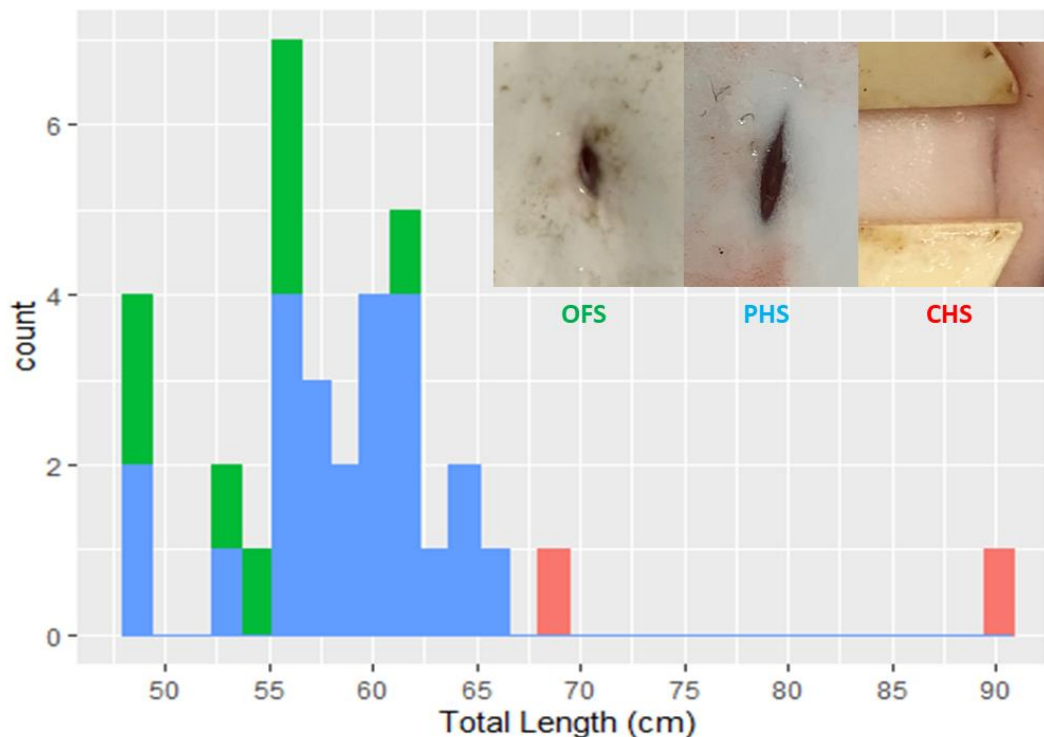
Figure 1: Total length frequency distribution of the sandbar sharks recorded in 2019 and 2020 in Cervia's landings. Colours indicate the umbilical scar condition of each individual: green for an open fresh scar (OFS) where the opening is present through the skin and superficial muscle tissues, blue for a partially healed scar where only the two skin edges are unhealed (PHS), and red for a completely healed scar (CHS).

The number of operating fishers varied among days, up to a maximum of 7 in 2019 and 5 in 2020, and the length of gillnets ranged from 1.4 km to 6 km depending on the fisher's habits. The gillnet soaking time when a shark was caught was, on average, 10.6±4.2 hours (range 6-15 hours). The daily LPUE in the two years did not differ (W=697.5, p=0.121). Based on the fishing distribution of 101 monitored trips and catch coordinates data, captured newborn and juvenile sharks were distributed within 6 nautical miles (NM) eastward from the Italian shore and extended from north to south between Cervia and Marina di Ravenna (MDR). The area of catches has shallow and warm waters and is downstream of the productive Po River plume but is located along a calm current front. Furthermore, this nursery area showed a higher turbidity than the offshore waters (Fig. 2).
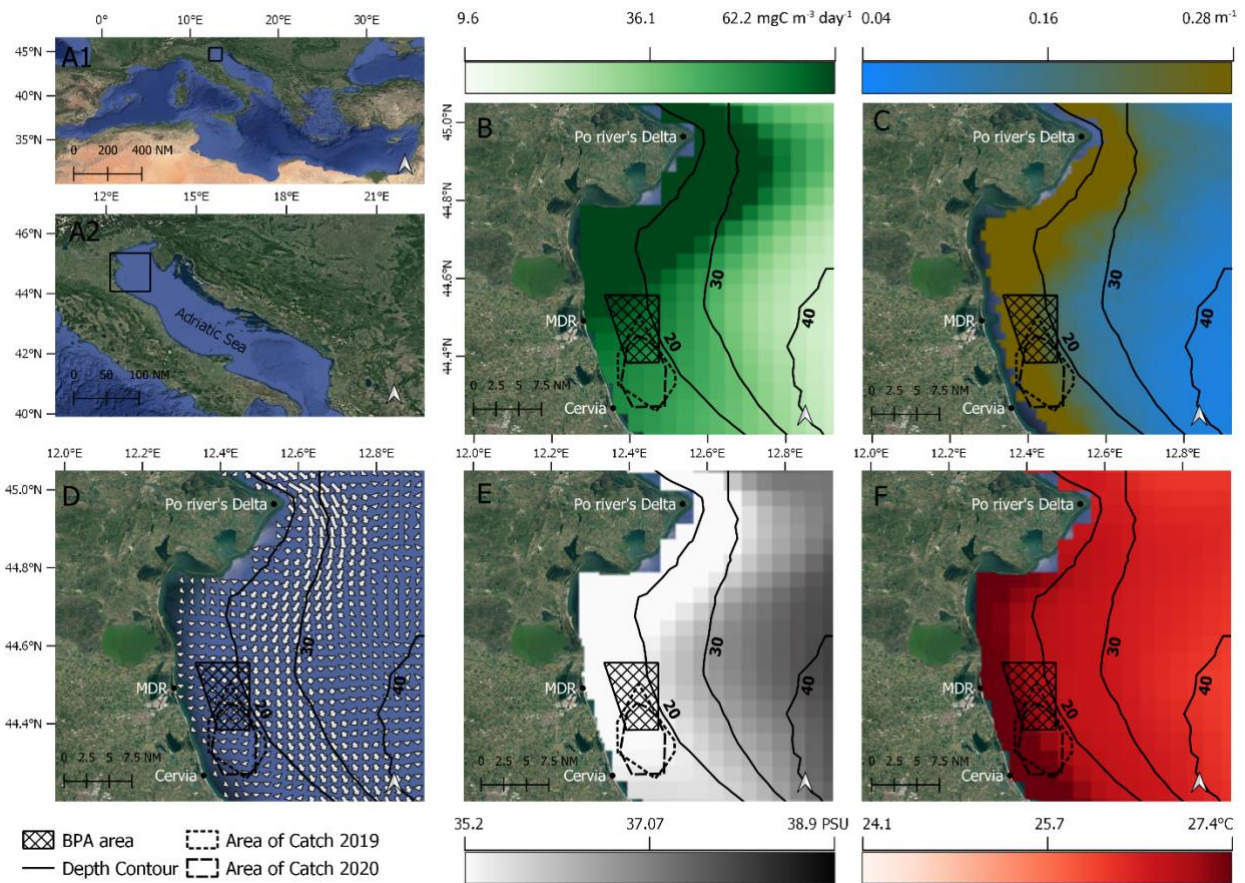
Figure 2: The novel sandbar shark birthing site in the northern Adriatic Sea, Mediterranean (A1, A2) identified between Cervia and Marina di Ravenna, and the local environmental conditions (B-F). Abiotic and biotic conditions were obtained from CMEMS (Tab A3.1) and include net primary production (B), turbidity (C), current direction and velocity (the latter is proportional to the arrow length; D), salinity (E) and sea surface temperature (F). At this site, a biological protection area (BPA), as shown on the map, was established in

2004 where trawling was banned, whereas artisanal fishery was still permitted (Tassetti et al., 2019). The shark catch distribution is

partially downstream of the productive Po River plume and the BPA, potentially allowing foraging ground and refuge effects.

3.3.2. Modelling and validation Mediterranean nurseries

In total, 14 studies over 2000-2020 met the criteria for the PA dataset, of which 4 reported the presence of newborns. Such studies, covering widely different Mediterranean subbasins (Annex II, Tab. S1; Fig. S1), included 52 areas from which 15,642 points (14,671 absence, 971 presence) were extracted to build the GLM. The density plot of the PA points showed a marked contrast in CUR and SST (Annex II, Fig. S3.1). KD was dropped from further analyses given its high correlation with NPP (Annex II, Fig. S3.2).

The ten lowest-AIC models were inspected for significance (Annex II, Tab. S3.1 and Tab S3.2). In the lowest-AIC GLM model (M64), which included all environmental factors as predictors, only the coefficient associated with salinity was non-significantly different from zero (p=0.072; Annex II, Tab. S3.1). However, the model with the second lowest AIC (M56), which excluded only salinity, had an AIC differing only by a negligible 1.2 points compared to M64; therefore, it was selected as the best model being more parsimonious (Burnam & Anderson, 2002).

Among the ten lowest-AIC GAM models, the M2 had the lowest AIC, but the smoother function of salinity was not significant (p=0.09, Tab S3.2). the second lowest-AIC model (M2) only differ of 0.4 from the first one and the smoother function of salinity was excluded among the predictor (AnnexII, Tab S3.2). So M2 was chosen for downstream analysis.

The comparison between the best GLM (M56) and GAM (M2) was executed according to the AIC calculated from the ten random 80% partitions of the original dataset. In all ten subsets, the GAM models had lower AIC than the GLM ones (Annex II, Tab. S3.3). However, when calculating the accuracy, type I and II errors, equal values of accuracy and type I and II errors were obtained, and the standard deviations were slightly larger for the GAM model compared to the GLM one (Annex II, Tab. S3.4). For this reason, the GLM model was chosen for its simplicity and was as follows:

$$logit(PA) = \beta_0 + \beta_1 \cdot cur + \beta_2 \cdot depth + \beta_3 \cdot ln(NPP) + \beta_4 \cdot ln(slope + 1) + \beta_5 \cdot SST$$

The best GLM model (Tab. 1) was highly reliable (Tab. 2) and its performance was validated as well (Annex II, Tab. S3.4) In addition, M56 indicated that nursery sites for early juveniles were more likely in areas with a shallow depth, a mild slope, calm and warm waters and relatively low primary production.

*Tabe*1: The coefficients of the best GLM model predicting the probability of nursery areas for early juveniles for the sandbar shark in the Mediterranean Sea.

Parameter	Coefficient	Standard Error (±)	p
β_0	15.143	2.352	< 0.001
β_1	-64.123	5.335	< 0.001
β_2	-0.079	0.007	< 0.001
β_3	-2.114	0.210	< 0.001
β_4	-15.042	0.989	< 0.001
β_5	0.301	0.083	< 0.001

Table 2: Model performance assessed by comparison of predicted and observed points of presence and absence of birthing sites.

Model outcome accuracy (percentage of correct predictions) was 99.2%, with a type I error rate of 0.6% and a type II error of 4.1%.

	Observed presence	Observed absence
Predicted presence	931	87
Predicted absence	40	14584

The map generated by projecting this model over the whole Mediterranean Sea predicted the Adriatic Sea and Gulf of Gabés had the largest suitable areas for nursery of early juveniles of *C. plumbeus*, while other suitable sites emerged, with different extents, in Libyan and Egyptian waters, south-eastern Turkish coasts, northern Aegean Sea, Ionian Sea, north-eastern Tyrrhenian Sea and along southern Spanish shores (Fig. 3).
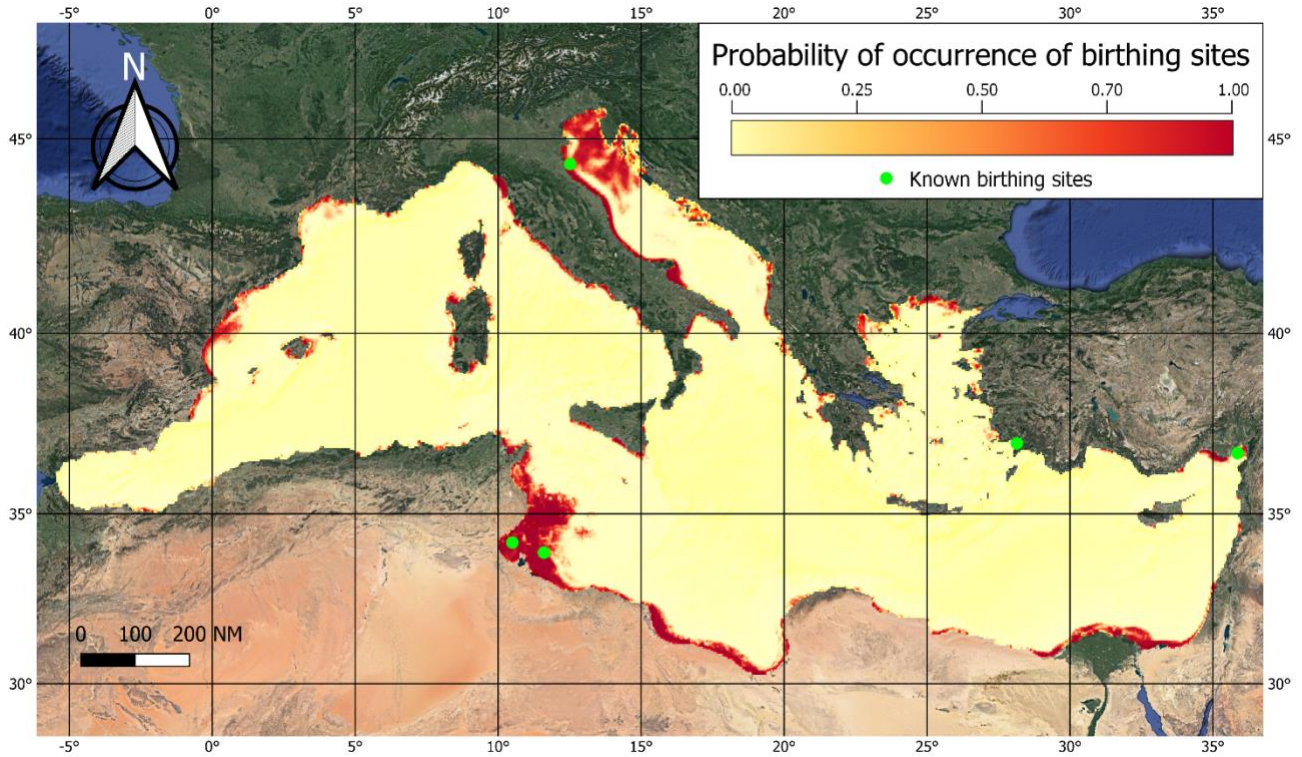
Figure 3: Predicted probability of birthing site suitability throughout the Mediterranean Sea.

3.4 Discussion

This study provides novel data that unequivocally confirm the occurrence of a multiyear birthing site of the sandbar shark in the northern Adriatic Sea and integrates sparse literature data as well as these new findings to robustly predict where other potential birthing sites may be located based on the environmental conditions in the Mediterranean Sea.

Several records (e.g., occasional catches, museum samples, citizen science data) of sandbar shark presence have been previously reported in the northern Adriatic Sea, suggesting the occurrence of nursery areas (Jambura et al., 2021). Our systematic study allows documenting the interannual persistence of newborn occurrences, hence a nursery for early juveniles, given the open or partially healed scar. Although no data about the persistence of the umbilical scar are available for the sandbar shark, in a congeneric species, the wild-caught neonates of the blacktip reef shark (*C. melanopterus*) had the healing capacity to reduce the umbilical scar area within 30 days in reared conditions (Chin et al., 2015), therefore a similar time period is assumed also for the sandbar shark.

Multiyear nursery site fidelity has been observed in many elasmobranch species (Chapman et al., 2015), and its evaluation through genetic and telemetry studies appears crucial for conservation. Indeed, even if limited to summer months, our data highlight the overlap between an Adriatic artisanal fishery and newborn occurrence. The environmental conditions of the Cervia-MDR site are comparable to those of the birthing and nursery areas found in the western Atlantic Ocean (Conrath & Musick, 2010; Baremore & Hale, 2012), thus confirming the importance of near-shore habitats, where the combination of shallow, calm and warm waters may contribute to creating refuge and ready-to-use foraging grounds for early juveniles.

In this study, an ecological modelling approach was applied for the first time to map suitable areas for the nursery of a neritic endangered juveniles in the Mediterranean Sea. The constructed model was simple, with few monotonic predictors, yet it performed exceptionally well in fitting the PA data. Overall, shallow, calm

and warm marine environments have been reported as ideal conditions for sandbar shark nursery in other oceans (Kinney, & Simpfendorfer, 2009; Conrath & Musick, 2010; Baremore & Hale, 2012) as well as for other shark species (Knip et al., 2010; Schlaff et al., 2014), supporting our model-based inference.

When compared with PA observations, an interesting component of the model emerges. High nursery probability is predicted in sites where scientific evidence for nursery is strong (Bradai et al., 2005) or where several anecdotal reports exist (Başusta et al., 2021; Jabura et al., 2021). In contrast, the model computes a low probability in Boncuk Cove (Turkey), where a nursery site was hypothesized after eyewitnesses reported a delivery and two stillborns (Clo & de Sabata, 2004). A subsequent study documented only adults, without early juveniles sights, forming a year-round aggregation there (Filiz, 2019). Our model agrees with the latter study, although model predictions could be biased by the limited spatial resolution available for environmental factors (Tab. A2.2). In general, additional PA data could enhance the predictive power of the model along Mediterranean coasts.

The large extent, compared to the documented locations, of modelled sandbar shark nursery sites in the Mediterranean may reflect several factors. Along coasts, the model predicts high nursery probability also in spots where no reports are available The lack of reports does not equate to evidence of juvenile absence given the widespread deficiency or ambiguity of landing data for endangered elasmobranchs in Mediterranean countries, which is related to a lack of dedicated systematic data collection (Cashion et al., 2019). Alternatively, some historical nursery sites might have disappeared due to the loss of shark behavioural knowledge (e.g., site fidelity and/or cultural transmission of parturition sites, Warner, 1988) following an abrupt population decline (Ferretti et al., 2016) or the onset of anthropogenic stressors such as habitat loss, pollution or noise (Coll et al., 2012), making sites unfit for nursering early juveniles. Finally, the model was constructed with data spanning twenty years, but its predictions were built on environmental data from 2016-2020. Due to climate change, the availability of suitable habitats for birthing and nursery may change, for example making suitable sites that were unsuitable in the past (Crear et al., 2020), yet these sies

may still remain unused. Indeed, the same authors concluded that the use of newly available habitats requires a behavioural shift otherwise they may remain unused.

Our findings provide essential information for Mediterranean decision-makers to protect nursery areas for early juveniles of the endangered sandbar shark. Given their coastal location, these predicted nursery sites in the Mediterranean Sea overlap with fishery exploitation, especially by small-scale fishery (Lloret et al.,2020) and the cumulative impacts of multiple anthropogenic factors (Coll et al., 2012). We provide evidence for early juvenile fishing mortality, whose estimate needs to be improved with more data at wider spatiotemporal scales, given the poor registration accuracy of elasmobranchs in fish market statistics and the low commercial value of these young individuals (Cashion et al., 2019). Landing monitoring (Cashion et al., 2019), protection schemes on nursery and aggregation sites (Cattano et al., 2021; Filiz, 2019), and improved fishery management through bycatch mitigation, fishing mortality reduction or catch restrictions on immature individuals (Brewster-Geisz & Miller, 2000) are pillars for defining conservation strategies for the sandbar shark and other endangered sharks in the Mediterranean and worldwide (Dulvy et al., 2017; Milazzo et al., 2021).

3.5.Conclusion

This study is the first to identify the key Mediterranean areas that are pivotal for the recruitment of sandbar sharks, where conservation actions are needed for well-known goals such as aggregation site protection and fishing pressure reduction across the whole Mediterranean. Our methodology could be applied to other elasmobranch species through multiyear systematic data collection to create a robust knowledge base to define nursery areas (Heupel et al., 2007). Conservation issues for large predators are often transboundary (Maguire et al., 2006), and given the fragmented jurisdictions across the Mediterranean Sea, an effective spatially explicit governance of Mediterranean migratory marine species is urgently needed.

Biblography

Barausse, A., Correale, V., Curkovic, A., Finotto, L., Riginella, E., Visentin, E., & Mazzoldi, C. (2014). The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. *ICES Journal of Marine Science*, *71*(7), 1593-1603. *doi*:10.1093/icesjms/fst222

Baremore, I. E., & Hale, L. F. (2012). Reproduction of the sandbar shark in the western North Atlantic Ocean and Gulf of Mexico. *Marine and Coastal Fisheries*, *4*(1), 560-572. *doi:10.1080/19425120.2012.700904*

Barton, K. (2020). MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Başusta, N., Başusta, A., & Ozyurt, C. E. (2021). Evidence of a second nursery area of the sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) in the Eastern Mediterranean Sea. *Mediterranean Marine Science*, *22*(1), 20-26. *doi*:10.12681/mms.24490

Brewster-Geisz, K. K., & Miller, T. J. (2000). Management of the sandbar shark, *Carcharhinus plumbeu*s: implications of a stage-based model. *Fishery Bulletin*, 98 (2), 236-249.

Bradai, M.N., Saidi, B., Bouaïn, A., Guélorget, O., & Capapé, C. (2005). The Gulf of Gabès (Central Mediterranean): Nursery area for the sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) (Chondrichthyes: Cacharhinidae). *Annales Series Historia Naturalis*, 15 (2), 187-194.

Burnham, K. P. & Anderson, D. R. (2002). *Model selection and multimodel inference. a practical information-theoretic approach*. (2nd ed.). New York: Springer.

Cashion, M. S., Bailly, N., & Pauly, D. (2019). Official catch data underrepresent shark and ray taxa caught in Mediterranean and Black Sea fisheries. *Marine Policy*, *105*, 1-9. *doi*:10.1016/j.marpol.2019.02.041

Cattano, C., Turco, G., Di Lorenzo, M., Gristina, M., Visconti, G., & Milazzo, M. (2021). Sandbar shark aggregation in the central Mediterranean Sea and potential effects of tourism. *Aquatic Conservation: Marine and Freshwater Ecosystem*, 1–9. *doi:*10.1002/aqc.3517

Chapman, D. D., Feldheim, K. A., Papastamatiou, Y. P., & Hueter, R. E. (2015). There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. *Annual Rreview of Marine Science*, *7*, 547-570. *doi:10.1146/annurev-marine-010814-015730*

Chin, A., Mourier, J., & Rummer, J. L. (2015). Blacktip reef sharks (*Carcharhinus melanopterus*) show high capacity for wound healing and recovery following injury. *Conservation Physiology*, *3*(1).

Clò, S., & de Sabata, E. In the sharks' cradle. In *8th European Elasmobranch Association Conference, 21st-23rd October 2004, London*, 45.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W. W., Christensen, V., ... & Pauly, D. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. *Global Ecology and Biogeography*, *21*(4), 465-480. *doi:10.1111/j.1466-8238.2011.00697.x*

Conrath, C. L., & Musick, J. A. (2010). Residency, space use and movement patterns of juvenile sandbar sharks (*Carcharhinus plumbeus*) within a Virginia summer nursery area. *Marine and Freshwater Research*, *61*(2), 223-235. *doi: 10.1071/MF09078*

Costantini, M., & Affronte, M. (2003). Neonatal and juvenile sandbar sharks in the northern Adriatic Sea. *Journal of Fish Biology*, *62*(3), 740-743. *doi:10.1046/j.1095-8649.2003.00045.x*

Crear, D. P., Latour, R. J., Friedrichs, M. A., St-Laurent, P., & Weng, K. C. (2020). Sensitivity of a shark nursery habitat to a changing climate. *Marine Ecology Progress Series*, *652*, 123-136.

Dulvy, N. K., Simpfendorfer, C. A., Davidson, L. N., Fordham, S. V., Bräutigam, A., Sant, G., & Welch, D. J. (2017). Challenges and priorities in shark and ray conservation. *Current Biology*, *27*(11), R565-R572. *doi:10.1016/j.cub.2017.04.038*

Ebert, D. A., & Dando, M. (2020). *Field Guide to Sharks, Rays & Chimaeras of Europe and the Mediterranean*. Oxfordshire Princeton University Press.

FAO. 2018. The State of Mediterranean and Black Sea Fisheries. General Fisheries Commission for the Mediterranean. Rome. 172 pp. Licence: CC BY-NC-SA 3.0 IGO.

Ferretti, F., Myers, R. A., Serena, F., & Lotze, H. K. (2008). Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology*, *22*(4), 952-964. *doi: 10.1111/j.1523-1739.2008.00938.x*

Ferretti, F., Walls, R.H.L., Musick, J., Stevens, J., Baum, J.K., Bradai, M.N., Fergusson, I., Grubbs, D., Soldo, A., Vacchi, M. & Vooren, C.M. 2016. *Carcharhinus plumbeus*. The IUCN Red List of Threatened Species 2016: e.T3853A16527809. Downloaded on 18 February 2021.

Filiz, H. (2019). Year-Round Aggregation of Sandbar Shark, *Carcharhinus plumbeus* (Nardo, 1827), in Boncuk Cove in the southern Aegean Sea, Turkey (Carcharhiniformes: Carcharhinidae). *Zoology in the Middle East*, *65*(1), 35-39. *doi:10.1080/09397140.2018.1540148*

Fortibuoni, T., Libralato, S., Raicevich, S., Giovanardi, O., & Solidoro, C. (2010). Coding early naturalists' accounts into long-term fish community changes in the Adriatic Sea (1800–2000). *PloS one*, *5*(11), e15502. *doi:10.1371/journal.pone.0015502*

Grati, F., Aladzuz, A., Azzurro, E., Bolognini, L., Carbonara, P., Çobani, M., ... & Ikica, Z. (2018). Seasonal dynamics of small-scale fisheries in the Adriatic Sea. *Mediterranean Marine Science*, *19*(1), 21-35. *doi:10.12681/mms.2153*

Heupel, M. R., Carlson, J. K., & Simpfendorfer, C. A. (2007). Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series*, *337*, 287-297. *doi: 10.3354/meps337287*

Hijmans R. J. (2021). raster: Geographic Data Analysis and Modeling. R package version 3.5-2. https://CRAN.R-project.org/package=raster

Humphries, A. T., Gorospe, K. D., Carvalho, P. G., Yulianto, I., Kartawaijaya, T., & Campbell, S. J. (2019). Catch composition and selectivity of fishing gears in a multi-species Indonesian coral reef fishery. *Frontiers in Marine Science*, *6*, 378. *doi:10.3389/fmars.2019.00378*

Jambura, P. L., Cétkovic, I., Kriwet, J., & Turtscher, J. (2021). Using historical and citizen science data to improve the knowledge about the occurrence of the elusive sandbar shark *Carcharhinus plumbeus* (Chondrichthyes–Carcharhinidae) in the Adriatic Sea. *Mediterranean Marine Science*.*22*(1), 169-179. *doi*:10.12681/mms.24624

Kinney, M. J., & Simpfendorfer, C. A. (2009). Reassessing the value of nursery areas to shark conservation and management. *Conservation letters*, *2*(2), 53-60. *doi:10.1111/j.1755-263X.2008.00046.x*

Knip, D. M., Heupel, M. R., & Simpfendorfer, C. A. (2010). Sharks in nearshore environments: models, importance, and consequences. *Marine Ecology Progress Series*, *402*, 1-11. *doi:10.3354/meps08498*

Kuhn M. (2021). caret: Classification and Regression Training. R package version 6.0-90. https://CRAN.R-project.org/package=caret

Lloret, J., Biton-Porsmoguer, S., Carreño, A., Di Franco, A., Sahyoun, R., Melià, P., ... & Calò, A. (2020). Recreational and small-scale fisheries may pose a threat to vulnerable species in coastal and offshore waters of the western Mediterranean. *ICES Journal of Marine Science*, *77*(6), 2255-2264. *doi:10.1093/icesjms/fsz071*

Lotze, H. K., Coll, M., & Dunne, J. A. (2011). Historical changes in marine resources, food-web structure and ecosystem functioning in the Adriatic Sea, Mediterranean. *Ecosystems*, *14*(2), 198-222. *doi:10.1007/s10021-010-9404-8*

Maguire J.J., Sissenwine M., Csirke J., & Grainger R. (2006). The state of the world highly migratory, straddling and other high seas fish stocks, and associated species. FAO Fisheries Technical Paper. No. 495. Rome: FAO. 2006. 84p.

Merson, R. R., & Pratt, H. L. (2001). Distribution, movements and growth of young sandbar sharks, Carcharhinus plumbeus, in the nursery grounds of Delaware Bay. *Environmental Biology of Fishes*, *61*(1), 13-24.

Milazzo, M., Cattano, C., Al Mabruk, S. A., & Giovos, I. (2021). Mediterranean sharks and rays need action. *Science*, *371*(6527), 355-356. *doi:10.1126/science.abg1943*

Myers, R. A., Baum, J. K., Shepherd, T. D., Powers, S. P., & Peterson, C. H. (2007). Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science*, *315*(5820), 1846-1850. *doi:10.1126/science.1138657*

Pacoureau, N., Rigby, C. L., Kyne, P. M., Sherley, R. B., Winker, H., Carlson, J. K., ... & Dulvy, N. K. (2021). Half a century of global decline in oceanic sharks and rays. *Nature*, *589*(7843), 567-571. *doi:10.1038/s41586-020-03173-9*

RStudio Team 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Saïdi, B., Bradaï, M. N., Bouaïumln, A., Guéacutelorget, O., & Capapé, C. (2005). The reproductive biology of the sandbar shark, *Carcharhinus plumbeus* (Chondrichthyes: Carcharhinidae), from the Gulf of Gabès (southern Tunisia, central Mediterranean). *Acta Adriatica: International Journal of Marine Sciences*, *46*(1), 47-62.

Schlaff, A. M., Heupel, M. R., & Simpfendorfer, C. A. (2014). Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. *Reviews in Fish Biology and Fisheries*, *24*(4), 1089-1103. *doi:10.1007/s11160-014-9364-8*

Sguotti, C., Lynam, C. P., García-Carreras, B., Ellis, J. R., & Engelhard, G. H. (2016). Distribution of skates and sharks in the North Sea: 112 years of change. *Global Change Biology*, *22*(8), 2729-2743. *doi:10.1111/gcb.13316*

Tassetti, A. N., Ferrà, C., & Fabi, G. (2019). Rating the effectiveness of fishery-regulated areas with AIS data. *Ocean & Coastal Management*, *175*, 90-97. *doi:10.1016/j.ocecoaman.2019.04.005*

Warner, R. R. (1988). Traditionality of mating-site preferences in a coral reef fish. *Nature*, 335, 719-721. *doi:10.1038/335719a0*

Wood, S.N. (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society (B) 73(1):3-36

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution*, *1*(1), 3-14.

Acknowledgement

Chapter 4: Seasonal use of habitat of smooth-hound sharks (*Mustelus* spp.) in the northern-central Adriatic Sea.

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Abstract

The overexploitation of marine resources is causing a marked decline of the population abundances. Especially in the last century, an extended overfishing period has been demonstrated to deplete the stocks of many marine commercial species in the Mediterranean Sea. By the direct or accidental catch, elasmobranch species have been impacted consistently by the fishing pressure, leading to the majority of the species threatened by extinction. Among the commercial-relevant species, the smooth hounds represent highly exploited species in the Mediterranean Sea, and their decline has been documented over the last century. For elasmobranch species, the use of space during different periods of their life cycle can influence the seasonal and local density given the occurrence of site fidelity properties such as philopatry and natal homing. In the northern and central Adriatic Sea, some indications from fishery-dependent and independent research highlighted the likely occurrence of periodic movements of the two main smooth hound species occurring in the area, *Mustelus mustelus* and *M. punctulatus*. In this study, the use of fishery-dependent data allowed to define at finer spatial and temporal resolution the movement patterns and the occurrence of nursery areas where young-of-the-year were observed.

Keywords

4.1 Introduction

Fishery overexploitation is globally driving the decline of several marine resources, and this is particularly marked for some large-sized predatory species like elasmobranchs (Walls &Dulvy 2021; Dulvy et al., 2021; Pacoureau et al., 2021). While some elasmobranch species are targeted due to the trade of highly valuable parts, as fins, most species are exploited for their meat. As a consequence of the decline of several stocks of more valuable commercial species, elasmobranch species not only are more retained but also are more targeted by fishers, leading to an increase of fishing effort on these species (Dulvy et al., 2014). Because of elasmobranch size, their accidental catch, also called by-catch, is often inevitable since fishing gears are designed to capture smaller commercial target species, thus, posing elasmobranchs in an extended state of overfishing (Dulvy et al., 2021).

A factor of vulnerability to overfishing is represented by the life history traits of elasmobranchs that typically show a slower growth rate compared to many teleost fish. Reaching the first reproductive size takes much longer than other marine species (Dulvy et al., 2017). A further factor of vulnerability is constituted by the multi-year (e.g., site fidelity or natal homing) and multi-generational (e.g., philopatry) use of space in specific life cycle periods (e.g., birthing, nursery or foraging; Chapman et al., 2015). The periodic use of different sites for different purposes also implies periodic movements or migrations, driven by both environmental features, often related to species-specific physiological needs (Schalf et al., 2014), and behaviours, including mating, parturition or egg deposition, feeding, sexual and size segregation (Jacoby et al., 2011; Chapman et al., 2015). A high fishing pressure on elasmobranchs in repeatedly used areas can further impact their life cycle and, lastly, impair the population. In addition, degradation of these essential habitats may pose a further threat to elasmobranch conservation (Dulvy et al., 2014). While stock assessment has often represented the main tool for developing management strategies, in species characterized by the recurrent use of different habitats, the inclusion of spatial information is essential to develop spatially explicit strategies (Braccini et al., 2016).

Among the commercial species, the smooth hound sharks (*Mustelus* spp.), a globally distributed genus, represent an example of global fishery relevance (Carpentieri et al., 2021). In this genus, complex behavioural traits, including philopatry and site fidelity for delivery and reproduction, and periodic movements between different areas were demonstrated by different methods (Klein et al., 2021; Brevè et al 2016). The integration of these behaviours and the use of space in conservation strategies allowed successful achievements after many years of spatial-explicit management in Australia for *M. antarticus* fishery (Prince, 2002; Kinney & Simpfendorfer, 2009).

The Mediterranean Sea is considered a hotspot of elasmobranch biodiversity (Coll et al., 2012) and, at the same time an area where elasmobranch species are particularly threatened, with several species showing a worsening condition in the Mediterranean Sea respect to other oceans (Walls & Dulvy, 2021). Among the Mediterranean basins, the Adriatic Sea has a particularly long history of resource exploitation and anthropogenic impacts (Lotze et al., 2006, 2011) in particular trawling (Pitcher et al., 2022), with a consequent decline of several elasmobranch species (Ferretti et al., 2008, 2013; Barausse et al., 2014). Three smooth hound species (*M. mustelus*, *M. punctulatus* and *M. asterias*) can be found in the Mediterranean Sea (Serena et al., 2020). Among the three species, *M. asterias* is considered very rare based on fishery independent data in the GSA17 (Follesa et al., 2019; Ferretti et al., 2013) and from landing data collected in Chioggia's fish market (Barausse et al., 2014). Evidence of fishery-driven overexploitation have been documented in these shark species in the Adriatic Sea (Ferretti et al., 2008, 2013; Fortibuoni et al., 2010; Barausse et al., 2014) as well as in the whole Mediterranean Sea (Colloca et al., 2017). Therefore, these species are considered vulnerable to extinction (Walls & Dulvy, 2021). While life history traits and diet habits are available in some Mediterranean areas (Saïdi et al., 2008, 2009; Di Lorenzo et al., 2020; Riginella et al., 2020), their use of space and movements patterns are still poorly investigated. The occurrence of some areas of aggregations of smooth hounds has been reported in different Mediterranean sites by fishers (Colloca et al., 2020; Barbato et al., 2021), while nursery areas, in particular in the Gulf of Gabés (Enajjar et al., 2015;

Saïdi et al., 2016), and seasonal movements in the Adriatic Sea have been suggested by fishery-dependent data (Bonanomi et al., 2018) and local ecological knowledge (Barbato et al., 2021).

In the Mediterranean Sea, spatial management for nursery protection has been proposed already for many commercial species, including two elasmobranch species (Colloca et al., 2015). Currently in the Italian coasts, spatial or temporal closures have been implemented since mid-2010 (EC No. 1967/2006). These closures consist in the year-round trawling fishing ban within the 3 nautical miles from shore and a temporary trawling closure of roughly 30 days every year. However, no spatially explicit management plans have been developed for elasmobranch conservation, possibly in relation also to the lack of data on the use of space.

To provide information on the use of space in the Adriatic Sea of *Mustelus* spp., representing the main commercial species of the area (Barusse et al., 2014) with the goal to support the development of a spatially explicit management, this study aims at: (i) contributing to the spatial characterization of *Mustelus* spp. movements; (ii) the spatial identification of key areas in *Mustelus* spp. life stages across the most important periods of their life cycle.

4.2 Material and Method

4.2.1 Study area

The FAO-GFCM geographical sub-area 17 (GSA 17) includes the northern and central Adriatic Sea (Resolution GFCM/33/2009/2; FAO, 1990-2021). Italy, Slovenia and Croatia boarder this semi-enclosed Mediterranean subbasin. Its physical and environmental features manly consist in a relative shallow depth, strong seasonal fluctuations and an important river runoff. The variation of the main biotic factors like primary production and the abiotic ones such as water temperature, salinity and currents are influenced by seasonal wind-driven currents that move large body masses from south to north, along the Croatian coasts, and from north to south, along the Italian shore (Russo & Artegiani, 1997). Coupled with this, seasonal rainfall discharges, dominated by the Po River inputs, bring in an important amount of nutrients that, along with temperature

and salinity variation with season, promote the primary production and fuel the productivity of the GSA 17 (Cozzi & Giani, 2011).

4.2.2 Periodic movement pattern

To investigate the recurrent movement pattern of *Mustelus* spp., the official fishery data by the Ministry of Agricultural, Food and Forestry Policies (Italy) from 2014 to 2019 were used. Such dataset consisted in the *Mustelus* spp. landing from the fisher's logbooks, compiled for each fish trip with catches and fish trip date. The fishing effort dataset reported the name of the fishing vessel, EU identification number, port of departure and arrival, the date of departure and arrival for each fish trip, and vessel gross tonnage. Because the *Mustelus* spp. are a by-catch of different types of gear, the fishing effort was considered for all vessels cumulatively. The choice to use this data collection relied on the fact it can give an indication of the seasonality and periodicity of the catch rate in space, throughout the northern-central Adriatic Sea, and in time, on a monthly period, averaging the years to counterbalance the possible bias due to the nature of the by-catch registration (Cashion et al., 2019). Due to the misidentification issues about the two main smooth hound species occurring in the area (Marino et al., 2018), landings are considered aggregated for the genus *Mustelus* spp.

The input time series consisted in a monthly LPUE for five regions, i.e, Friuli V. Giulia, Veneto, Emilia-Romagna, Marche and Abruzzo (Fig. 1) calculated as the sum of landing in each fish trip divided by the fishing effort expressed as the gross tonnage of each boat multiplied by the number of fish trips (Eq.1).

$$(Eq . 1) \ LPUE_m = \frac{\sum_i \ fish \ trip \ landing}{\sum_i GT_i \times N_i \ of \ monthly \ fish \ trip}$$

Data were analysed applying the Continuous Wavelet transformation (Torrence & Compo, 1998) by PAST v.4.03 (Hammer et al., 2001). Since the landing data of August was not used because of the temporary trawling ban, PAST software allows carrying out statistical analysis when missing entry are found in a time

series. From the same dataset, monthly average of LPUE and confidence interval (CI) at 95% were calculated and plotted using Microsoft Excel.

4.2.3 Characterization of the seasonal use of space

Fishery dependent data were obtained within the monitoring CAMPBIOL program through scientific boarding data. The choice to use these data depended on fact that this program is the only one available monthly and covering most of the northern and central Adriatic Sea. Fishery independent surveys, like the MEDITS program, are carried out only during limited periods of the year (Follesa et al., 2019). In the CAMPBIOL program, observers onboard of commercial fishing boats collected data on fishing operations (progressive number of registered hauls in each fish trip, fishing gear, haul start and stop coordinates, fishing time, registered port), length frequency distribution and total weight and subsample weight of both retained and discarded bycatch; no data on sex were registered. Due to the difficulties in species identification (Marino et al., 2018), and considering that different observers were involved in data collection, the data for the smooth hound species were conservatively pooled. Only data from otter bottom trawling (OTB) were used because OTB is the main contributor to the smooth hound landing in the northern- central Adriatic Sea (Barausse et al., 2014).

The number of registered hauls was inspected by visualising the number of hauls by month and year using ggplot2 R package, separately in the northern (Chioggia and Pila ports) and central Adriatic Sea (Ancona, Cattolica, Cesenatico, Giulianova, Pescara, Rimini, San Benedetto and Termoli).

For each haul, catch data were transformed into catch per unit effort (CPUE) as number of individuals divided by fishing effort index given by the fishing hours. CPUE has been considered a proxy of density. Giving the protocol of data collection in CAMPBIOL program for discarded data, density of discarded *Mustelus* spp. must be intended as minimum registered density applying a conservative and cautionary approach. Indeed, if the subsampled weight was lower than the total weight of discard, it meant that a high number of discarded

individuals (often individuals below 60 cm of TL) was caught, and the measured individuals were not representative of the entire catch (Santojanni unpublished data).

To test the effect of fishing vessel length (length overall -LOA), that can be considered a proxy of the horizontal net opening, on density, a non-parametric Kruskal-Wallis test (RStudio Team, 2021) was applied to compare three LOA size classes (LOA < 15 m, 15 < LOA < 24 m and LOA > 24 m) of surveyed fishing vessels. Such test was necessary since the data were not normally distributed (Shapiro test, RStudio Team, 2021; significant level below 0.05), even after logarithmic transformation.

To plot the observed density of all the individuals, without life stage division, here after referred as unstructured density, the hauls were divided according to the seasonality inferred from the landings (Fig. 1). The three identified periods consisted in: period A: December, January, February, and March; Period B: April, May, June and July; Period C: September, October, November. From the length frequency distribution of the smooth hounds, the density of three life stages were plotted in the study area according to the recorded total length (TL): young-of-the-year (YoY) below 60 cm TL; juveniles between 61 and 99.5 cm; adults above 99.5 cm. These sizes were chosen according to the minimum sizes at sexual maturity of the two main species, *M. mustelus* and *M. punctulatus* (Riginella et al., 2020), the size at birth (Saïdi et al., 2008, 2009), and the growth rate during the first few months, from tag-recapture data (Barbato, unpublished data).

Observed densities, attributed to an average point coordinate according to the haul start and stop coordinates, were plotted according the three periods and proportional to the density value, expressed in number of individuals per fishing hour, using QGIS3 (https://qgis.org). Such approximation was chosen to account for the different sampling effort across the study area, years and through time.

4.3 Results

4.3.1 Periodic movement pattern

The monthly LPUE of *Mustelus* spp. in each region indicated how a recurrent seasonality of catches occurred approximately in a time period between 10 and 12 months (Fig. 1). This repeated pattern appeared to be evident, from north to south, in Friuli V. Giulia, Veneto, Marche and Abruzzo. Only in Emilia Romagna region, such recurrent periodicity did not emerge according to the data collected by the national landings. The peak of the catch rate in the northern part of the Adriatic, Friuli V. Giulia and Veneto, occurred in May, then decreased in the summer months and boosted in September. From September until December the level of catches declined constantly and in the Winter months and early Spring, the level of catch remained low. In the two southern regions, Marche and Abruzzo, the trend in catch rates appeared to be generally reversed. The late Spring and Summer months seemed to be the period when the lowest LPUE occurred whereas the Autumn and Winter months presented the highest amount of LPUE. In Emilia Romagna, LPUE appears to be the lowest throughout the year and no seasonality emerged.
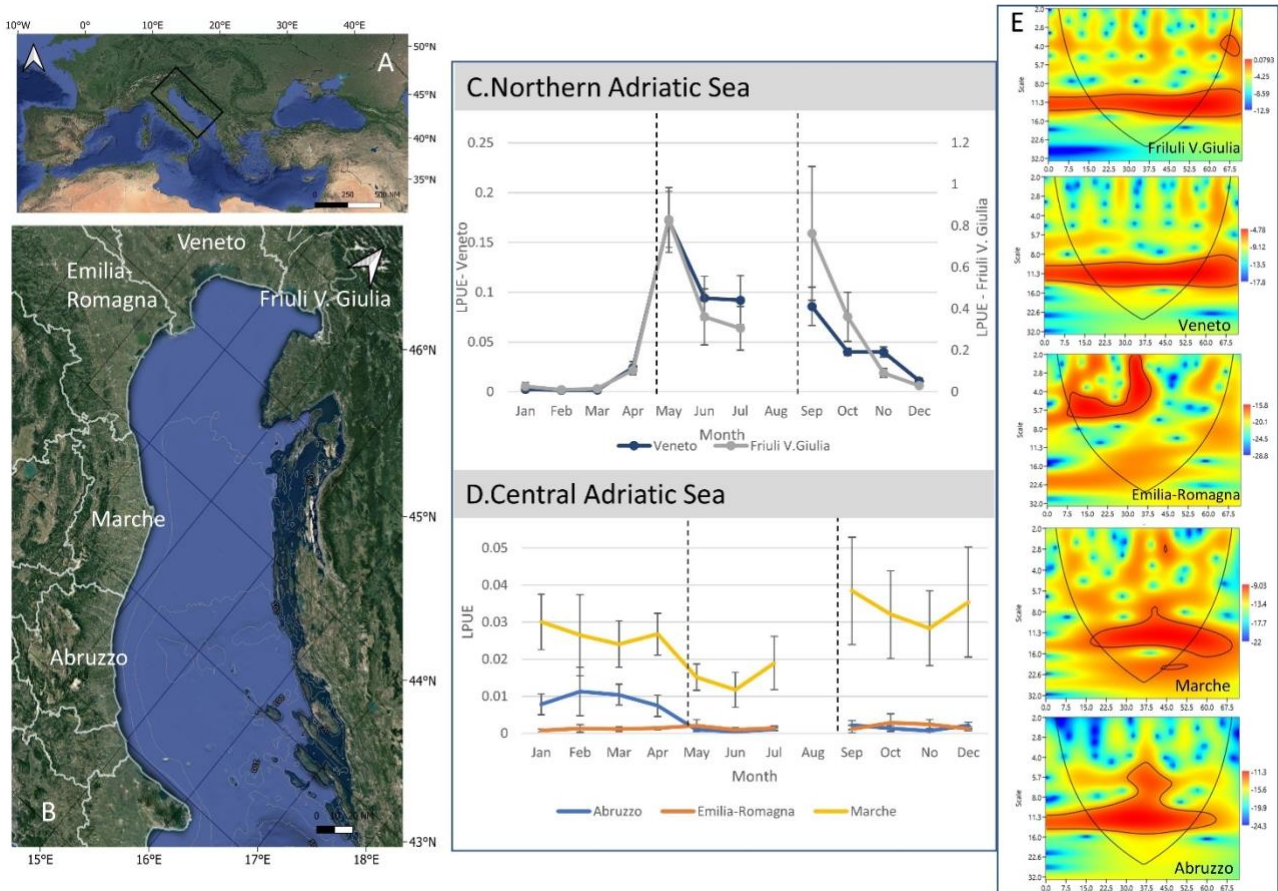
Figure 1: The northern-central Adriatic Sea is shown at low (A) and high (B) resolution with the bordering Italian regions in the western side. The seasonality of catch rate of Mustelus spp. is reported in the northernmost regions, Veneto and Friuli V. Giulia (C) and in the central part, Emilia Romagna, Marche and Abruzzo (D). Dashed line reports the seasonality periods. The wavelet in the graphs E are represented for each region individually. The vertical axis of wavelet indicates the monthly time scale given by the input monthly LPUE. In the wavelet graph, the red area marked with the black continuous line indicates statistically significant level of periodicity.

4.3.2 Seasonal use of space

In total, 1892 hauls from 371 fish trips, between 2011-2019 in the northern-central Adriatic Sea (GSA 17), were considered for the analyses within the CAMPBIOL program. Among registered hauls, 146 reported the catch occurrence of *Mustelus* spp. in 83 fish trips. Regarding the sampling effort, the number of registered hauls and fish trip did not have an even monthly distribution and spatial distribution across the years (Fig. 2). Kruskal-Wallis' test indicated that the CPUE were not statistically different between the LOA classes (p-value = 0.2099).
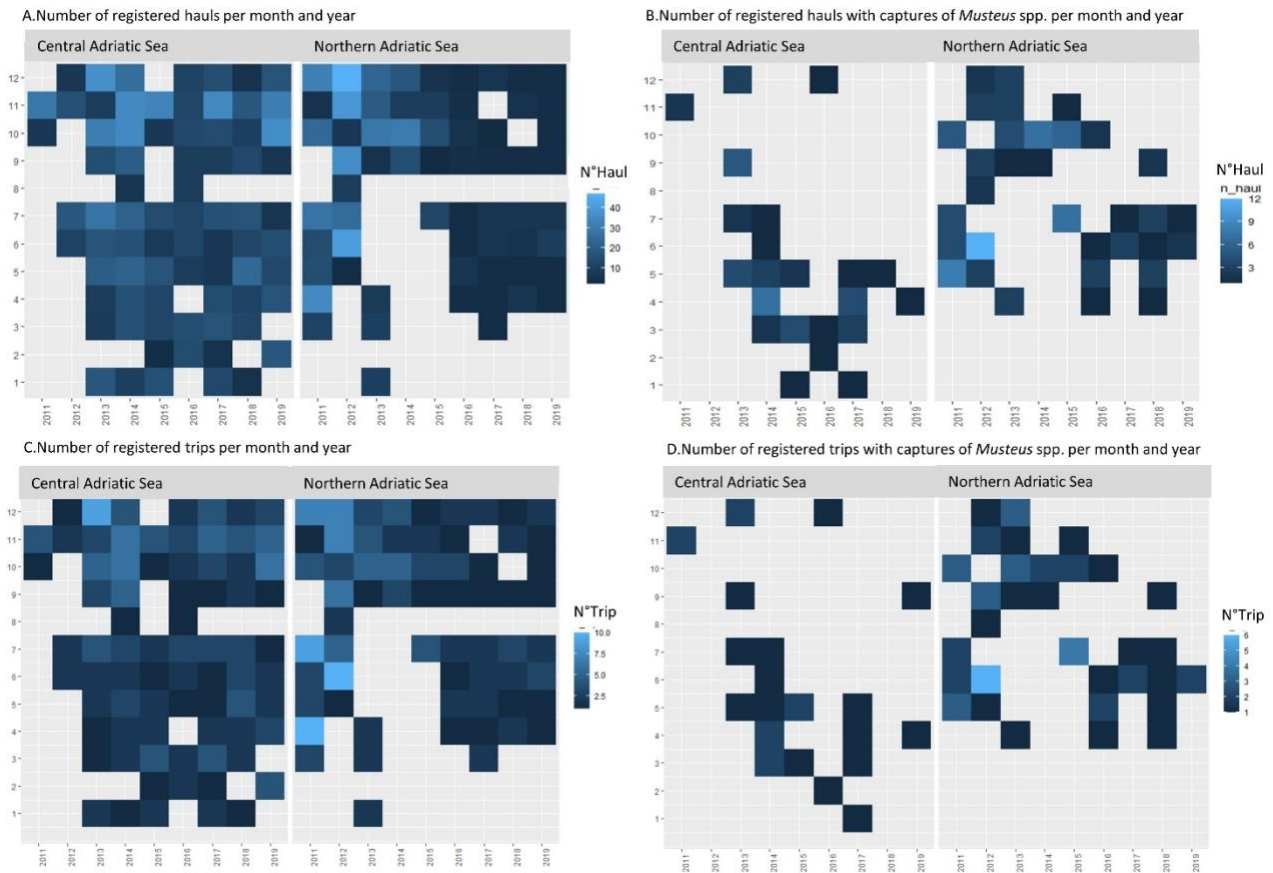
Figure 2: Number of all hauls (A) and trips (C) registered in the CAMPBIOL program, number of hauls (B) and number of trips (D) with catches of Mustelus spp. in the northern and central Adriatic Sea

The total number of registered individuals was 472 among which 257 YoY, 178 juveniles and 37 adults. The overall length frequency distribution showed that the highest counts of individuals were YoY, followed by juveniles and adults (Fig. 3). Among hauls, the highest number of individuals registered in one haul per fishing hour was approximately 8 YoY, 7 juveniles and 1 adult and 12 for unstructured density.
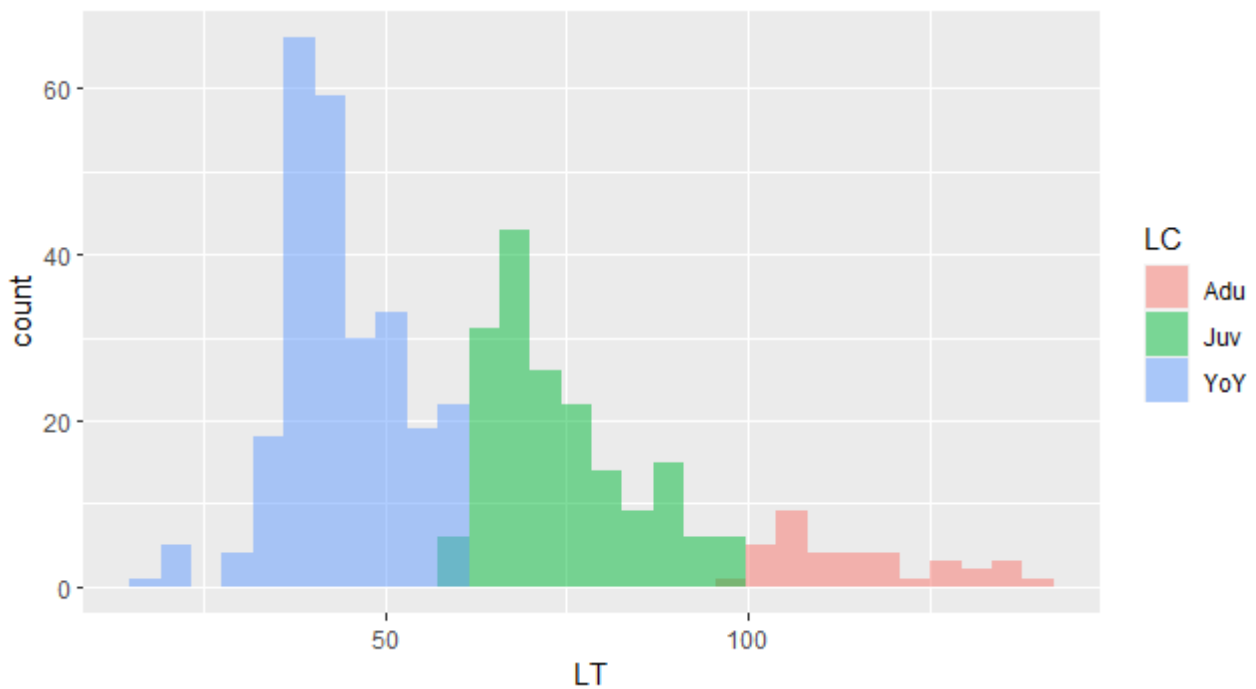
Figure 3: length frequency distribution of Mustelus spp. caught by monitored bottom trawlers. Registered total length (TL) in cm of adults (Adu), juveniles (Juv), YoY (young-of-the-year).

The observation of the unstructured density showed that the northern part of the Adriatic Sea is characterized by a large number of individuals per fishing hour close to the shore in the Gulf of Venice (< 50 m of depth) in the period from May to July (Fig. 4B), followed by the period from September to November (Fig. 4C). In the Winter months and in the beginning of the Spring, smooth hounds are found in the central part of the Adriatic far from the shore in deeper waters (> 50 m) with generally low-density values (Fig. 4A)
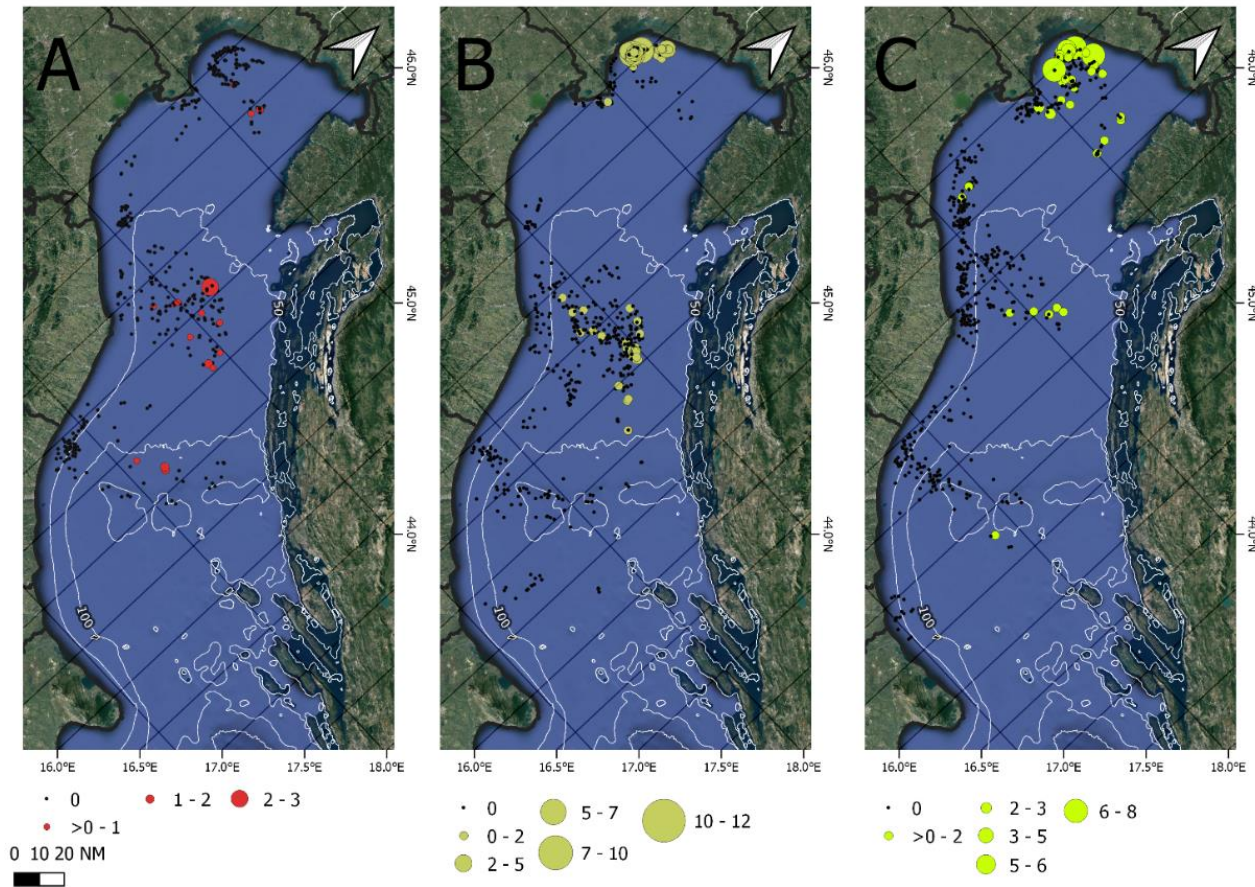
Figure 4: Unstructured density of Mustelus spp. from December to March(A), April to July (B), and September to November (C), dots size indicates the number of caught individuals per fishing hour.

Regarding the observation of the structured density of *Mustelus* spp., a high density of YoY and juveniles was registered in the shallow area in front of the Gulf of Venice, persistently throughout the Summer and Autumn periods, while in the period from December to March the number of reported YoY was scatter and lower than the other two periods. In all the three periods, very low but constant density was reported in the central area of the Adriatic Sea between 50 and 100 m, close to Pomo pit boundary (Fig. 5 and 6). Very low values of adult density emerged. However, the same pattern of YoY and juveniles in the use of space emerged for the adults, in particular the difference between the Winter and the Summer periods was striking (Fig. 7).
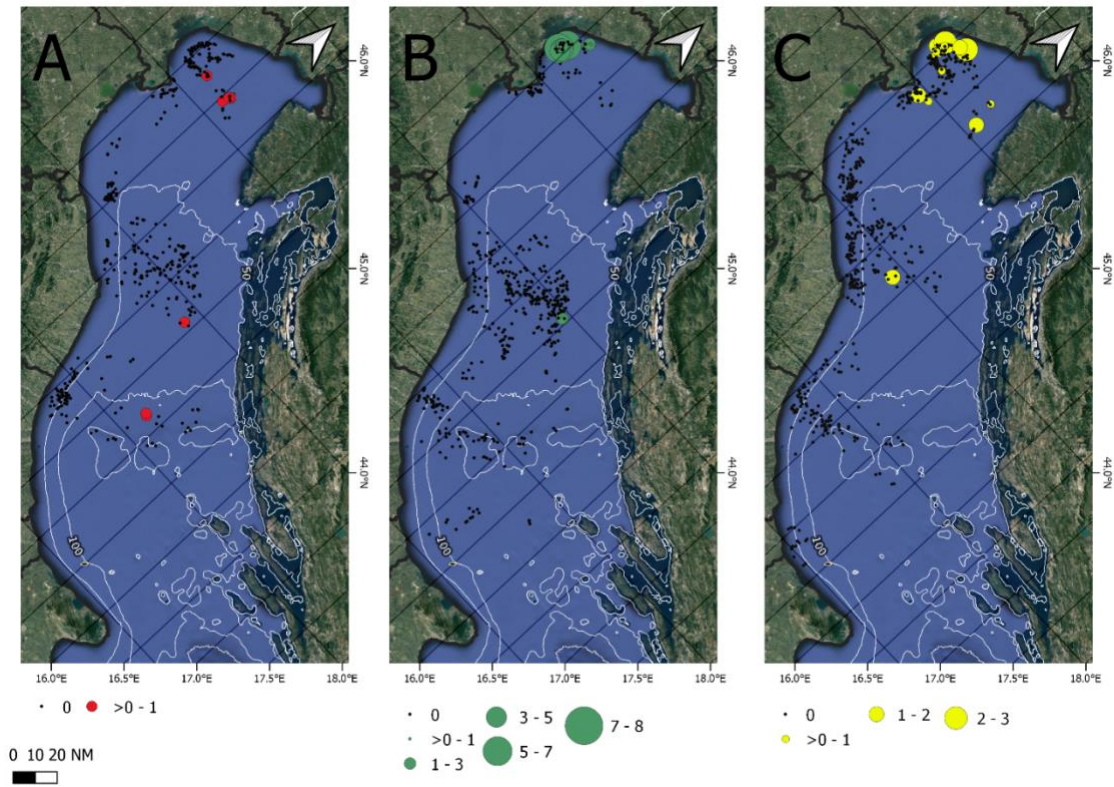
Figure 5: Desnity of Young of the Year from December to March(A), April to July (B), and September to November (C), dots size indicates the number of caught individuals per fishing hour.
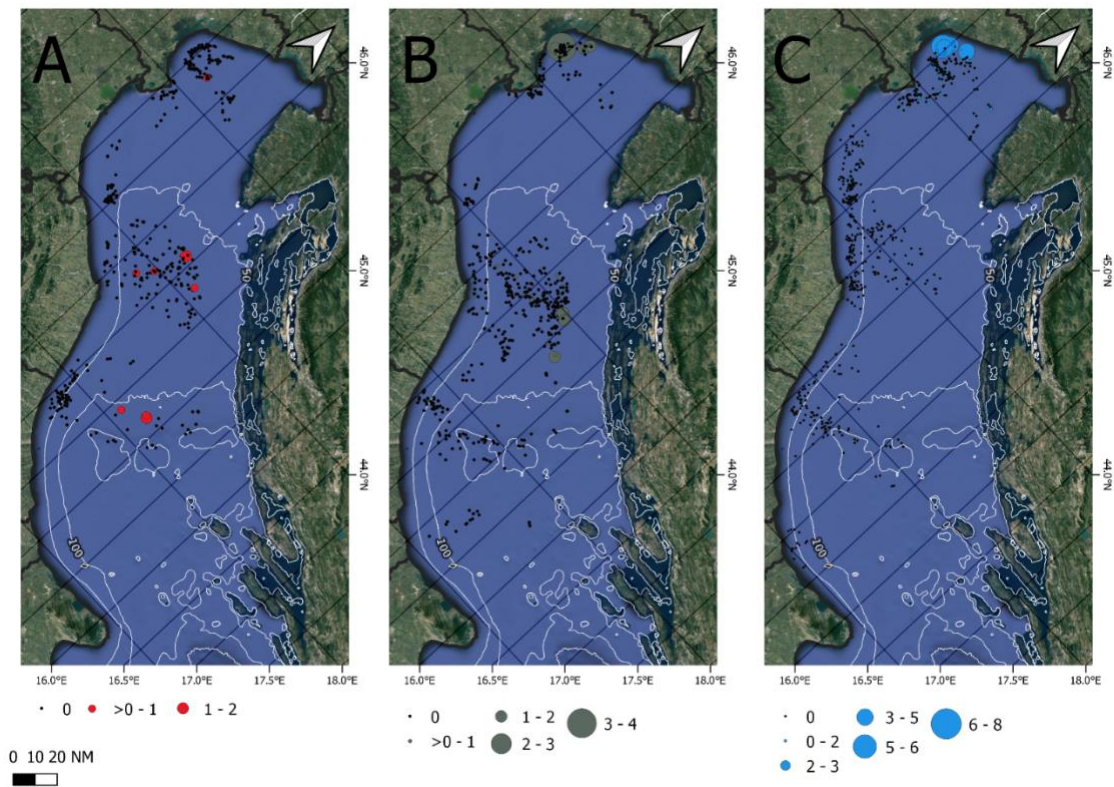
Figure 6: Density of juveniles from December to March(A), April to July (B), and September to November (C), dots size indicates the number of caught individuals per fishing hour.
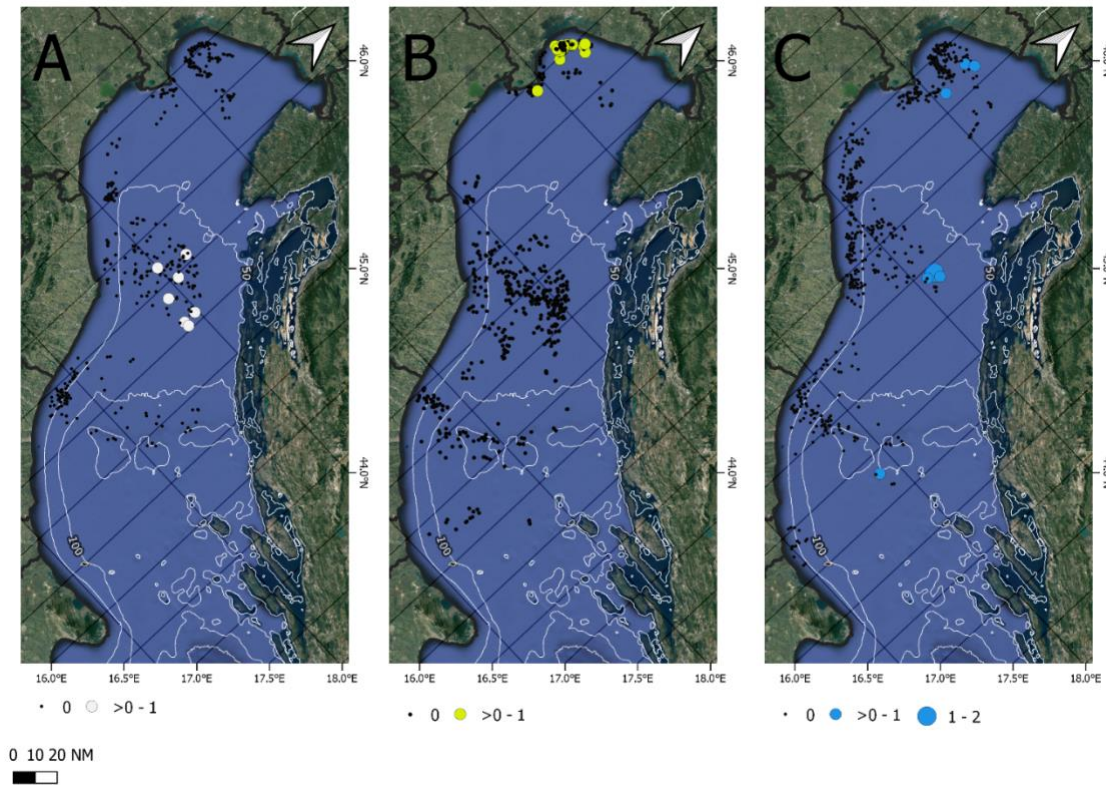
Figure 7: Density of adults from December to March(A), April to July (B), and September to November (C), dots size indicates the number of caught individuals per fishing hour.

4.4 Discussion

4.4.1 Recurrent movements and seasonal use of space

In this study, a recurrent periodicity of movements has been highlighted for *Mustelus* spp., with the occurrence of three periods during which *Mustelus* spp. may have a different use of space.

Landing data and CAMPBIOL observations highlighted that in the Winter and early Spring periods, the smooth hounds tend to be in the central part of the Adriatic Sea, far from the shore, while in the late Spring and in the summer, there may be a tendency to remain mainly in the areas of the Gulf of Venice (Veneto and Friuli V. Giulia, Italy), but their occurrence in the central Adriatic Sea was documented as well. Throughout the

Autumn, the persistence within the Gulf of Venice is supported by the observations. However, scattered presence of smooth hounds was reported in the central Adriatic Sea as well.

This movement pattern of *Mustelus* spp. has already emerged from previous research based on fishery dependent data recovered from mid-water trawlers (Bonanomi et al., 2018) and fishery independent data (Manfredi et al., 2010) data. Despite the very rare occurrence of the *M. asterias* in the Adriatic Sea, both studies did not tackle the misidentification issue between the *M. mustelus* and *M. punctulatus*, so findings were reported only for the *M. mustelus*, but they should be intended for *Mustelus* spp. Regardless the source of the data, the observation from the wavelet and life graph and from CAMPBIOL data are in line with the results in both studies (Manfredi et al., 2010; Bonanomi et al., 2018). However, in the latter study, the high period of catches seemed to be between April and June and were dominated by individuals of about 90 cm of TL. In this study, the bottom trawling data provided additional observations in relation to the young-of-the-year (< 60 cm in TL), confirming also the previous publication for larger individuals (Bonanomi et al., 2018). In the most recent study, the magnitude of the bycatch event was used as an index of the presence of mating and nursery areas (Bonanomi et al., 2018) which seemed to be in line with the CAMPBIOL observations, especially during the late Spring and summer period when the co-occurring presence of adults, juvenile and YoY was reported. However, the finer temporal resolution of the movement patterns, combined with the observations of YoY occurrence, may have shed light on a specific use of space for parturition purposes in shallow coastal areas like the Gulf of Venice. It is noteworthy that movement pattern and use of space across the years may be similar in the eastern Croatian side of the northern Adriatic Sea, in particular no data were available to see whether the deeper Croatian waters may be relevant for the smooth hound during the winter periods. More research is needed using fishery dependent data from both sides of the Adriatic Sea or fishery independent methodologies such as acoustic telemetry or conventional tagging.

In the Mediterranean Sea, other indications of nursery areas occurrence for *M. mustelus* and *M. punctulatus* emerged in the Gulf of Gabés (Tunisia), in the southern Mediterranean Sea, where young-of-the-year have

been found from April to July while juveniles were present throughout the year (Enajjar et al., 2015; Saidi et al., 2016). In such areas, the marine environment is mainly characterized by shallow and productive ecosystem (Saidi et al., 2008) which resemble the ecosystem in the Gulf of Venice. In fact, specific environmental features were found in relation to the occurrence of the different life stages in a congeneric species, the *M. schmitti,* with the presence of young-of-the-year and juveniles influenced by depth, temperature and salinity, especially in nearshore environment. The presence of adults was influenced by depth and temperature. During non-reproductive seasons, adults were found in deeper waters whereas shallow coastal habitats were typical for adults during reproduction season. Marked sexual segregation was also found associated with the pre- and post- mating periods (Cortés et al., 2011)

The recurrent movement pattern can also determine the fidelity of individuals to a specific site to carry out part of the life cycle. In other oceans, the site fidelity of *M. mustelus* has been reported at fine spatial scale (Klein et al., 2021) and acoustic telemetry has revealed that the same species movement was influenced by temperature, with a preference between 18° and 22°C (da Silva et al., 2021). In other congeneric species that are not found any longer in the Adriatic Sea or very rare, like *M. asterias,* the philopatric behaviour of both males and females was reported in eastern Atlantic Ocean and different migrating ranges emerged between mature and immature individuals (Brevé et al., 2016), Moreover, changes in the use of habitats according to the seasons emerged (Griffiths et al., 2020). In *M. lenticulatus* in New Zeland coastal water, juveniles were found to have wider habitat range in autumn compared to summer (Francis et al., 2013).

The *Mustelus* spp. movements are likely to be related to the seasonal variation in environmental conditions in the northern-central Adriatic Sea, which has been found to influence the seasonal movements of other marine species (Papetti et al., 2014; Morello & Arneri, 2009). During Winter and early Spring periods, mild and stable temperature present in the central Adriatic Sea could drive the smooth hound in the offshore area of the central Adriatic Sea, especially the eastern side that is characterized by the warmer and saltier water body mass coming from the southern Adriatic Sea (Russo & Artegiani, 1996). On the contrary, in late Spring

and summer, the strong river input in the northern part fuels the primary productivity creating the favourable conditions to sustain the physiological and foraging needs (Schalff et al., 2014).

4.4.2 Future directions: a modelling approach for seasonal use of space

To demonstrate the occurrence of nursery areas and different seasonal use of space, a spatial modelling could be a possible approach (Zuur et al., 2009). However, there are several obstacles and pitfalls to overcome in applying such methodology.

Generally, the use of fishery-dependent data consisted in a proxy of biomass or density expressed as the kilogram of landing or number of individuals divided by an index of effort (e.g., fishing hours, number of hooks, etc.) and in some cases, a spatial component was added in the standardization factor (e.g., fishing surface, grid cell, etc.). However, to make any spatial inference in unfished areas (e.g., where the fishers did not fish), some extrapolation modelling was necessary where the biomass or density proxy was modelled based on some predictors (e.g., environmental conditions, latitude, longitude or season) (Walter, 2003).

Fishery-dependent boarding data for elasmobranchs are often zero inflated since the elasmobranch catches are not very common and the fishing gear is not designed to target them. In various modelling approaches (Zuur et al 2009; Punt et al., 2000), the use of the zero inflated negative binomial distribution helps to deal with large amounts of zero catches, possibly being false zeros or true zeros. However, such distribution is used for discrete variable, so it often required rounding approximation (often expressed as real number in the use of CPUE) to the closest integer values.

To deal with zero-inflated continuous data, another approach is to use a two-parts regression model, either a generalized liner model (GLM), in case linear relationships are assumed between the response and the independent variables, or a general additive model (GAM), in case non-liner relationships are assumed (Zuur et al., 2009). In both models, the two parts consisted in modelling the presence and absence value with a

Bernoulli distribution and the non-zero catch by a gamma distribution which allows greater variation for large mean value (e.g., more tolerance for outliers in the response variable) (Zuur et al., 2010).

However, when it comes to use fishery-dependent data and environmental conditions in different areas of the same basin, in different seasons or sometimes in different years, a great deal of improvement has been evidenced by the application of spatial and temporal autocorrelation factor. Autocorrelation factor can help to reduce the variation at spatial or temporal level for those observations that are in close distance and with similar values, or that the same variable is measured in lagged interval series (Zuur & Ieno, 2018).

In the case of the CAMPBIOL boarding data, the only data series where seasonal boarding data are available, the different sampling effort across the sampling years, seasons and the Adriatic Sea surely contribute to make the application of spatial modelling challenging. However, a promising application could be including the spatial and temporal autocorrelation fact when inferring the seasonal use of space of such demersal sharks.

4.5 conclusion

The smooth hound are severely exploited in the Adriatic Sea, especially in the northern Adriatic part and this requires these species to be managed according to scientific based evidence. However, there are still some knowledge gaps in the biology (e.g., growth rate). This work was aimed at shedding light on the movement ecology and essential habitat delineation. In particular in the Gulf of Venice, the period raging from April to the late summer seemed to be relevant for the reproduction of this species some management measures should be tested and eventually implemented to sustainably manage such shark fishery. However the result in this chapter should be taken as an indication since more evaluation is necessary on movement range and essential habitat use through modelling the fishery data with environmental predictors and other fishery independent methodologies

Bibliography

Barausse, A., Correale, V., Curković, A., Finotto, L., Riginella, E. *et al.*, 2014. The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. *ICES Journal of Marine Science*, 71(7), 1593-1603.

Barbato, M., Barría, C., Bellodi, A., Bonanomi, S., Borme, D., Ćetković, I., ... & Mazzoldi, C. (2021). The use of fishers' Local Ecological Knowledge to reconstruct fish behavioural traits and fishers' perception of conservation relevance of elasmobranchs in the Mediterranean Sea. *Mediterranean Marine Science*, *22*(3), 603-622.

Bonanomi, S., Pulcinella, J., Fortuna, C. M., Moro, F., & Sala, A. (2018). Elasmobranch bycatch in the Italian Adriatic pelagic trawl fishery. *PloS one*, *13*(1), e0191647.

Braccini, M., Aires-da-Silva, A., & Taylor, I. (2016). Incorporating movement in the modelling of shark and ray population dynamics: approaches and management implications. *Reviews in Fish Biology and Fisheries*, *26*(1), 13-24.

Brevé, N. W. P., Winter, H. V., Van Overzee, H. M. J., Farrell, E. D., & Walker, P. A. (2016). Seasonal migration of the starry smooth-hound shark Mustelus asterias as revealed from tag-recapture data of an angler-led tagging programme. *Journal of fish biology*, *89*(2), 1158-1177.

Carpentieri, P., Nastasi, A., Sessa, M. & Srour, A., eds. 2021. Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries – A review. Studies and Reviews No. 101 (General Fisheries Commission for the Mediterranean). Rome, FAO. https://doi.org/10.4060/cb5405en

Cashion, M. S., Bailly, N., & Pauly, D. (2019). Official catch data underrepresent shark and ray taxa caught in Mediterranean and Black Sea fisheries. *Marine Policy*, *105*, 1-9.

Chapman, D.D., Feldheim, K.A., Papastamatiou, Y.P., & Hueter, R.E. (2015). There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. *Annual Review of Marine Science*, 7: 547-570.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W. W., Christensen, V., ... & Pauly, D. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. *Global Ecology and Biogeography*, *21*(4), 465-480. *doi:10.1111/j.1466-8238.2011.00697.x*

Colloca, F., Garofalo, G., Bitetto, I., Facchini, M. T., Grati, F., Martiradonna, A., ... & Spedicato, M. T. (2015). The seascape of demersal fish nursery areas in the North Mediterranean Sea, a first step towards the implementation of spatial planning for trawl fisheries. *PloS one*, *10*(3), e0119590.

Colloca, F., Enea, M., Ragonese, S., Di Lorenzo, M., 2017. A century of fishery data documenting the collapse of smooth-hounds (*Mustelus* spp.) in the Mediterranean Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27(6), 1145-1155.

Colloca, F., Carrozzi, V., Simonetti, A., & Di Lorenzo, M. (2020). Using Local Ecological Knowledge of fishers to reconstruct abundance trends of elasmobranch populations in the Strait of Sicily. *Frontiers in Marine Science*, 7: 508

Cortés, F., Jaureguizar, A. J., Menni, R. C., & Guerrero, R. A. (2011). Ontogenetic habitat preferences of the narrownose smooth-hound shark, Mustelus schmitti, in two Southwestern Atlantic coastal areas. *Hydrobiologia*, *661*(1), 445-456.

Council Regulation (EC) No 1967/2006 of 21 December 2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, amending Regulation (EEC) No 2847/93 and

repealing Regulation (EC) No 1626/94. [Viewed on 07/01/2022] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1967-20190814

Cozzi, S., & Giani, M. (2011). River water and nutrient discharges in the Northern Adriatic Sea: current importance and long term changes. *Continental Shelf Research*, *31*(18), 1881-1893.

Di Lorenzo, M., Vizzini, S., Signa, G., Andolina, C., Boscolo Palo, G., Gristina, M., Mazzoldi, C., & Colloca, F. (2020). Ontogenetic trophic segregation between two threatened smooth-hound sharks in the central Mediterranean Sea. *Scientific Reports*, 10: 11011.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., ... & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *elife*, *3*, e00590.

Dulvy, N.K., Simpfendorfer, C.A., Davidson, L.N., Fordham, S.V., Bräutigam, A. et al., (2017). Challenges and priorities in shark and ray conservation. *Current Biology*, 27 (11): R565-R572

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., ... & Simpfendorfer, C. A. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, *31*(21), 4773-4787.

Enajjar, S., Saïdi, B., &Bradaï, M. N. (2015). The Gulf of Gabes (Central Mediterranean Sea): a nursery area for sharks and batoids (Chondrichthyes: Elasmobranchii). *Cahiers de Biologie Marine*, 56: 143-150;

FAO 1990-2021. FAO Major Fishing Areas. MEDITERRANEAN AND BLACK SEA (Major Fishing Area 37). CWP Data Collection. In: FAO Fisheries Division [online]. Rome. Updated. [Cited 22 July 2021].

Ferretti, F., Myers, R. A., Serena, F., Lotze, H. K., 2008. Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology*, 22(4), 952-964

Ferretti, F., Osio, G.C., Jenkins, C. J., Rosenberg, A.A., Lotze, H.K., 2013. Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact.*Scientific Reports*, 3, 1057.

Follesa, M. C., Marongiu, M. F., Zupa, W., Bellodi, A., Cau, A., Cannas, R., ... & Carbonara, P. (2019). Spatial variability of Chondrichthyes in the northern Mediterranean. *Scientia Marina*, *83*(S1), 81-100.

Fortibuoni, T., Libralato, S., Raicevich, S., Giovanardi, O., & Solidoro, C. (2010). Coding early naturalists' accounts into long-term fish community changes in the Adriatic Sea (1800–2000). *PloS one*, *5*(11), e15502.

Francis, M. P. (2013). Temporal and spatial patterns of habitat use by juveniles of a small coastal shark (Mustelus lenticulatus) in an estuarine nursery. *PloS one*, *8*(2), e57021.

Griffiths, C. A., Wright, S. R., Silva, J. F., Ellis, J. R., Righton, D. A., & McCully Phillips, S. R. (2020). Horizontal and vertical movements of starry smooth-hound Mustelus asterias in the northeast Atlantic. *Plos one*, *15*(10), e0239480.

Hammer, Ø., Harper, D. A., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, *4*(1), 9.

Jacoby, D.M.P., Croft, D.P., & Sims D.W. (2011). Social behaviour in sharks and rays: analysis, patterns and implications for conservation. *Fish and Fisheries*, 13: 399-417.

Kinney, M. J., & Simpfendorfer, C. A. (2009). Reassessing the value of nursery areas to shark conservation and management. *Conservation letters*, *2*(2), 53-60.

Klein, J.D., Asbury, T.A., da Silva, C., Hull, K.L., Dicken M.L., Gennari, E., Maduna, S.N., Bester-van der Merwe A. (2021) Site fidelity and shallow genetic structure in the common smooth-hound shark Mustelus mustelus confirmed by tag-recapture and genetic data. *Journal of Fish Biolo*gy, doi: 10.1111/jfb.14926

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., & Jackson, J. B. C. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, 312 : 1806-1809.

Lotze, H. K., Coll, M., & Dunne, J. A. (2011). Historical changes in marine resources, foodweb structure and ecosystem functioning in the Adriatic Sea, Mediterranean. *Ecosystems*, 14: 198-222.

Manfredi, C., Ciavaglia, E., Isajlovic, I., Piccinetti, C., & Vrgoc, N. (2010). Temporal and spatial distribution of some elasmobranchs in the northern and central adriatic sea. *Biologia Marina Mediterranea*, *17*(1), 254.

Marino, I. A. M., Finotto, L., Colloca, F., Di Lorenzo, M., Gristina, M., Farrell, E. D., ... & Mazzoldi, C. (2018). Resolving the ambiguities in the identification of two smooth-hound sharks (Mustelus mustelus and Mustelus punctulatus) using genetics and morphology. *Marine Biodiversity*, *48*(3), 1551-1562.

Morello, E. B., and Arneri, E. 2009. Anchovy and sardine in the Adriatic Sea—an ecological review. *Oceanography and Marine Biology: An Annual Review*, 47: 209–256.

Pacoureau, N., Rigby, C. L., Kyne, P. M., Sherley, R. B., Winker, H., Carlson, J. K., ... & Dulvy, N. K. (2021). Half a century of global decline in oceanic sharks and rays. *Nature*, *589*(7843), 567-571. *doi:10.1038/s41586-020-03173-9*

Papetti, C., Di Franco, A., Zane, L., Guidetti, P., De Simone, V. *et al.*, 2013. Single population and common natal origin for Adriatic *Scomber scombrus* stocks: evidence from an integrated approach. *ICES Journal of Marine Science*, 70(2), 387-398.

Pitcher, C. R., Hiddink, J. G., Jennings, S., Collie, J., Parma, A. M., Amoroso, R., ... & Hilborn, R. (2022). Trawl impacts on the relative status of biotic communities of seabed sedimentary habitats in 24 regions worldwide. *Proceedings of the National Academy of Sciences*, *119*(2).

Prince, J. D. (2002). Gauntlet fisheries for elasmobranchs–the secret of sustainable shark fisheries. *Journal of Northwest Atlantic Fishery Science*, *35*, 407-416.

Punt, A.E., Walker T.I., Taylor B.L., Pribac F. (2000) Standardization of catch and effort data in a spatially-structured shark fishery. Fish Res 45, 129–145.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Riginella, E., Correale, V., Marino, I. A., Rasotto, M. B., Vrbatovic, A., Zane, L., & Mazzoldi, C. (2020). Contrasting life-history traits of two sympatric smooth-hound species: implication for vulnerability. *Journal of fish biology*, *96*(3), 853-857.

RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Russo, A., & Artegiani, A. (1996). Adriatic sea hydrography. *Scientia Marina*, *60*, 33-43.

Saïdi, B., Bradaï, M. N., & Bouaïn, A. (2008). Reproductive biology of the smooth-hound shark *Mustelus mustelus* (L.) in the Gulf of Gabès (south-central Mediterranean Sea). *Journal of Fish Biology*, 72: 1343-1354.

Saïdi, B., Bradaï, M. N., & Bouaïn, A. (2009). Reproductive biology and diet of Mustelus punctulatus (Risso, 1826) (Chondrichthyes: Triakidae) from Gulf of Gabès, central Mediterranean Sea. *Scientia Marina*, 73: 249-258.

Saïdi, B., Enajjar, S., & Bradaï, M. N. (2016). Elasmobranch captures in shrimps trammel net fishery off the Gulf of Gabés (Southern Tunisia, Mediterranean Sea). *Journal of Applied Ichthyology*, 32: 421-426.

Schlaff, A. M., Heupel, M. R., & Simpfendorfer, C. A. (2014). Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. *Reviews in Fish Biology and Fisheries*, *24*(4), 1089-1103.

Serena, F., Abella, A. J., Bargnesi, F., Barone, M., Colloca, F., Ferretti, F., ... & Moro, S. (2020). Species diversity, taxonomy and distribution of Chondrichthyes in the Mediterranean and Black Sea. *The European Zoological Journal*, *87*(1), 497-536.

da Silva, C., Kerwath, S. E., Winker, H., Lamberth, S. J., Attwood, C. G., Wilke, C. G., & Næsje, T. F. (2021). Testing the waters to find the 'goldilocks' zone: fine-scale movement of Mustelus mustelus in relation to environmental cues. *Marine and Freshwater Research*, *73*(1), 110-124.

Torrence, C. & G.P. Compo. 1998. A practical guide to wavelet analysis. Bulletin of the American Meteorological Society 79:61-78.

Walls RHL, & Dulvy NK. (2021) Tracking the rising extinction risk of sharks and rays in the Northeast Atlantic Ocean and Mediterranean Sea. *Scientific Reports* 11(1):15397.

Walters, C. (2003). Folly and fantasy in the analysis of spatial catch rate data. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*(12), 1433-1436.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution*, *1*(1), 3-14.

Zuur, A. F., & Ieno, E. N. (2018). *Gam and zero-inflated models* (Vol. II). Highland Statistics Ltd.

Chapter 5: Genetic structure and indication of female philopatry at regional scale in the commercially relevant demersal sharks, *Mustelus mustelus* and *M. punctulatus* (Triakidae), from two Mediterranean areas.

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Abstract

Elasmobranch species can be the direct or accidental catch of various fisheries and have a substantial commercial importance. In the Mediterranean Sea, an extensive period of overfishing has brought several stocks to be severely depleted. For this reason, the majority of elasmobranch species in the Mediterranean is threatened by extinction, especially because of the vulnerability related to their life history traits. In addition, complex behavioural traits can influence high localized abundance for those individuals showing specific site fidelity properties. In this study, focused on two heavily exploited smooth-hound species, *Mustelus mustelus* and *M. punctulatus*, the combination of nuclear and mitochondrial marker applications allowed to highlight that both species present a significant genetic structure and to suggest that females showed a philopatric behaviour while males could be the likely dispersing sex. Sexual differences in behaviour may influence the genetic structure in both species. The results of this study can contribute to the definition of management units for the conservation strategy in the Mediterranean Sea of the two shark species.

Keywords

5.1 Introduction

Elasmobranchs decline in the Mediterranean Sea is worsening in the last decades (Walls & Dulvy, 2021) after extensive overexploitation of many shark, skate, and ray species (Ferretti et al., 2013; Barausse et al., 2014). Their life history traits, and in particular their slower growth rate and lower fecundity compared to other marine species, make elasmobranchs particularly vulnerable to overfishing (Dulvy et al., 2017). In addition, complex behaviours, with non-random use of space and migratory movements, can lead to localized aggregations or high density of individuals that show philopatric or natal homing behaviours (Heuter et al., 2004; Chapman et al., 2015), determining additional vulnerability, especially in specific contexts characterized by high fishing pressure like the Mediterranean Sea (Coll et al., 2012).

To sustainably manage any fishery, it is crucial to know both biological and behavioural traits of commercial species such as recruitment, growth, size and age at sexual maturity, natural and fishing mortality rates, and movements. In particular, the latter determines dispersal and population connectivity, a key feature for maintaining genetic diversity and determining the management unit of a species (Oveden, 2013; Cadrin et al., 2014), and its knowledge is therefore critical for population conservation especially in commercially exploited elasmobranch species (Domingues et al., 2018). Species dispersal is highly variable among species and can be related to specific life stages (larval, juvenile, adult stages) or sex. While in teleost fish dispersal occurs mainly passively at egg, larval or juvenile stages, in elasmobranchs, the lack of a larval stage implies that active movements of adults play a crucial role (Ovenden et al., 2013). In elasmobranchs, sex-biased dispersal has been documented in several species, and where present, males have higher dispersal (Phillips et al., 2021). Genetic connectivity can be shaped by philopatric behaviours, i.e., the tendency of individuals to return or stay at their natal sites (Chapman et al., 2015). In elasmobranchs, this behaviour has been documented to be related to parturition and mating which may highly reduce population connectivity even in highly mobile species (Hueter et al., 2004; Chapman et al., 2015).

Different methods can be used to evaluate movements, dispersal, philopatry and ultimately connectivity. At short-medium time scale, there is an increasing number of studies using different types of tags to track animal

movements whereas at long time scale, the most used approach to evaluate connectivity is represented by genetics which can unveil recurrent patterns of migration, sex-biased dispersal or philopatric behaviour (Hueter et al., 2005; Chapman et al., 2015). In the genetic approach, combining the application of nuclear (nDNA) and mitochondrial DNA (mtDNA) molecular markers has often been successful in studying the various aspects of population connectivity since their difference in inheritance mode, evolution rate and between sex (Dudgeon et al 2012; Philips et al 2021). Microsatellite markers have been often used as nDNA markers and are based on short and repeated motif of nucleotides inherited by both parents (Dudgeon et al 2012; Portnoy & Heist, 2012). In mtDNA, non-coding control region (CR) is often used as marker since it is uniparentally inherited and presents variable regions less constrained by selection then other protein-coding mtDNA genes (Portnoy & Heist, 2012).

In the Mediterranean Sea, two species of smooth-hounds, *Mustelus mustelus* and *M. punctulatus*, are among the most important shark species landed in the Mediterranean Sea (Carpentieri et al., 2021). These species are mainly by-catch of fishery targeting more valuable species, but also a seasonal target of small-scale artisanal fishery, operating mainly in the northern Adriatic Sea and Strait of Sicily which are the two Mediterranean areas where the smooth-hounds contribute consistently to the elasmobranch landings (Barausse et al., 2014; Colloca et al., 2017; Carpentieri et al., 2021). The common smooth-hound (*M. mustelus*) and the black spotted smooth-hound (*M. punctulatus*) have cumulatively suffered of a strong decline in the last century, with a strong contraction of their range of occurrence (Colloca et al., 2017). The morphological similarities between the two smooth-hounds often represented an obstacle for species identification, and consequently for species-specific data collection, until a genetic approach contributed significantly to identify key features for distinguishing the two species (Marino et al., 2015a; Marino et al., 2018). *M. mustelus* and *M. punctulatus* share similar life-history and ecological traits (Riginella et al., 2020; Di Lorenzo et al., 2020), and the reproductive strategy with the occurrence of multiple paternity (Marino et al., 2015b). In addition, genetic studies have helped to find out that hybridization may occur, though it has been detected in a single clutch of embryos (Marino et al., 2015b). In several GSAs of the Mediterranean Sea, these benthic sharks tend to form reproductive aggregations composed mainly by large pregnant females

(Barbato et al., 2021). These aggregations represent locally and seasonally a target for artisanal fishery (Colloca et al., 2017). In *Mustelus* species, philopatric behaviours of both sexes (Brevé et al., 2016) or female philopatric behaviours and male dispersal have been reported (Sandoval-Castillo & Beheregaray, 2015), with consequences in terms of population structures. In South-African sites, the evidence of site fidelity, based on conventional tagging data, was not coupled with a significant genetic structure, evaluated through genetic analyses in *M. mustelus* (Klein et al., 2021).

Despite their decline, the reduction of range of occurrence and the need to effectively manage the smooth-hound populations (Colloca et al., 2017), up to now no studies were conducted to evaluate population connectivity, here intended as "the number of self-sustaining population" (Sinclar, 1988), in the Mediterranean Sea. With this study, we aim at assessing the population connectivity and sex-biased dispersal of *M. mustelus* and *M. punctulatus* in two subareas of the Mediterranean Sea, the Adriatic Sea and the Strait of Sicily, using 17 nuclear markers (microsatellites) and one mitochondrial marker (CR) for each species.

5.2 Material and Method

5.2.1 Sample collection and DNA extraction

During scientific or onboard surveys of professional fishing vessels, 325 muscle tissue samples were collected opportunistically from retained and discarded smooth-hounds in the Strait of Sicily (FAO-GFCM geographical sub area – GSA- 16) and in two areas of the Adriatic Sea (Figure); catches were obtained with various fishing gears (i.e., pelagic midwater trawler, bottom otter trawler and gillnet). The two sampling areas within the Adriatic Sea are the Italian western coasts of the GSA 17, herein defined as northern-central Adriatic Sea, and the eastern Montenegrin coasts of the GSA 18, herein defined as southern Adriatic Sea. All the analysed samples were collected between 2016 and 2020 across the sampling sites; in the southern Adriatic Sea samples were collected only in 2020. Sampled individuals were identified using the latest diagnostic morphological features (Marino et al., 2018) and total length (TL), by a measuring tape, in centimetres (cm), sex (according to the presence of claspers in males, Conrath, 2005), and haul coordinates were recorded. A muscle sample was then collected and immediately stored in pure grade ethanol at 4° Celsius (°C) until

further analyses. Length frequency distribution of individuals and sampling years were created by R package ggplot2 (Wickham, 2016).

Genomic DNA was extracted from 30-40 mg tissue samples by salting-out protocol (Patwary et al. 1994) and the extract quality was checked by 1% agarose gel in TBE buffer (1X) electrophoresis, with 0.025 μL/mL of GelRed stain (Biotium). Among extracted samples, 25 did not achieve a satisfactory quality in extracted genomic DNA and were discarded. Extracted DNA was conserved at -20°C.

5.2.2 Nuclear DNA amplification and genotyping

From a batch previously tested microsatellites (Marino et al., 2015; Maduna et al., 2017), a total number of 19 microsatellites were considered for achieving this study objectives (Annex III, Tab. S1). The amplification by polymerase chain reaction (PCR) of each microsatellite primer pairs on 4 randomly selected samples of both species was individually checked by the expected amplicon length in 1.8% agarose gel in TBE buffer (1X) electrophoresis, with 0.025 μL/mL of GelRed stain (Biotium). Two pairs of both species-specific and polymorphic microsatellites were included such as Mmu9 and Gg22 in *M. mustelus* and MaND5 and Mh29 in *M. punctulatus* (Marino et al 2015a) to obtain greater inference power to identify any hybrid either based on species-specific amplification or allelic frequency.

The microsatellites were divided into three groups (Tab.S1) and amplified by Mulitplex PCR using the following settings: initial denaturation 15 min 95 °C, followed by 30 cycles of 30 sec denaturation at 94 °C, 1:30 min annealing (T_a) at 63 °C for Mix 2 and 57 °C for Mix1 and Mix3 and 1 min extension at 72 °C, final extension at 60 °C for 30 min. Multiplex PCR reactions were prepared in 10 μL of total volume containing the following reagents: 1X QIAGEN Multiplex PCR Master Mix (QIAGEN, HotStarTaq DNA Polymerase, Multiplex PCR Buffer, dNTPs Mix), 0.2 μM primer mix and roughly 100 ng of extracted DNA. For each microsatellite, fluorophores were chosen according to length to avoid overlapping. Prior to analyse all samples, we selected randomly 8 individuals, 4 for each species, to compare the profiles of each microsatellite (i.e., allelic peaks) when amplified individually or in multiplex PCR.

A separate PCR for *Mmu11* locus was carried out and its PCR product was eventually assembled before genotyping service. This was necessary because that microsatellite did not amplify in multiplex PCR while those included in multiplex PCR were successfully amplified in multiple and matching hetero or homo-zygosity was found when allelic peak was compared in single or multiplex PCR. The single PCR was performed with the following reagents: 1x PCR Buffer (Solis BioDyne), 0.2 mM of deoxynucleotide triphosphates (dNTPs; Solis BioDyne), 1.5 mM MgCl$_2$ (Solis BioDyne), 0.2 µM of forward and reverse primers, 0.05 U/µL Taq polymerase (Solis BioDyne) and roughly 100 ng of extracted DNA, for a total volume of 10 µL. The PCR reaction profile consisted in: initial denaturation 2 min 95 °C, followed by 35 cycles of 30 sec denaturation at 94 °C, 30 sec annealing at 56 °C and 30 sec extension at 72 °C, final extension at 72 °C for 5 min.

After checking successful amplification in 1.8% agarose gel in TBE buffer (1X) electrophoresis, with 0.025 µL/mL of GelRed stain (Biotium), PCR products were assembled and sent to the BMR Genomics (Padua, Italy) for genotyping service where an ABI Prism 3100 Genetic Analyzer and LIZ500 as size standard were used. Scoring to examine the allelic profiles was performed for each sample by the software PEAK SCANNER v1.0 (Applied Biosystem). The binning was done by FLEXIBIN (Amos et al., 2007).

Samples with a high percentage (> 80%) of missing loci were discarded from downstream analyses. Then, microsatellite genotypes were first checked for null alleles using MicroChecker v2.2.3 (Van Oosterhout et al., 2004). GENEPOP ON THE WEB v4.2 was used for testing linkage disequilibrium (LD) and deviations from Hardy–Weinberg Equilibrium (HWE). For statistical significance of multiple tests, Benjamini–Hochberg (B-H, Benjamini and Hochberg, 1995) correction was applied.

5.2.3 Mitochondrial DNA amplification

For the CR amplification, the primers pair MaCYB/MaDLP (5'-TAA CTT GAA TTG GRG GRC AAC-3'/5'-GCA TTA ATC AGA TGY CAG RT-3') was used (Hull et al., 2019), following protocols reported therein. After confirming the amplification by 1.8% agarose gel in TBE buffer (1X) electrophoresis, with 0.025 µL/mL of GelRed stain (Biotium), amplicons were purified by EuroSAP - PCR Enzymatic Clean-up (Euroclone, Italy) and then sent for Sanger sequencing at the Eurofins Genomics (Colone, Germany). All sequences were manually checked for

quality by FinchTV v1.4 (Geospiza Inc.), then trimmed, edited and forward and reverse primers removed. All

sequence were then visualized in Mega 6 (Tamura et al., 2013) and aligned by Muscle algorithm (Edgar, 2004)

with default parameters.

5.2.4 Hybrid identification

To identify any possible hybrid among the sampled individuals, two separate analyses were applied for the

nDNA and mtDNA data, respectively.

An exploratory Bayesian structure analysis by STRUCTURE software (Pritchard et al., 2000) was run to obtain

the most reliable species identification for each specimen and identify possible hybrid based on allele

frequency from all considered microsatellites (N=19) including the species-specific and polymorphic ones

(Mmu9 and Gg22 in *M. mustelus* and MaND5 and Mh29 in *M. punctulatus*). Assuming an admixture ancestry

model with independent allelic frequencies and ignoring a prior of sample origin, ten independent runs for

each K tested (K= 1-5) were performed using 10^6 Markov chain Monte Carlo (MCMC) replications, a burn-in

period of 10^5 steps. The best K was selected according to the Delta K, Ln Pr(X|K) (Evanno et al., 2005) and

plots were visualized using web-based software CLUMPAK (Kopelman et al., 2015). Following to this analysis,

hybrid individuals were identified and isolated from population connectivity analysis.

For the mtDNA, an exploratory haplotype network was created by using PopART (Leigh & Bryant, 2015) and

TCS network (Clement et al., 2002). The species identification from STRUCTURE results was used for the input

sequences as species label in the TSC network. A hybrid was detected if an individual whose species was

identified by nDNA, was found in a haplotype belonging to the other *Mustelus* species.

5.2.5 nDNA and mtDNA diversity

Using the species identification based on exploratory STRUCTURE results, the nDNA genetic diversity was

calculated using the R package DiveRsity (Keenan et al., 2013), as the number of alleles observed per locus

per population sample (A), the allelic richness (A_R), the observed and expected heterozygosity (H_O and H_E),

and the inbreeding coefficient (F_{IS}), together with its 95% confidence of interval (F_{IS} CI). For mtDNA, the CR

diversity was calculated by DNAsp (Rozas et al., 2017) based on the same species identification. Total number of haplotypes (H), haplotype diversity (h) and nucleotide diversity (π) were obtained. Tajima's D neutrality test (Tajima, 1989) was run by ARLEQUIN (Excoffier and Lischer, 2010).

Regarding the mtDNA, the sequenced fragment encompassed the last part of cytochrome b and the first of the CR (orientation 5'- 3') when compared with the full mitochondrial genome of *M. mustelus* available on GenBank (Accession number MH559351.1) by Blastn (Altschul et al., 1990) and Clustal Muscle alignment (Edgar, 2004). For this reason, all the downstream analyses were performed considering only the CR fragment (483 bp) or using the full-length fragment including part of the cytochrome b (713 bp).

5.2.6 Population connectivity

To assess the level of genetic structure among the sampling sites in the two species a Bayesian clustering analysis by STRUCTURE was performed with correlated allelic frequencies as above. The prior information of sample origin was also tested in separate trials.

For both nDNA and mtDNA, genetic differentiation among sampling areas was determined in ARLEQUIN (Excoffier & Lischer,2010) by pairwise F_{ST} test with 10^4 permutation and a significance level set for p-value < 0.05. B-H correction for multiple comparisons was applied when necessary. To further investigate whether the population connectivity was shaped by a sex biased dispersal, separate trials were run dividing the nuclear genotypes and CR sequences according to sex and TL. A minimum threshold of 60 cm of TL was chosen since below 60 cm individuals are likely to be young of the year and therefore less likely to disperse (Barbato pers. obs).

Two methods were used to indirectly assess a sex-mediated gene flow, but they account for the dispersal effect rather than a true migration estimation. The assignment method was applied by GeneClass v2.0 (Piri et al., 2004) with assignment threshold score set at 95%. The assigned site was considered as such when above 10% of probability and as discarded when below 5%.

Given the different inheritance mode and evolution rate, the comparison of F_{ST} derived either by nDNA or mtDNA can be obtained and being informative of possible sex-biased dispersal. To do this, despite the strong assumptions, the Wright's Island model (Wright 1987) could provide the absolute number of migrants per generation by nDNA-derived F_{ST} and the female migrants per generation from mtDNA-derived F_{ST} (Salvato et al., 2002). The absolute number of migrating females per generation was calculated based on Wright's Island model (Wright 1987) as: $N_F m_F = ((1/F_{ST}) -1) / 2$ where N_F is the effective female population size and m_F is the migration rate of females. Whilst the absolute number of individuals exchanged can be obtained by the following formula: $Nm = ((1/F_{ST}) – 1)/4$. Finally, the two estimates were statistically compared by calculating the confidence of interval (CI) at 95% by calculating the F_{ST} CI.

To assess and sibship among sampled individuals, Colony v2.0.6.7 (Jones and Wang, 2010) was used to assess: (*i*) whether a philopatric behaviour could be reflected in parentage link among sampled individuals; (*ii*) seasonal localization could be inferred from sibship of individual sampled in close distance and period of the year. For each species, separate runs were performed selecting for female and male polygamy with inbreeding and cloning, by full-likelihood method at 95% and no prior.

5.3 Results

5.3.1 Hybrid identification

Based on the microsatellite data, STRUCTURE cluster analysis allowed identifying 1 hybrid, moreover 3 samples had too many missing loci (Annex III, Fig. S1). These samples were removed from the analyses. The genetic divergence between the two species is 0.63 of F_{ST} (p-value < 0.001). The identified hybrid (samples #1103) was a morphologically identified *M. mustelus* male of 91 cm of TL from the northern-central Adriatic Sea, sexually immature. Among the species-specific microsatellite, Mmu9, Gg22, specific for the *M. mustelus,* and Mh29, specific for the other one, were amplified and detected in that sample while the CR fragment resulted to belong to the *M. mustelus*.

After constructing the haplotype network and occurrence table across all samples, one sample (#S301) was identified, presenting a *M. punctulatus* haplotype, in an individual morphologically-identified as *M. mustelus* and sexually mature male of 135 cm of TL. In this specimen, only the species-specific microsatellite *Mmu9* was amplified and detected. Even though the sample was excluded in the analysis, its haplotype was reported in the tables of haplotype occurrence (Annex III, Tab. S4 and S5).

5.3.2 Nuclear DNA diversity

From the preliminary STRUCTURE analysis for species identification, the total number of samples included in downstream analyses were 86 *M. mustelus* and 214 *M. punctulatus*. For *M. mustelus*, 48 sampled individuals from the northern-central Adriatic Sea ranged from 34 to 163 cm of TL, and 24 samples for each sex were collected. The 38 samples from the Strait of Sicily ranged from 53.5 to 148 cm of TL, of which 21 females and 17 males were sampled. Only 3 samples were collected from the southern Adriatic Sea but were not included in the analyses due to the small sample size. For *M. punctulatus,* 126 samples were collected from northern-central Adriatic Sea, with individuals ranging from 31.6 to 142 cm of TL, among which 68 males and 58 females. In the southern Adriatic Sea, only 37 females were sampled, ranging in size from 92 to 138 cm of TL. In the Strait of Sicily, among the 51 sampled individuals which ranged between 35.5 and 92 cm of TL, 21 males and 30 females were analysed (Fig1).
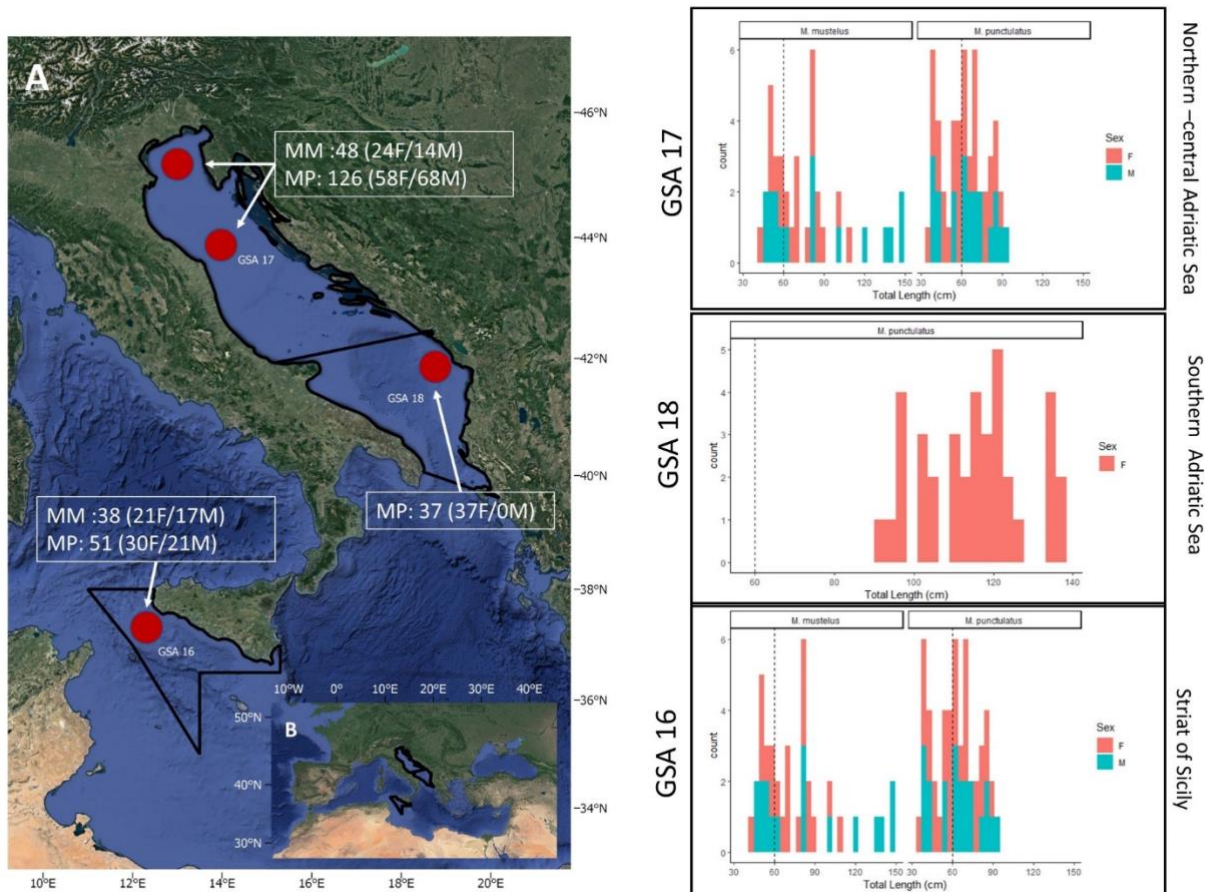
Figure 1: A) Map reporting the macro locations of the Adriatic Sea (GSA17 and GS18) and the Strait of Sicily (GSA16) in the Mediterranean Sea (Google Maps, 2021); B) length frequency distribution of the sampled M. mustelus (MM) and M. punctulatus (MP) females (red) and males (green) from in each sampling locations (red circles in the map. A.

All microsatellites did not show null alleles within and between origin sites, and deviation from HWE and LD (Annex III, Tab. S2, S3, S4). So, all loci were considered for the analyses. In *M. mustelus*, moderate genetic diversity emerged in both locations in allele number, allelic richness and observed heterozygosity, whereas *M. punctulatus* samples showed a lower nuclear diversity in the three considered parameters. Inbreeding coefficient and its related CI spanned between small negative and positive values (Tab.1). The mean global F_{IS} for each population sample resulted to be not significant given the 95% CI.

Table 1: Overall nuclear diversity for each species in the sampling area. Number of samples (N), number of alleles as a mean across loci (A), allelic richness as a mean across loci(A_R), observed heterozygosity as a mean across loci (Ho), expected heterozygosity as a mean across loci (He), fixation index as a mean across loci (Fis) and confidence interval of fixation index as a mean across loci (Fis CI).

	M. mustelus		*M. punctulatus*		
	Northern-central Adriatic Sea	Strait of Sicily	Northern—central Adriatic Sea	Southern Adriatic Sea	Strait of Sicily
N	48	38	126	37	51
A	77	67	45	37	40
A_R	3.42	3.2	2.33	2.14	2.22
Ho	0.31	0.37	0.26	0.22	0.25
He	0.35	0.36	0.26	0.23	0.25
F_{is}	0.1165	-0.0262	-0.0118	0.0519	0.0076
F_{is} CI	-0.0036 - 0.2633	-0.1792 - 0.2069	-0.0588 - 0.0365	-0.0475 - 0.1503	-0.058 - 0.0712

5.3.3 Mitochondrial DNA diversity

The mtDNA fragment was obtained from 83 samples of *M. mustelus* and 207 samples of *M. punctulatus* (Table 2). For this mtDNA analyses, 7 *M. punctulatus* (N=3 from the northern-central Adriatic, 1 from the southern Adriatic and 3 from the Strait of Sicily) and 3 *M. mustelus* (N=3 from the Strait of Sicily) samples were excluded due to the poor sequencing quality and failed amplification (N=9) and hybrid (N=1). No evidence of divergence was detected in the genetic differentiation between the full sequenced fragment and the uniquely trimmed CR sequence. So, all mtDNA analyses were executed with only the CR fragment. The number of haplotypes ranged from 3 to 5 and the segregating sites between 2 and 5 with lowest diversity values (h, π, *Pi*) found mainly in *M. punctulatus* across the three sites. All Tajima's D values are not statistically significant (Tab.2).

Table 2: CR diversity parameters according to sampling location and species. Number of samples (N), number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), segregating sites (S), mean number of pairwise difference (Pi), Tajima's D and Tajima's D p-values.

	M. mustelus		M. punctulatus		
	Northern-central Adriatic Sea	Strait of Sicily	Northern-central Adriatic Sea	Southern Adriatic Sea	Strait of Sicily
N	48	35	123	36	48
H	4	4	5	3	4
h	0.542857	0.522689	0.314941	0.164706	0.295213
π	0.00132	0.001246	0.000706	0.000348	0.001098
S	3	2	5	2	4
Pi	0.63755	0.60168	0.3396	0.16807	0.53014
Tajima's D	-0.09937	0.46792	-1.30605	-1.28069	-0.94279
Tajima's D p-value	0.4772	0.74010	0.0732	0.06940	0.19630

In *M. mustelus,* the haplotype N° 3 was the most frequent followed by the N° 4. The two sampling areas have nearly 10% difference in haplotype of the sequenced CR. Haplotype N° 2 and N° 10 were only found in the Strait of Sicily and the northern-central Adriatic Sea, respectively (Figure). *M. punctulatus* has the 83% of similar haplotypes (N° 1), present in the hybrid individual as well, and the N° 7 was the second most frequent haplotype. *M. punctulatus* presented 3 unique haplotypes in the northern-central Adriatic Sea (N° 11, 12,

13), one in the southern Adriatic Sea (N° 9), and 2 in the Strait of Sicily (N° 6 and 8) (Fig.2; Annex III, Tab. S4 and S5).
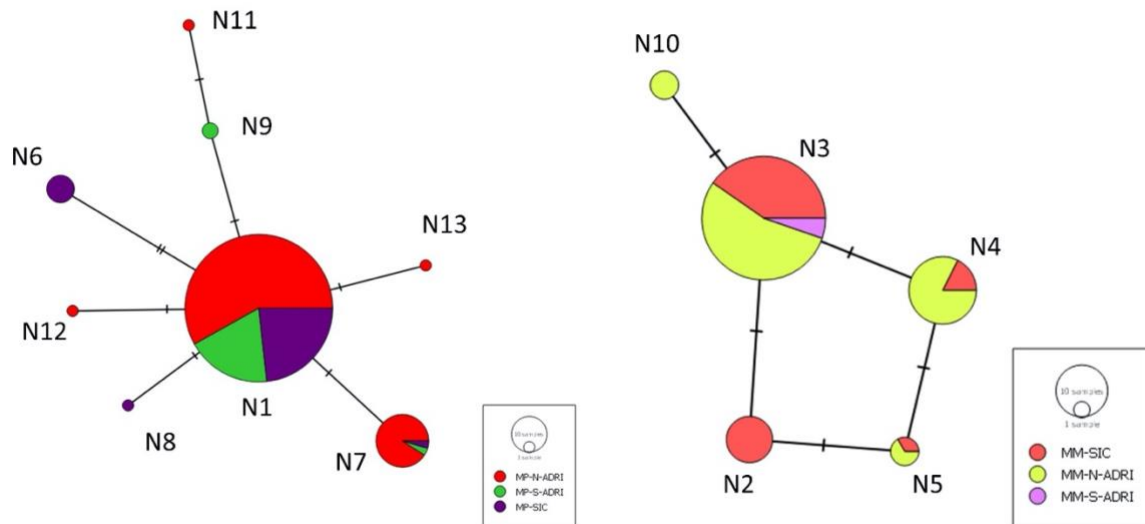
Figure 2: Haplotype network of the sequenced control region fragment of M. punctulatus in the left and of M. mustelus in the right. Hybrid sample was not reported in the figure. The 3 M. mustelus individuals from the southern Adriatic Sea were included only in the network.

5.3.4 Population connectivity

Without giving a prior information of sample origin the cluster analysis in STRUCTURE did not evidence the presence of structure between the two sampling areas in both species (Annex III, Fig. S2). However, based on ARLEQUIN software, microsatellite derived F_{ST} values showed a statistically significant differentiation in the individuals from the two sampling areas in both species (F_{ST} = 0.02, p-value = 0.005, in *M. mustelus* and F_{ST} = 0.03, p-value = 0.003 in *M. punctulatus*). When splitting the samples according to the size (only those above 60 cm of TL) and sex, a difference in microsatellite derived F_{ST} between individuals sampled in the Strait of Sicily and the Adriatic Sea emerged only in *M. punctulatus* males and females. On the contrary, no

statistically significant differences were found between males and females in *M. mustelus* and between the three sampling areas in *M. punctulatus* (Tab. 3). CR derived F_{ST} values showed significant differentiation in both species sampled in the Adriatic Sea and the Strait of Sicily (F_{ST} = 0.12, p-value = 0.001, in *M. mustelus* and F_{ST} = 0.086, p-value < 0.001 in *M. punctulatus*). After B-H correction, moderate genetic divergence emerged only between the northern-central Adriatic Sea and the Strait of Sicily, but no statistical significance was observed between the two Adriatic parts (northern-central vs southern) and between the southern Adriatic Sea and the Strait of Sicily (Tab.3). When calculating the CR derived F_{ST} only in the individuals above 60 cm of TL, the female F_{ST} in both species showed a strong mitochondrial structure compared to the males whose F_{ST} did not result statistically significant. No differences were found in females between the two parts of the Adriatic Sea (Tab.3).

Table 3: F_{ST} values calculated according to sampling origins (Adri: Adriatic Sea; N-C Adri: northern central Adriatic Sea; S-Adri: southern Adriatic Sea; SIC: Strait of Sicily), size and sex. p-values are reported in bold when statistically significant. Statistical significance is set by ARLEQUIN default setting at p-value < 0.05 and, after B-H correction, significance was set at p-values < 0.005 when applied and marked by an asterisk. N refers to the sample size.

			M. mustelus	*M. punctulatus*			
			ADRI-SIC	ADRI/SIC	N-C-ADRI/S-ADRI	N-C-ADRI/SIC	S-ADRI/SIC
Microsatellite derived F_{ST}	**All individuals**	F_{ST}	**0.02**	**0.03**	0.002	**0.032**	**0.026**
		p-value	**0.005**	**0.003**	0.23*	**<0.001***	**<0.001***
		N	48:38	163:51	126:37	126:51	37:51
	Male TL> 60 cm	F_{ST}	0.009	-	-	**0.045**	-
		p-value	0.293	-	-	**< 0.001**	-
		N	17:11	-	-	47:14	-
	Female TL> 60 cm	F_{ST}	0.015	**0.03**	0.005	0.031	0.030
		p-value	0.0708	**0.003**	0.14*	0.006*	0.01*
		N	20:14	76:15	39:37	39:15	37:15
CR derived F_{ST}	**All individual**	F_{ST}	**0.122**	**0.086**	0.039	**0.091**	0.063
		p-value	**0.001**	**<0.001**	0.026*	**<0.001***	0.026*
		N	48:35	160:48	123:36	123:48	36:48
	Male TL> 60 cm	F_{ST}	0.13	-	-	-0.01	-
		p-value	0.12	-	-	0.59	-
		N	17:10	-	-	45:7	-
	Female TL> 60 cm	F_{ST}	**0.31**	**0.30**	0.09	**0.26**	**0.26**
		p-value	**<0.001**	**< 0.001**	0.015	**< 0.001***	**0.002***
		N	20:14	74:14	40:34	40:14	34:14

Between the Adriatic Sea and the Strait of Sicily, the numerical outputs of Wright's Island did not reveal a strongly unbalanced migration rate according the F_{ST} comparison. In *M. mustelus*, the female migration rate

consisted of 4 individual per generation while the absolute number of migrants appeared to be of 11 individuals per generation. In *M. punctulatus*, 5 female migrants per generation emerged while the absolute number of migrants was attributed to be of 8 individuals per genration.

The assignment test found one male and one female of unknow origins in *M. mustelus*, while 2 females of unknown origins in the northern-central Adriatic and 2 females sampled in the southern Adriatic assigned to the northern-central Adriatic Sea, in *M. punctulatus*.

The COLONY analysis revealed only two full-sibship pairs of individuals above 95% likelihood in *M. mustelus*: two Adriatic females (#1003 and #1050), both caught in 2018 and in northern Adriatic Sea (Gulf of Venice) at 34 kilometres of distance, one measuring 41 cm of TL in July and the other one measuring 68 cm of TL in November; two individuals of 90 and 53.5 cm of TL respectively (one female and one male; #S303 and #S305) from the Strait of Sicily, caught in two separate hauls within the same scientific survey campaign in 2017, thus sampled in the same month. In *M. punctulatus*, only two females (#S302 and #S308), one measuring 85 and 50.5 cm in TL respectively, from the Strait of Sicily and caught in the 2017 in separate hauls, were found to be with 97.5% probability to be full siblings. No individuals were found to be half-siblings in both species.

5.4 Discussion

This is the first study that investigated the intra-Mediterranean population connectivity (Sinclair, 1988) of the two *Mustelus* species in areas where the occurrence and landing of these species are still abundant (Carpentieri et al., 2021; Colloca et al., 2017). In our study, the use of cumulatively 17 microsatellites combined with a CR fragment for both species brought to the first assessment of a statistically significant population structure among the Adriatic Sea and the Strait of Sicily according to the nDNA and mtDNA-CR genetic difference based on F_{ST}, in both species. Given the F_{ST} divergence and unique CR haplotype in different sampling location, our study also suggested the occurrence of female philopatry and male dispersal in *M. mustelus*, a species widely studied in other oceans (Hull et at., 2019; Klein et al 2021), and in *M. punctulatus*, a species for which no data are available, in the Mediterranean Sea. In addition, the identification through microsatellites of one hybrid is in line with previously reported hybridization and multiple paternity in the

Adriatic Sea (Marino et al., 2015b). However, for the first time, a maternal hybrid was identified by the CR sequence in the Strait of Sicily. The use of CR for maternal hybridization has been previously applied on *Sphyrna* species (Barker et al., 2019; Pazmiño et al., 2019) or together with other mitochondrial markers in *Carcharhinus* species (Morgan et al., 2012). Hybridization can lead to a source of genetic variation for threatened species (Allendorf et al., 2011) or to the introduction of adaptive variation to overcome environmental change (Becker et al., 2013). However, if hybridization regardless the cause (e.g., fishery or other anthropogenic pressures) could induce a reduction in reproductive fitness, this will clearly represent an additional threat to the species conservation (Rhymes and Simberloff, 1996). For these reasons, more detailed research is needed on the consequence of hybridization in the two *Mustelus* species.

5.4.1 Population connectivity

The genetic divergence between the Adriatic Sea and the Strait of Sicily found in our study in a benthic elasmobranch species is in line with other investigations among the Mediterranean basins, where genetic structure has emerged (Gubili et al., 2014; Catalano et al., 2021). In the small spotted catshark (*Schyliorhinus canicula)*, besides the strong isolation from the Atlantic population, the Adriatic population of this species has resulted to be genetically different respect to the other investigated locations (Balearic, Sardinia and Crete waters), with microsatellite derived F_{ST} ranging between 0.02 to 0.06.(Gubili et al., 2014). Recently, the connectivity of the starry skate (*Raja asterias*) has been evaluated by the combined use of microsatellite and cytochrome c oxidase subunit I (COI) (Catalano et al., 2021). In this benthic skate, the individuals from 8 Mediterranean locations have been compared in their level of genetic divergence and, only in areas where a substantially sample size was available (30-50 samples), statistically significant differences have been reported: between the northern Adriatic individuals compared with, respectively, Sardinian waters (F_{ST} = 0.12), northern Tyrrhenian (F_{ST} = 0.21), and central Tyrrhenian (F_{ST} = 0.16). In other marine species whose dispersal occurs at larval stage, genetic structure has been evidenced in relation with oceanographic features for demersal species like in *Solea Solea* (Sabatini et al., 2018) and *Mullus* spp. (Matić-Skoko et al., 2018). Regarding differences between northern and southern Adriatic, the occurrence of fragmented populations has been suggested for teleost species like the Atlantic mackerel (*Scomber Scombrus)* (Papetti et al., 2014)

and *Mullus* spp. (Matić-Skoko et al., 2018). In both study cases, the restricted connectivity has been suggested to be related to the presence of constant gyres in the central Adriatic Sea and oceanic currents which flow from south to north in the eastern Adriatic coast and from north to south in the western shore (Russo & Artegiani, 1996). Within the Adriatic locations, this study has detected an overall panmixia in *M. punctulatus*, supported by both the nDNA and the CR, also considering only females with more dispersal capability (> 60 cm of TL). The intra-Adriatic water circulation and seasonal gyres (Russo & Artegiani, 1996) does not reduce the connectivity between North and South Adriatic likely because of the active dispersal capability by both males and female to overcome those ecological barriers that shape other marine species with larval dispersal (Papetti et al., 2014; Matić-Skoko et al., 2018). In the Mediterranean Sea, there is no information about the movement range of the two studied *Mustelus* species, but findings of the three full sibling pairs in close distance or in the same haul may represent a seasonal localization and residency in the two study areas. Indeed, a seasonal movement has been highlighted in the northern-central Adriatic Sea in a fishery-based study (Bonanomi et al., 2018) and potential difference in seasonal movement appeared in a local-ecological-knowledge based study (Barbato et al., 2021). However, such results may be also due to either a limited diversity in the microsatellite, which may overestimate the relatedness, or a very low effective and census population size which may raise serious concern on the conservation of the two species. The creation of a simulated dataset based on the microsatellite data of the two *Mustelus* species may clarify if the observed relatedness is due to a limited diversity in the microsatellite markers used in this study.

While no connectivity research is available in *M. punctulatus* from published literature, *M. mustelus* has been subjected to several study in other oceans (Mann & Bullen, 2009; da Silva et al., 2013; Maduna et al., 2017; Hull et al., 2019; Klein et al., 2021). At vast spatial scale, *M. mustelus* displayed a high level of isolation and the connectivity among populations seemed to be shaped by the effect of oceanic currents or other biogeographical barriers (Hull et al., 2019). On the contrary, at smaller spatial scale, a weak structure emerged (Maduna et al., 2017). In *M. mustelus*, a conventional tagging study in the South African waters highlighted a strong site fidelity, but no evidence of structure emerged (Klein et al., 2021). However, based on tag and recapture studies, long distance movements had been reported, with a registered distance of

1404 km of movement (Mann & Bullen, 2009). On the other hand, results obtained combing tagging and acoustic methodology suggested that the potential to undertake long migrations seemed unlikely (da Silva et al., 2013). It is also important to highlight that movement and gene flow do not always represent a straight link. In other words, an individual movement may occur without the success of mating in another population considering also the fact the immigrating individual will have to overcome behavioural knowledge to success in mating (Oveden, 2013)

When looking at the population connectivity studies on other congeneric species, in the brown smooth-hound (*M. henlei*), a strong genetic structure has been evidenced at fine regional scale (Sandoval-Castillo and Beheregaray, 2015). A different genetic methodology called single nucleotide polymorphism (SNP) has been able to show that the gummy shark (*M. antarcticus*) is structured in two discrete populations in the eastern and southern coasts of Australia, respectively (Petrolo et al., 2021). However, in *M. antarcticus*, acoustic telemetry in Australian waters showed wide movement range (238-900 km) (Braccini et al., 2017).

5.4.2 Philopatry and sex biased dispersal

The CR derived genetic divergence, when all samples are considered, highlighted higher level of genetic structure than nDNA-derived one. However, when looking at the F_{ST} comparison of all sampled individuals, Wright's Island indicates that both sexes seem to contribute to the similar exchange rate per generation. The latter aspect emerged from the Wright's Island may be a masked effect since in elasmobranchs migration is likely to occur at older age when sexual maturity is reached (Oveden, 2013). Indeed, when isolating the individuals with more dispersal capacity (above 60cm of TL), females showed very strong CR structure compared to males suggesting the occurrence of female philopatric behaviour in the sampling areas and in both species. Moreover, the presence of unique haplotype in different sampling sites further suggested the genetic structure driven by female philopatry in these species. A lack of significant divergence emerged between the Southern Adriatic Sea and the Strait of Sicily when all the sampled individuals of *M. punctulatus* were considered. Once the CR sequences for those individuals with more dispersal capacity were isolated, statistically significant structure was obtained even between the southern Adriatic Sea and the Strait of Sicily,

suggesting the occurrence of local philopatry as well. However, this findings should be considered as an indication given the low samples size (N= 14) of individual above 60 cm from the Strait of Sicily, which may have influenced the significance.

Evidence of philopatry was not demonstrated in other studies for *M. mustelus* and *M. punctulatus*. Hull et al. (2019) did not find such mitochondrial divergence between the Strait of Sicily and the Adriatic Sea neither for the nDNA nor the mtDNA. Although using the same mitochondrial marker but lower number of microsatellites, the life stage was not considered, and the sample size was considerably low (N = 15) in both sites. This may have affected the statistical significance as well. In other *Mustelus* species, the occurrence of sex biased dispersal has been reported before (Sandoval-Castillo and Beheregaray, 2015). In the brown smooth-hound (*M. henlei*), female philopatry and a male-mediated gene flow have been highlighted (Sandoval-Castillo and Beheregaray, 2015). Additionally, indications of male dispersal and female philopatry have been highlighted by the level of differentiation of sequenced CR in the other demersal elasmobranchs such as *S. canicula* (Gubili et al., 2014) and other species (Heuter et al., 2004).

From the methodological point of view in the sex-biased investigation, the robustness of our results stems from the number of microsatellites employed and the overall large sample size, which both appeared to be enough even for investigating sex biased dispersal (Phillips et al., 2021). One weakness might be due to the small sample size of the individuals with more dispersal capacity (>60 cm), especially for the samples from the Strait of Sicily. Nonetheless, statistically significant signal was obtained. On the other hand, long CR fragment (~1000 bp) for fine-scale differentiation has been demonstrated to be more appropriate (Phillips et al., 2021). In this study, statistically significant value of mtDNA differentiation was obtained between the two areas, only with 483 bp fragment (roughly 43% of the whole CR fragment). Sequencing the whole fragment could likely increase the inference power of genetic divergence and sex-biased dispersal at fine regional scale. The availability of biometric data allowed additional analyses separating the pre-dispersal young-of-the-year and those individual with more dispersing potential as such approach is thought to increase the inference power in sex-biased dispersal investigations (Phillips et al., 2021).

5.4.3 Management implication

From the management standpoint, genetic studies can be useful to provide essential information for conserving the genetic diversity of species or also called the portfolio effect (Schindler et al., 2010), especially of those that have been exploited for long periods (Heuter et al., 2004; Domingues et al., 2018).

In the Mediterranean Sea there is a lack of definition in population unit boundaries for elasmobranch species that are subjected to harvesting (Catalano et al., 2021). Given the vast importance of *Mustelus* species in the landings from the Mediterranean Sea (Barausse et al., 2014; Colloca et al., 2017; Carpentieri et al., 2021), the definition of evolutionary significant unit ("ESU should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci", Moritz, 1994) and management unit (MU is "therefore recognized as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles", Moritz, 1994) is important. The evidence of nuclear structure of *M. mustelus* and *M. punctulatus* suggests that more research is needed. For instance, tagging-recapture data could allow the estimation of the true migration rate between Mediterranean subbasins, intra- and inter-generational site fidelity and philopatry. Also, it is important to understand if the detected genetic structure could be a consequence of a historical isolation or due to the recent reduction in abundance (Colloca et al., 2017) clarifying the aspect of gene flow as well.

Since the optimal habitat distribution of these two *Mustelus* species is between 0-50 meters (Serena, 2005) the high trawling fishing effort in the coastal areas and the Mediterranean (Coll et a., 2012) may have degraded habitats that are essential to maintain the portfolio effect of genetic diversity (Yates et al., 2012).

5.5 Conclusion

The genetic connectivity of *Mustelus* species are the results of the combination of gene flow and behavioural traits, as highlighted not only by genetic studies, as the present one, but also by the combination of genetic results with mark and recapture tagging (Klein et al., 2021). On one hand, *Mustelus* species may show wide ranges of movements performing often seasonal movement (Mann & Bullen, 2009; da Silva et al., 2013; Braccini et al., 2017). On the other hand, behavioural traits as female philopatry may support genetic population structure (Sandoval-Castillo and Beheregaray, 2015; Petrolo et al., 2021). This study allowed to conclude that the genetic structure emerged between individuals form the Adriatic Sea and the Strait of Sicily and it suggested that female philopatric behaviour and male-mediate dispersal may have influenced such structure. So far, only two MUs, the Adriatic Sea and Strait of Sicily units, can be defined for a short-term management. Nonetheless, it should also be important to identify "a number of discrete homes in the sea" (Heuter et al., 2005) such as areas relevant for delivery and nursery where the local abundance may be influenced by philopatric individuals (Heuter et al., 2004; Chapman et al., 2015).

Bibliography

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: setting conservation guidelines. *Trends in ecology & evolution*, *16*(11), 613-622.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. (1990). "Basic local alignment search tool." *Journal of Molecular Biology*. 215:403-410.

Amos W., Hofffman J. I., Frodsham, A., Zhang L., Best S., & Hill A. V. S. (2007) Automated binning of microsatellite alleles: problems and solutions. *Molecular Ecology Notes* 7:10–14.

Barausse, A., Correale, V., Curkovic, A., Finotto, L., Riginella, E., Visentin, E., & Mazzoldi C. (2014). The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. *ICES Journal of Marine Science*, 71: 1593-1603.

Barker, A. M., Adams, D. H., Driggers III, W. B., Frazier, B. S., & Portnoy, D. S. (2019). Hybridization between sympatric hammerhead sharks in the western North Atlantic Ocean. *Biology letters*, *15*(4), 20190004.

Becker, M., Gruenheit, N., Steel, M., Voelckel, C., Deusch, O., Heenan, P. B., ... & Lockhart, P. J. (2013). Hybridization may facilitate in situ survival of endemic species through periods of climate change. *Nature Climate Change*, *3*(12), 1039-1043.

Benjamini, Y., Hochberg, Y., (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological), 57(1), 289-300.

Bonanomi, S., Pulcinella, J., Fortuna, C. M., Moro, F., & Sala, A. (2018). Elasmobranch bycatch in the Italian Adriatic pelagic trawl fishery. *PloS one*, *13*(1), e0191647.

Braccini, M., Rensing, K., Langlois, T., & McAuley, R. (2017). Acoustic monitoring reveals the broad-scale movements of commercially important sharks. *Marine Ecology Progress Series*, *577*, 121-129.

Brevé, N. W. P., Winter, H. V., Van Overzee, H. M. J., Farrell, E. D., & Walker, P. A. (2016). Seasonal migration of the starry smooth-hound shark *Mustelus asterias* as revealed from tag- recapture data of an angler-led tagging programme. Journal of Fish Biology, 89, 1158–1177

Cadrin, S. X., Karr, L. A., & Mariani, S. (2014). Stock identification methods: an overview. *Stock identification methods*,2nd 1-5.

Carpentieri, P., Nastasi, A., Sessa, M., & Srour, A., eds. 2021. Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries – A review. Studies and Reviews No. 101 (General Fisheries Commission for the Mediterranean). Rome, FAO.

Chapman, D.D., Feldheim, K.A., Papastamatiou, Y.P., & Hueter, R.E. (2015). There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. *Annual Review of Marine Science*, 7: 547-570.

Catalano, G., Crobe, V., Ferrari, A., Baino, R., Massi, D., Titone, A., ... & Cariani, A. (2021). Strongly structured populations and reproductive habitat fragmentation increase the vulnerability of the Mediterranean starry ray Raja asterias (Elasmobranchii, Rajidae). *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Clement, M., Snell, Q., Walker, P., Posada, D., & Crandall, K. (2002, April). TCS: estimating gene genealogies. In *Parallel and Distributed Processing Symposium, International* (Vol. 3, pp. 0184-0184). IEEE Computer Society.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W. W., Christensen, V., ... & Pauly, D. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. *Global Ecology and Biogeography*, *21*(4), 465-480.

Colloca, F., Enea, M., Ragonese, S., & Di Lorenzo, M. (2017). A century of fishery data documenting the collapse of smooth hounds (*Mustelus* spp.) in the Mediterranean Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27 (6): 1145-1155.

Conrath, C. L. (2005). Reproductive biology. In Management Techniques for Elasmobranch Fisheries, pp. 103–126. Ed. by J. A Musick, and R. Bonfil. Fisheries Technical Report 474. Food and Agriculture Organization, Rome.

Di Lorenzo, Manfredi, Salvatrice Vizzini, Geraldina Signa, Cristina Andolina, Gabriele Boscolo Palo, Michele Gristina, Carlotta Mazzoldi, and Francesco Colloca. "Ontogenetic trophic segregation between two threatened smooth-hound sharks in the Central Mediterranean Sea." *Scientific Reports* 10, no. 1 (2020): 1-15.

Domingues, R. R., Hilsdorf, A. W. S., & Gadig, O. B. F. (2018). The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics*, *19*(3), 501-525.

Dulvy, N.K., Simpfendorfer, C.A., Davidson, L.N., Fordham, S.V., Bräutigam, A. et al., (2017). Challenges and priorities in shark and ray conservation. *Current Biology*, 27 (11): R565-R572.

Dudgeon, C. L., Blower, D. C., Broderick, D., Giles, J. L., Holmes, B. J., Kashiwagi, T., ... & Ovenden, J. R. (2012). A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of fish biology*, *80*(5), 1789-1843.

Edgar, R. C. (2004), MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Research*, 32(5), 1792-97.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

Excoffier, L., & H.E. L. Lischer (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources,* 10: 564-567.

Ferretti, F., Osio, G.C., Jenkins, C. J., Rosenberg, A.A., & Lotze, H.K. (2013). Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact. *Scientific Reports*, 3, 1057.

Gubili, C., Sims, D. W., Veríssimo, A., Domenici, P., Ellis, J., Grigoriou, P., ... & Griffiths, A. M. (2014). A tale of two seas: contrasting patterns of population structure in the small-spotted catshark across Europe. *Royal Society open science*, *1*(3), 140175.

Hueter, R. E., Heupel, M. R., Heist, E. J., & Keeney, D. B. (2004). Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, 35, 239–247.

Jones, O. and Wang, J. (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10: 551–555

Hull, K. L., Asbury, T. A., da Silva, C., Dicken, M., Veríssimo, A., Farrell, E. D., ... & Bester-van der Merwe, A. E. (2019). Strong genetic isolation despite wide distribution in a commercially exploited coastal shark. *Hydrobiologia*, *838*(1), 121-137.

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., & Prodöhl, P.A. (2013). diveRsity: An R package for the estimation of population genetics parameters and their associated errors, *Methods in Ecology and Evolution,* doi: 10.1111/2041-210X.12067

Klein, J.D., Asbury, T.A., da Silva, C., Hull, K.L., Dicken M.L., Gennari, E., Maduna, S.N., Bester-van der Merwe A. (2021) Site fidelity and shallow genetic structure in the common smooth-hound shark Mustelus mustelus confirmed by tag-recapture and genetic data. *Journal of Fish Biolo*gy, doi: 10.1111/jfb.14926

Kopelman, N. M., J. Mayzel, M. Jakobsson, N. A. Rosenberg & I. Mayrose, 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15: 1179–1191.

Leigh, J. W., & Bryant, D. (2015). popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110-1116.

Maduna, S. N., & Bester-van der Merwe, A. E. (2017). Molecular research on the systematically challenging smoothhound shark genus Mustelus: a synthesis of the past 30 years. *African Journal of Marine Science*, *39*(4), 373-387.

Marino, I. A., Riginella, E., Cariani, A., Tinti, F., Farrell, E. D., Mazzoldi, C., & Zane, L. (2015a). New molecular tools for the identification of 2 endangered smooth-hound sharks, Mustelus mustelus and Mustelus punctulatus. *Journal of Heredity*, *106*(1), 123-130.

Marino, I. A., Riginella, E., Gristina, M., Rasotto, M. B., Zane, L., & Mazzoldi, C. (2015b). Multiple paternity and hybridization in two smooth-hound sharks. *Scientific reports*, *5*(1), 1-11.

Marino, I. A. M., Finotto, L., Colloca, F., Di Lorenzo, M., Gristina, M., Farrell, E. D., ... & Mazzoldi, C. (2018). Resolving the ambiguities in the identification of two smooth-hound sharks (Mustelus mustelus and Mustelus punctulatus) using genetics and morphology. *Marine Biodiversity*, *48*(3), 1551-1562.

Matić-Skoko, S., Šegvić-Bubić, T., Mandić, I., Izquierdo-Gomez, D., Arneri, E., Carbonara, P., ... & Tzanatos, E. (2018). Evidence of subtle genetic structure in the sympatric species Mullus barbatus and Mullus surmuletus (Linnaeus, 1758) in the Mediterranean Sea. *Scientific reports*, *8*(1), 1-14.

Mann, B. Q., & E. M. Bullen (2009). ORI/WWF-SA Tagging Project: Summary of Tag and Recapture Data for Smoothhoundsharks (*Mustelus mustelus*) Caught Along the Southern African Coast from 1984 to 2008. Oceanographic Research Institute, Durban.

Morgan, J. A., Harry, A. V., Welch, D. J., Street, R., White, J., Geraghty, P. T., ... & Ovenden, J. R. (2012). Detection of interspecies hybridisation in Chondrichthyes: hybrids and hybrid offspring between Australian (Carcharhinus tilstoni) and common (C. limbatus) blacktip shark found in an Australian fishery. *Conservation Genetics*, *13*(2), 455-463.

Moritz, C. (1994). Defining 'evolutionarily significant units' for conservation. *Trends in ecology & evolution*, *9*(10), 373-375.

Ovenden, J. R. (2013). Crinkles in connectivity: combining genetics and other types of biological data to estimate movement and interbreeding between populations. *Marine and Freshwater Research*, *64*(3), 201-207.

Pazmiño, D. A., van Herderden, L., Simpfendorfer, C. A., Junge, C., Donnellan, S. C., Hoyos-Padilla, E. M., ... & Maes, G. E. (2019). Introgressive hybridisation between two widespread sharks in the east Pacific region. *Molecular Phylogenetics and Evolution*, *136*, 119-127.

Papetti, C., Di Franco, A., Zane, L., Guidetti, P., De Simone, V., Spizzotin, M., ... & Mazzoldi, C. (2013). Single population and common natal origin for Adriatic Scomber scombrus stocks: evidence from an integrated approach. *ICES Journal of Marine Science*, *70*(2), 387-398.

Patwary M. U., Kenchington E.L., Bird C.J., & Zouros E. (1994). The use of random amplified olymorphic DNA (RAPD) markers in genetic studied of the sea scallop *Placopecten magellanicus* (Gmelin, 1971). *Journal of Shellfish Research* 13: 547-553.

Petrolo, E., Boomer, J., Hare, J. O., Bilgmann, K., & Stow, A. (2021). Stock structure and effective population size of the commercially exploited gummy shark Mustelus antarcticus. *Marine Ecology Progress Series*, *678*, 109-124.

Phillips, N. M., Devloo-Delva, F., McCall, C., & Daly-Engel, T. S. (2021). Reviewing the genetic evidence for sex-biased dispersal in elasmobranchs. *Reviews in Fish Biology and Fisheries*, *31*(4), 821-841.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L., & Estoup, A. (2004). GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, *95*(6), 536-539.

Pritchard, J. K., Stephens M., & Donnelly P. (2000). Inference of population structure using multilocus genotype data. *Genetics,* 155: 945–959.

Portnoy, D. S., & Heist, E. J. (2012). Molecular markers: progress and prospects for understanding reproductive ecology in elasmobranchs. *Journal of Fish Biology*, *80*(5), 1120-1140.

Rhymer, J. M., & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual review of ecology and systematics*, *27*(1), 83-109.

Riginella, E., Correale, V., Marino, I. A., Rasotto, M. B., Vrbatovic, A., Zane, L., & Mazzoldi, C. (2020). Contrasting life-history traits of two sympatric smooth-hound species: implication for vulnerability. *Journal of fish biology*, *96*(3), 853-857.

Rozas, J., Ferrer-Mata, A., Sànchez-Del Barrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., & Sànchez-Gracia,A. (2017) DnaSP v6: DNA Sequence Polymorphism Analysis of Large Datasets. *Molecular Biology and Evolution. 34:3299-3302.*

Russo, A., & Artegiani, A. (1996). Adriatic sea hydrography. *Scientia Marina*, *60*, 33-43.

Sabatini, L., Bullo, M., Cariani, A., Celić, I., Ferrari, A., Guarniero, I., ... & Scarcella, G. (2018). Good practices for common sole assessment in the Adriatic Sea: Genetic and morphological differentiation of Solea solea (Linnaeus, 1758) from S. aegyptiaca () and stock identification. *Journal of Sea Research*, *137*, 57-64.

Salvato, P., Battisti, A., Concato, S., Masutti, L., Patarnello, T., & Zane, L. (2002). Genetic differentiation in the winter pine processionary moth (Thaumetopoea pityocampa—wilkinsoni complex), inferred by AFLP and mitochondrial DNA markers. *Molecular Ecology*, *11*(11), 2435-2444.

Sandoval-Castillo, J., & Beheregaray, L. B. (2015). Metapopulation structure informs conservation management in a heavily exploited coastal shark (*Mustelus henlei*). Marine Ecology Progress Series, 533, 191–203.

Schindler, D. E., Hilborn, R., Chasco, B., Boatright, C. P., Quinn, T. P., Rogers, L. A., & Webster, M. S. (2010). Population diversity and the portfolio effect in an exploited species. *Nature*, *465*(7298), 609-612.

Serena, F. (2005). Field identification guide to the sharks and rays of the Mediterranean and Black Sea. Rome, Italy: FAO Species Identification Guide for Fishery Purposes, 97 p.

da Silva, C., S. E. Kerwath, C. G. Attwood, E. B. Thorstad, P. D. Cowley, F. Økland, C. G. Wilke & T. F. Næsje, (2013). Quantifying the degree of protection afforded by a no-take marine reserve on an exploited shark. *African Journal of Marine Science* 35: 57–66.

Sinclair, M., & Iles, T. D. (1988). Population richness of marine fish species. *Aquatic Living Resources*, *1*(1), 71-83.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, *30*(12), 2725-2729.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535-538.

Walls RHL, & Dulvy NK. (2021) Tracking the rising extinction risk of sharks and rays in the Northeast Atlantic Ocean and Mediterranean Sea. *Scientific Reports* 11(1):15397.

Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Wright S (1978) Evolution and the Genetics of Populations, Vol. 4: Variability Within and Among Natural Populations. University of Chicago Press, Chicago.

Yates, P. M., Heupel, M. R., Tobin, A. J., & Simpfendorfer, C. A. (2012). Diversity in young shark habitats provides the potential for portfolio effects. *Marine Ecology Progress Series*, *458*, 269-281.

Chapter 6: General Discussion

Dealing with the environmental issues is not an easy task especially when it involves many different stakeholders at different levels of the society. In addition, the scientific research on elasmobranch further complicates the feasibility of the study given the elusive nature and abundance compared to other marine species. So, many aspects involving the elasmobranch ecology and biology in the Mediterranean Sea still need to be understood. The main objectives of this thesis were to study the movement ecology and the delineation of essential habitats of elasmobranch species in the Mediterranean Sea with a particular focus on one endangered species, the sandbar shark (*C. plumbeus*), and two commercially relevant species (*M. mustelus* and *M. punctulatus*) in the Adriatic Sea. Within the context of the use of space and the site fidelity proprieties the thesis aimed at shedding light on implication of elasmobranch vulnerability and how this thesis results can be helpful for conservation and management strategies. The specific objectives that each chapter has attempted to address were: (*i*) to reconstruct behaviour traits such as movement and aggregation in the Mediterranean Sea through the use of local ecological knowledge (LEK) and the fisher's perception on the relevance of elasmobranchs to fishery and conservation importance; (*ii*) to assess the occurrence of multi-year nursery site of the sandbar shark (*C. plumbeus*) in the northern Adriatic Sea and to map its suitable nursery area of early juveniles in the Mediterranean Sea; (*iii*) to define the seasonal use of space in the northern-central Adriatic Sea of *Mustelus* spp; (*iv*) to evaluate the population connectivity of two species of smooth hounds (*M. mustelus* and *M. punctulatus*) and the sex-biased dispersion;

Using fisher's LEK, several elasmobranch species were found to have a seasonality of catch, indicating the possibility of seasonal movement within the Mediterranean subbasins. Such findings were supported by available literature, but new insights based on the LEK emerged as well. Also, LEK allowed to gather new information on spatial occurrence and description of species aggregations and evidenced how well the aggregation phenomenon is known by fishers. Last, LEK highlighted the importance of bottom-up approach for an integrated management including fisher's perception on elasmobranch conservation and relevance

for fishery (Moller et al., 2004). The use of LEK can be cost-effective and useful especially for elasmobranch that are difficult to study (Colloca et al., 2020).

In the case of endangered species like the sandbar shark, the evidence of a multi-year birthing site in the northern Adriatic Sea during pupping period in summer, highlight the importance of the Adriatic subbasin as a key area for the life cycle of this coastal shark species. In addition, the ecological modelling reports other key areas in the Mediterranean Sea that are essential for the sandbar shark recruitment. Together with the control of the fishing mortality on sub-adults close to the first length of maturity, the nursery protection seems to be another crucial pillar for the conservation and management of large coastal shark (Kinney & 2009).

The seasonal use of space in *Mustelus* spp. in the northern Adriatic has indicated that recurrent movement takes place across the seasons and how higher density can be found in the northern Adriatic Sea, within the Gulf of Venice, during late spring and summer periods than in other areas. The co-dominant high density of adult juvenile and young-of-the-year during that period, pointed out that the Gulf of Venice may serves an important nursery and mating area, where aggregation event may occur as well. This information can be useful for assessing where and how much the fishing effort has been impacting these commercially relevant shark species and for defining spatially explicit management and conservation actions (Colloca et al., 2015). Lastly, such evidence can be used collectively to achieve similar management results as for the *M. antarticus* (Prince, 2002) where, size limit to the landing catch was implemented throughout the year and inside and outside the nursery area, together with the integration of the movement components into the stock assessment (Prince, 2002; Kinney & Simpfendorfer, 2009).

The study of population connectivity and sex-biased dispersal has shed light on the genetic structure of the individuals sampled in two Mediterranean areas, the Adriatic Sea and the Strait of Sicily, that are still relevant for the abundance and fishery of such species (Carpentieri et al., 2021). In addition, a philopatry and a male-mediated dispersal have been suggested according to the results in population divergence. These findings brought to the definition of two management units for a short-term conservation strategy (Morits, 1994) and

pointed to the importance of considering areas of mating and reproduction where local abundance may also be the result of philopatric individuals (Heuter et al., 2004).

The last two chapters, regarding population connectivity and the seasonal use of space, provide essential information at different time scales for the spatially-explicit management of commercially exploited sharks (Colloca et al., 2015; Domingues et al., 2018). Together, the results provide at different temporal and spatial scale new insights on the possible seasonal site fidelity, inferred by the fishery data, but also the identification of two populations that present some degree of genetic structure. Although the two species were aggregated in the fishery data given the species-misidentification issue (Marino et al., 2018), the genetic results indicated for both species a seasonal localization of the individuals since full sibling pairs were identified for both species in the two subbasins. Fishery data also pointed out a seasonal high density of individuals where the presence of newborns, juveniles and adults. This is in line with the strong site fidelity found in *M.mustelus* from South African water (Klein et al, 2021) even though from the same study sub-populations structure from the genetic analysis was not inferred from all sampling locations. In agreement with the seasonal high density in the Gulf of Venice from the fishery data, the genetic inferences also suggested a strong philopatry of female individuals. All together these results may represent a first picture of the spatial ecology of the *Mustelus* species in the Adriatic Sea. However, there knowledge gap on the migration exchange or temporal and spatial movement

The Mediterranean Sea requires dramatically scientific-evidence-based management for the fishery resources where most of the stocks have been demonstrated to be in an overfishing state (FAO, 2020). While the protection of nursery has been proposed in the Mediterranean waters for some commercially-relevant species (Colloca et al., 2015) and that spatial-temporal closures have been in force for the last 10 years (EC No. 1967/2006), the only elasmobranch management in force is only bounded to the landing ban for those species listed the in the Annex II of the Barcellona convention.

Within the framework of a spatially explicit management, the four research chapters in this thesis indicated scientific evidence in the Mediterranean Sea for the elasmobranch species under study. In the context of the

Adriatic Sea, a future spatial-explicit management should be considering the period, ranging from the late spring and throughout the summer, that has been suggested in this thesis to play an important role for the reproduction and mating of the three shark species under study.

At Mediterranean level, a spatially-explicit management needs to be particularly articulated by integrating all the scientific information about the movement ecology and essential habitats of endangered and commercially-exploited elasmobranch species and challenging since fisher perspectives and their socio-economic context should be considered to facilitate a bottom-up approach.

Based on the most abundant landed species and the endangered one listed by international agency for conservation (e.g. IUCN) it is important to investigate the movement ecology and the essential habitat delineations of other elasmobranch species, although different availability in sample and data may occur ample for commercially-relevant or endangered, thus relatively more rare. However, the applied approaches in this thesis are adjustable according to the target species under study and could provide scientific information eventually, crucial for a spatial-explicit managment.

Although it was beyond the scope of the thesis research, several bottom-up measures (e.g., seasonal closure during reproduction, early-juveniles ore size-landing limits), as also suggested in the questionnaire from the second chapter, could be tested in combination or as stand-alone to several species having different biological characteristics (e.g., growth rate or gestation period) and movement features (e.g., showing seasonal movement). This kind of investigation could simulate whether a similar management to the successful one applied in Australian waters (Prince,2002), is applicable and with an equal performance in the Mediterranean subbasins from a population dynamic and fishery perspectives.

Lastly, the stakeholders such as the European commission and the general fishery commission of the Mediterranean Sea (FAO-GFCM) could be engaged and advised for such applied research to tailor specific management on elasmobranch species listed in the annex III which required to be regulated.

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Bibliography

Carpentieri, P., Nastasi, A., Sessa, M. & Srour, A., eds. 2021. Incidental catch of vulnerable species in Mediterranean and Black Sea fisheries – A review. Studies and Reviews No. 101 (General Fisheries Commission for the Mediterranean). Rome, FAO. https://doi.org/10.4060/cb5405en

Colloca, F., Garofalo, G., Bitetto, I., Facchini, M. T., Grati, F., Martiradonna, A., ... & Spedicato, M. T. (2015). The seascape of demersal fish nursery areas in the North Mediterranean Sea, a first step towards the implementation of spatial planning for trawl fisheries. *PloS one*, *10*(3), e0119590.

Colloca, F., Carrozzi, V., Simonetti, A., & Di Lorenzo, M. (2020). Using local ecological knowledge of fishers to reconstruct abundance trends of elasmobranch populations in the Strait of Sicily. *Frontiers in Marine Science*, *7*, 508.

Domingues, R. R., Hilsdorf, A. W. S., & Gadig, O. B. F. (2018). The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics*, *19*(3), 501-525.

Council Regulation (EC) No 1967/2006 of 21 December 2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, amending Regulation (EEC) No 2847/93 and repealing Regulation (EC) No 1626/94. [Viewed on 07/01/2022] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1967-20190814

FAO. 2020. The State of Mediterranean and Black Sea Fisheries 2020. General Fisheries Commission for the Mediterranean. Rome. https://doi.org/10.4060/cb2429en

Hueter, R. E., Heupel, M. R., Heist, E. J., & Keeney, D. B. (2005). Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, 35, 239–247.

Klein, J.D., Asbury, T.A., da Silva, C., Hull, K.L., Dicken M.L., Gennari, E., Maduna, S.N., Bester-van der Merwe A. (2021) Site fidelity and shallow genetic structure in the common smooth-hound shark Mustelus mustelus confirmed by tag-recapture and genetic data. *Journal of Fish Biolo*gy, doi: 10.1111/jfb.14926

Kinney, M. J., & Simpfendorfer, C. A. (2009). Reassessing the value of nursery areas to shark conservation and management. *Conservation letters*, *2*(2), 53-60.

Marino, I. A. M., Finotto, L., Colloca, F., Di Lorenzo, M., Gristina, M., Farrell, E. D., ... & Mazzoldi, C. (2018). Resolving the ambiguities in the identification of two smooth-hound sharks (Mustelus mustelus and Mustelus punctulatus) using genetics and morphology. *Marine Biodiversity*, *48*(3), 1551-1562.

Moller, H., Berkes, F., Lyver, P. O. B., & Kislalioglu, M. (2004). Combining science and traditional ecological knowledge: monitoring populations for co-management. *Ecology and society*, *9*(3).

Moritz, C. (1994). Defining 'evolutionarily significant units' for conservation. *Trends in ecology & evolution*, *9*(10), 373-375.

Prince, J. D. (2002). Gauntlet fisheries for elasmobranchs–the secret of sustainable shark fisheries. *Journal of Northwest Atlantic Fishery Science*, *35*, 407-416.

ANNEX I : Supplementary Materials Chapter 2

Fig. S1 Questionnaire template

PERSONAL DATA

Name and surname (voluntary):			
Residence:	_____	City_____	Province_____
Age and years of fishing experience:			
Fisheries	☐ Gill nets ☐ Longline	☐ Bottom trawl ☐ Otter beam trawl	☐ Others_____
Targeted species:			
Fishing frequency:	Number of times/year		

Questions

1. Have you ever fished, in the past or the present, sharks, skates or rays?

	PAST		PRESENT	
Sharks	☐ YES	☐ NO	☐ YES	☐ NO
Skates and Rays	☐ YES	☐ NO	☐ YES	☐ NO

2. In which areas have you fished in the past? (point in the map)

3. In which areas do you fish nowadays? If it has changed regarding the past, why?

4. Have the shark fisheries varied over time? In which way?

CATCHES

	1940-1960	**1960-1980**	**1980-2000**	**2000-present**
Total catches in the past regarding the present	☐ Very abundant (more than 3 times in comparison to the present)	☐ Very abundant (more than 3 times in comparison to the present)	☐ Very abundant (more than 3 times in comparison to the present)	☐ Very abundant (more than 3 times in comparison to the present)
	☐ Abundant (twice more	☐ Abundant (twice more	☐ Abundant (twice more	☐ Abundant (twice more

Personal assessment	than in the present)	than in the present)	than in the present)	than in the present)
	☐ They remain the same	☐ They remain the same	☐ They remain the same	☐ They remain the same
	☐ Less abundant	☐ Less abundant	☐ Less abundant	☐ Less abundant
	☐ No assessment	☐ No assessment	☐ No assessment	☐ No assessment
Which ones used to be the most abundant species?	1)	1)	1)	1)
	2)	2)	2)	2)
	3)	3)	3)	3)
	4)	4)	4)	4)
	5)	5)	5)	5)

5. Which shark species are not caught anymore, or have dramatically declined?

6. Are there any new shark species that have been caught nowadays, but not in the past? Which ones?

7. Have you noticed any change (increase or decrease) in the size of any shark species over time?

Specie	Increase	Decrease

8. Have the rays and skates' fisheries varied over time? In which way?

CATCHES

	1940-1960	1960-1980	1980-2000	2000-present
Total catches in the past regarding the present Personal assessment	☐ Very abundant (more than 3 times in comparison to the present)	☐ Very abundant (more than 3 times in comparison to the present)	☐ Very abundant (more than 3 times in comparison to the present)	☐ Very abundant (more than 3 times in comparison to the present)
	☐ Abundant (twice more than in the present)	☐ Abundant (twice more than in the present)	☐ Abundant (twice more than in the present)	☐ Abundant (twice more than in the present)
	☐ They remain the same	☐ They remain the same	☐ They remain the same	☐ They remain the same
	☐ Less abundant	☐ Less abundant	☐ Less abundant	☐ Less abundant

	☐ No assessment	☐ No assessment	☐ No assessment	☐ No assessment
Which ones used to be the most abundant species?	1)	1)	1)	1)
	2)	2)	2)	2)
	3)	3)	3)	3)
	4)	4)	4)	4)
	5)	5)	5)	5)

9.   Which ray/skate species are not caught anymore, or have dramatically declined?

10. Are there any new ray/skate species that have been caught nowadays, but not in the past? Which ones?

11.   Have you noticed any change (increase or decrease) in the size of any ray/skate species over time?

Specie	Increase	Decrease

12. In your opinion, has the way of fishing sharks, skates and rays changed over time? (Type of fishery, seasons…)

13. Are there seasons in which you caught more or fewer sharks and/or skates and rays? (Please indicate the season(s))

Species	Spring	Summer	Autumn	Winter

14. In your opinion, is this seasonality related to migratory movements? For which species do you think this is true?

15. According to your knowledge, how do the sharks, skates and rays move in your fishing area?

16. Have you ever seen or fished any time aggregation of sharks and/or skates/rays? Of which species?

17. Has the occurrence of such aggregations changed over time? For which species?

<div align="center">AGGREGATIONS</div>

	1940-1960	1960-1980	1980-2000	2000-present
Species	☐ Never observed ☐ Rarely ☐ Frequently	☐ Never observed ☐ Rarely ☐ Frequently	☐ Never observed ☐ Rarely ☐ Frequently	☐ Never observed ☐ Rarely ☐ Frequently

18. Description of the aggregations: dimension, sex and abundance, in the past and present.

<div align="center">AGGREGATIONS</div>

	1940-1960	1960-1980	1980-2000	2000-present
Species	☐ Few ☐ Many (more than 10 specimens) ☐ A lot of them (more than 50 specimens)	☐ Few ☐ Many (more than 10 specimens) ☐ A lot of them (more than 50 specimens)	☐ Few ☐ Many (more than 10 specimens) ☐ A lot of them (more than 50 specimens)	☐ Few ☐ Many (more than 10 specimens) ☐ A lot of them (more than 50 specimens)
	☐ Big ☐ Medium ☐ Small	☐ Big ☐ Medium ☐ Small	☐ Big ☐ Medium ☐ Small	☐ Big ☐ Medium ☐ Small
	☐ Males ☐ No pregnant females ☐ Pregnant females ☐ Mixed	☐ Males ☐ No pregnant females ☐ Pregnant females ☐ Mixed	☐ Males ☐ No pregnant females ☐ Pregnant females ☐ Mixed	☐ Males ☐ No pregnant females ☐ Pregnant females ☐ Mixed

19. Could you indicate in which areas these aggregations occurred (map)? Inshore or offshore? Any location in particular?

20. In which period of the year?

21. Do you believe that sharks, rays and skates are important for the marine environment?

22. Do you believe that sharks, skates and rays are important for fisheries?

23. Do you think that is important to conserve sharks, skates and rays? If so, how would you do it?

Tables

Tab. S1: *Percentage of interviewed fishers answering about the seasonality of each species for the different sampling points (Ancona (ANC), Chioggia (CHIO), Marano Lagunare (ML), Northern Istria (NI), Southern Istria (SI), Eastern Adriatic coast (EAC) and Montenegro (MON) in the Adriatic Sea, above the 25% threshold.*

	ANC	CHIO	ML	NI	SI	EAC	MON
Total of fishers	15	12	13	23	21	8	10
Mustelus spp.	-	**83**	**92**	**100**	**52**	**100**	**40**
M. aquila	-	-	**92**	**100**	-	**75**	-
Raja spp.	**53**	**33**	**85**	**75**	**62**	**100**	**50**
S. acanthias	**27**	**42**	**100**	**100**	**57**	**25**	**30**
S. stellaris	-	-	**62**	**60**	-	-	-

Tab. S2: *Percentage of interviewed fishers answering about the seasonality question of each species for the different sampling areas in other GSAs (Italy (ITA), Turkey (TUR), Spain (SPA)), above the 25% threshold.*

GSA	9	9	22/28	11.2	16	6	19	10
Country	ITA	ITA	TUR	ITA (SAR)	ITA (SIC)	SPA	ITA(CAL)	ITA(CAL)
Tot. fishers	12	9	10	14	15	15	6	6
Raja spp.	**40**	**33**	**44**	**57**	-	-	-	-
P. glauca	-	**33**	-	-	-	**28**	**50**	-
S. canicula	-	-	-	**42**	-	-	-	-
Torpedo spp.	-	-	-	**28**	-	-	**33**	-
Mustelus spp.	-	-	-	-	**40**	-	-	-
P. violacea	-	-	-	-	-	**42**	-	-
I. oxyrhincus	-	-	-	-	-	-	-	**33**

Tab. S3: *Percentage of interviewed fishers answering to each question (QN: question number) for each species indicating aggregation features in the Adriatic Sea during different time periods: B) 1960-1980; C) 1980-2000; D) 2000-Present.*

	QN 17			QN 18A			QN 18B			QN 18C		
	1960-1980	1980-2000	2000-Present	1960-1980	1980-2000	2000-Present	1960-1980	1980-2000	2000-Present	1960-1980	1980-2000	2000-Present
Mustelus spp.	9	45	48	7	49	49	7	38	42	5	43	44
S. acanthias	5	30	35	4	22	26	4	17	20	5	17	19
M. aquila	4	22	23	2	19	16	2	15	13	2	10	9

	7	19	25	4	16	24	4	13	20	3	11	14
Raja spp.	7	19	25	4	16	24	4	13	20	3	11	14

Tab. S4: Percentage of interviewed fishers answering to each question (QN: question number) for each species indicating aggregation features in other Mediterranean GSAs during different time periods: B) 1960-1980; C) 1980-2000; D) 2000-Present.

	QN 17			QN 18A			QN 18B			QN 18C		
	1960-1980	1980-2000	2000-Present	1960-1980	1980-2000	2000-Present	1960-1980	1980-2000	2000-Present	1960-1980	1980-2000	2000-Present
Mustelus spp.	9	9	7	8	9	4	7	8	3	6	6	4
Raja spp.	9	11	9	9	13	10	8	13	9	8	10	9
S. canicula	9	14	11	7	12	12	5	12	11	5	9	8

Tab. S5: Percentage of interviewed fishers answering to the questions related to aggregations (QN 17 and QN 18) in each GSA.

GSA	GSA 6	GSA 9	GSA 10	GSA 11	GSA 16	GSA 19	GSA 22/28
Mustelus spp.	-	8	-	-	84	8	
Raja spp.	-	25	13	13	36	-	13
S. canicula	23	27	-	23	27	-	-

Tab. S6: Percentage of fishing gears used by interviewed fishers in the different sampling areas.

|	GSA 6	GSA 9	GSA 10	GSA 11	GSA 13	GSA 16	GSA 17	GSA 18	GSA 19	GSA 20	GSA 22	GSA 28
Gillnets (GNS)	0	40	30	34	41	17	29	17	31	8	45	0
Long Line (LLS)	11	21	25	35	5	20	8	25	31	33	45	50
Bottom trawl (OTB)	55	29	40	24	33	63	35	25	8	58	10	6
Mid-water Trawl (PTM)	7	0	0	0	0	0	10	17	0	0	0	6
Other beam trawl (TTB)	0	0	0	0	22	0	9	0	0	0	0	19
Other	27	9	5	7	0	0	10	17	31	0	0	19

ANNEX II: Supplementary materials Chapter 3

Table S1: Dataset based on meta-analysis of presence and absence of birthing site from literature. Sampled areas refers to number of each georeferenced areas; N° sampling points refers to the number of point extracted from each georeferenced areas; TB stands for reporting target and bycatch (TB) and B for only bycatch; PA for presence and absence of birthing site; FAO-GSA refers to the FAO- geographical sub area division by FAO-GFCM (FAO 1990-2021); Basin/Area refers to the name of the sampling location; Abbreviation of sampling method: gillnet(GNS); trammel net (GRT); set longline (LLS); bottom otter trawl (OTB); local ecological knowledge (LEK); underwater visuals sampling (UVS); baited underwater video (BUV); Sampling effort reports the number of replicates (hauls for fishery sampling); Time series refers to the sampling years. Aggregating site were considered absence

Reference	Label	N° sampling Areas	N° sampling points	T B	P A	FAO-GSA	Basin/Area	Reported sampling method	Sampling effort	Time series
Lloret et al., 2020	38	1	7	T B	0	6	Côte Bleue	LLS, GTR&GNS	272 hauls	whole year 2012-2015
Lloret et al., 2020	37	1	22	T B	0	7	Cap de Creus	LLS, GTR&GNS	572 hauls	Spring and Summer 20015
Ceyhan & Akyol, 2014	7-8	2	79	T B	0	22	Noth Eastern Aegean Sea	LLS	50 survey operations	2008-2013
Falsone et al., 2020	30-36	7	63	T B	0	16	Sicily channel (Mazara)	GTR	263 fishing operation	March to December 2009-2013
Present study	50 & 52	2	5	T B	0	9	Ligurian Sea	GNS	18 hauls	June to November 2020
Present study	48,49 &51	3	14	T B	0	17	North Western Adriatic Sea	GNS	36 hauls	June to October 2020
Cetkovic, 2018	6	1	268	B	0	18	South-Eastern Adriatic Sea	GNS&LLS	logbooks	March to November 2016, 2017 and 2018
Damalas & Megalofonou, 2012	10-24	15	13706	B	0	20,21,22,23,24, 25,26,	Eastern Mediterranean Sea	LLS (hook Size No.2)	1360 fishing sets	March to September: Only 2003–2005

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Morey et al., 2006	39-41 & 42-44	6	7	B	0	5	Balearic Sea	GTR	306 surveys	Spring summe 2000 & Spring summer 2003
Present study	45&46	2	49	B	1	17	Northern Adriatic Sea	GNS	-	July to August 2019 & 2020
Batusta et al., 2021	2	1	25	B	1	24	Gulf of Iskenderun	LLS	1 fishing day	July 2017
Clo & De Sabata 2004	9	1	3	B	1	22	Gökova's Boncuk Cove	UVS	NA	Summer 2001-2004
Bradai et al., 2005	3 & 4	2	316	B	1	14	Gulf of Gabés	OTB, LLS&GNS	NA	March-July 2001-2004
Cattano et al., 2021	5	1	1	al l	0	16	Lampione Islet	BUV	51 deployments	July& September 2019
Barash et al., 2018	1	1	492	B	0	27	Israel coast	sighting, LEK	na	na
Saidi et al., 2019	47	1	578	B	1	14	Gulf of Gabés	LLS (Hook size No.2)	35 LLS sets	July-September 2016-2017
De Ranieri et al., 2003a	25-27	3	5	T B	0	9	Northern Tyrrhenian sea	GTR	20 boardings	July-September 2003
De Ranieri et al., 2003b	28-29	2	2	T B	0	9	Northern Tyrrhenian sea	GNS	20 boardings	July-August 2003

Figure S1: The map shows the georeferenced area used to extract the PA areas. The label number for each area referes to the table S1
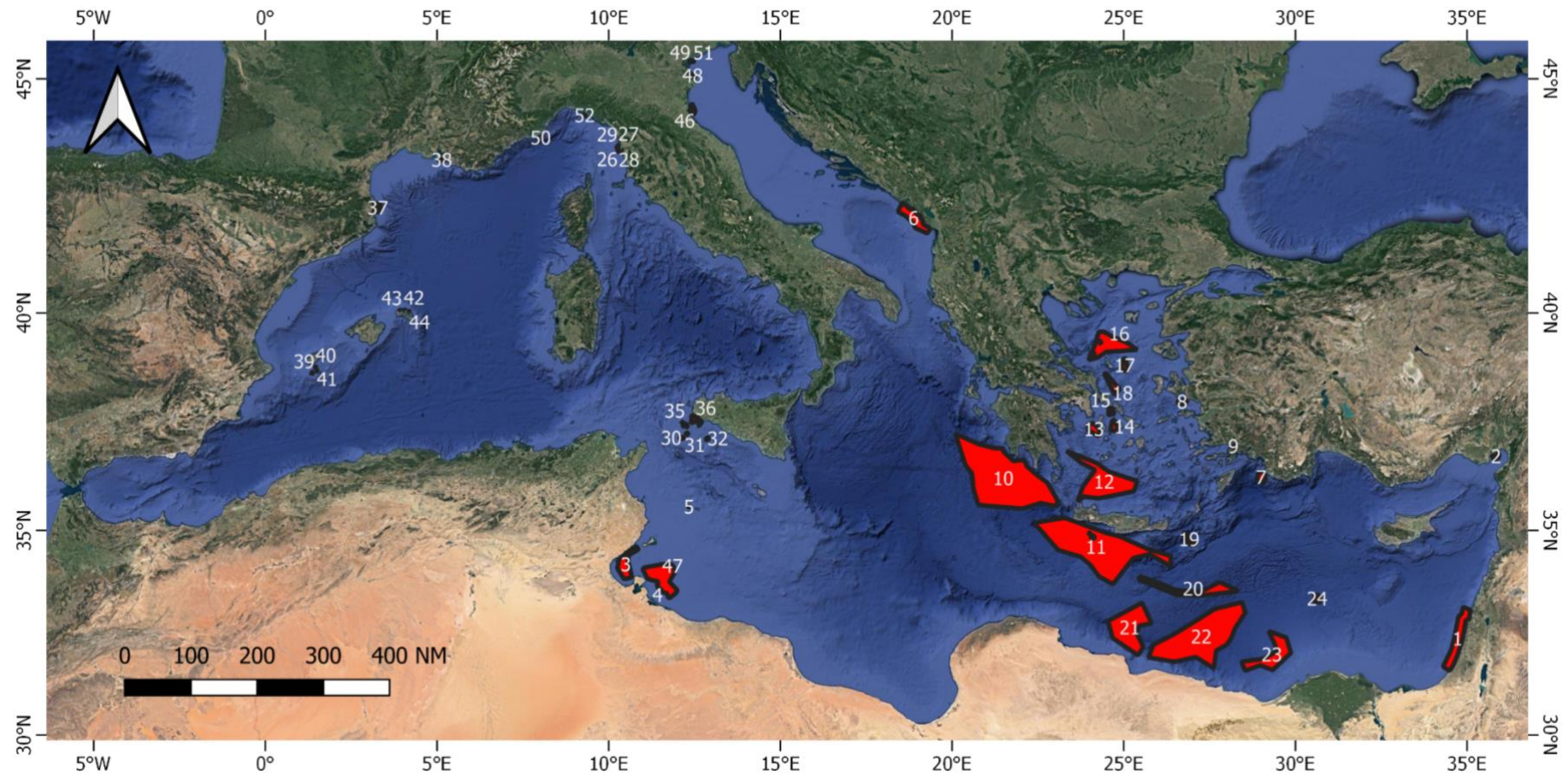
Reference

Barash, A., Pickholtz, R., Pickholtz, E., Blaustein, L., & Rilov, G. (2018). Seasonal aggregations of sharks near coastal power plants in Israel: an emerging phenomenon. *Marine Ecology Progress Series*, *590*, 145-154.

Basusta, N., BAŞUSTA, A., & Ozyurt, C. E. (2021). Evidence of a second nursery area of the sandbar shark, Carcharhinus plumbeus (Nardo, 1827) in the Eastern Mediterranean Sea. *Mediterranean Marine Science*, *22*(1), 20-26.

Cattano, C., Turco, G., Di Lorenzo, M., Gristina, M., Visconti, G., & Milazzo, M. Sandbar shark aggregation in the central Mediterranean Sea and potential effects of tourism. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Ceyhan, T., & Akyol, O. (2014). On the Turkish surface longline fishery targeting swordfish in the Eastern Mediterranean Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *14*(3), 825-830.

Ćetković I. (2018):Composition and abundance of shark by-catch in Montenegrin fisheries.Montenegrin Ecologists Society and Environment Programme, Podgorica, Montenegro.

Clò, S., & de Sabata, E. In the sharks' cradle. In *8th European Elasmobranch Association Conference*, *21st-23rd October 2004, London*, 45.

Damalas, D., & Megalofonou, P. (2012). Occurrences of large sharks in the open waters of the southeastern Mediterranean Sea. *Journal of natural history*, *46*(43-44), 2701-2723.

De Ranieri, S., Sbrana, M., Belcari, P., Sartor, P., Francesconi, B., Rossetti, I., Sartini M., Gambaccini, S., Vannucci, A. (2003a): Individuazione di maglie più selettive nella pesca con il tramaglio lungo il litorale livornese. Consorzio per il Centro Interuniversitario di Biologia Marina ed Ecologia, Livorno, Italy.

De Ranieri, S., Sbrana, M., Belcari, P., Sartor, P., Francesconi, B., Rossetti, I., Sartini M., Gambaccini, S., Vannucci, A., Reale, B., Ligas, A. (2003b): Individuazione di maglie più selettive nella pesca della sogliola, *solea vulgaris*, con reti ad imbrocco lungo il litorale livornese. Consorzio per il Centro Interuniversitario di Biologia Marina ed Ecologia, Livorno, Italy

Falsone, F., Scannella, D., Geraci, M. L., Vitale, S., Colloca, F., Di Maio, F., ... & Fiorentino, F. (2020). Identification and characterization of trammel net métiers: A case study from the southwestern Sicily (Central Mediterranean). *Regional Studies in Marine Science*, *39*, 101419.

FAO 1990-2021. FAO Major Fishing Areas. MEDITERRANEAN AND BLACK SEA (Major Fishing Area 37). CWP Data Collection. In: FAO Fisheries Division [online]. Rome. Updated.

Lloret, J., Biton-Porsmoguer, S., Carreño, A., Di Franco, A., Sahyoun, R., Melià, P., ... & Font, T. (2020). Recreational and small-scale fisheries may pose a threat to vulnerable species in coastal and offshore waters of the western Mediterranean. *ICES Journal of Marine Science*, *77*(6), 2255-2264.

Morey, G., Moranta, J., Riera, F., Grau, A. M., & Morales-Nin, B. (2006). Elasmobranchs in trammel net fishery associated to marine reserves in the Balearic Islands (NW Mediterranean). *Cybium*, *30*(4), 125-132.

Saidi, B., Enajjar, S., Karaa, S., Echwikhi, K., Jribi, I., & Bradai, M. N. (2019). Shark pelagic longline fishery in the Gulf of Gabes: Inter-decadal inspection reveals management needs. *Mediterranean Marine Science*, *20*(3), 532-541.

Table S2.1: Abiotic and biotic factor used to plot birthing site in the northern Adriatic Sea

Factor Name	Time series	Abbreviation	Unit	Reference
Sea surface temperature	July and Augsust 2019-2020	SST	Celsius degree	Clementi et al., 2019
Salinity	July and Augsust 2019-2020	SAL	PSU	Clementi et al., 2019
Current velocity and direction	July and Augsust 2019-2020	CUR	m/s	Clementi et al., 2019
Net primary production	July and Augsust 2019-2020	NPP	mg of C/ m^3 * Day	Bolzon et al., 2020
Diffuse attenuation coefficient at 490nm	July and Augsust 2019-2020	KD	m^{-1}	Volpe et al., 2019

Reference

Volpe, G., Colella, S., Brando, V. E., Forneris, V., Padula, F. L., Cicco, A. D., ... & Santoleri, R. (2019). Mediterranean ocean colour Level 3 operational multi-sensor processing. *Ocean Science*, 15(1), 127-146.

Clementi, E., Pistoia, J., Escudier, R., Delrosso, D., Drudi, M., Grandi, A.,Lecci R., Cretí S., Ciliberti S., Coppini G., Masina S., Pinardi, N. (2019). Mediterranean Sea Analysis and Forecast (CMEMS MED-Currents, EAS5 system) [Data set]. Copernicus Monitoring Environment Marine Service (CMEMS). DOI (Product): https://doi.org/10.25423/CMCC/MEDSEA_ANALYSIS_FORECAST_PHY_006_013_EAS5

Bolzon, G., Cossarini, G., Lazzari, P., Salon, S., Teruzzi, A., Feudale, L., Di Biagio, V., and Solidoro, C., "Mediterranean Sea Biogeochemical Analysis and Forecast (CMEMS MED-Biogeochemistry 2018-Present)." Copernicus Monitoring Environment Marine Service (CMEMS), 2020. DOI (Product): https://doi.org/10.25423/CMCC/MEDSEA_ANALYSIS_FORECAST_BIO_006_014_MEDBFM3.

Table S2.2: Biotic and abiotic factors for GLM construction

Factor Name	Time series	Abbr.	Unit	Horizontal Resolution	Reference
Depth	NA	Same	Meter	0.008°	Sbrocco & Barber 2013
Slope	NA	Same	Angle degree	0.008°	From Depth layer calculated by QGIS slope tool.
Sea surface temperature	2000-2018	SST	Celsius degree	0.04°	Clementi et al., 2019
Sea surface salinity	2000-2018	SAL	PSU	0.04°	Clementi et al., 2019
Current velocity	2000-2018	CUR	m/s	0.04°	Clementi et al., 2019
Sea surface temperature	2019-2020	SST	Celsius degree	0.04°	Escudier et al., 2020
Sea surface salinity	2019-2020	SAL	PSU	0.04°	Escudier et al., 2020
current velocity	2019-2020	CUR	m/s	0.04°	Escudier et al., 2020
Net primary production	2000-2018	NPP	mg of C / m^2 *Day	0.04°	Colella et al., 2020a
Net primary production	2018-2020	NPP	mg of C / m^2 *Day	0.04°	Colella et al., 2020b
Diffuse attenuation coefficient at 490nm	2000-2020	KD	m^{-1}	4 km	https://oceancolor.gsfc.nasa.gov/l3/

Reference

Sbrocco, E. J., & Barber, P. H. (2013). MARSPEC: ocean climate layers for marine spatial ecology: ecological archives E094-086. *Ecology*, *94*(4), 979-979.

Clementi, E., Pistoia, J., Escudier, R., Delrosso, D., Drudi, M., Grandi, A.,Lecci R., Cretí S., Ciliberti S., Coppini G., Masina S., Pinardi, N. (2019). Mediterranean Sea Analysis and Forecast (CMEMS MED-Currents, EAS5 system) [Data set]. Copernicus Monitoring Environment Marine Service (CMEMS). https://doi.org/10.25423/CMCC/MEDSEA_ANALYSIS_FORECAST_PHY_006_013_EAS5

Escudier, R., Clementi, E., Omar, M., Cipollone, A., Pistoia, J., Aydogdu, A., Drudi, M., Grandi, A., Lyubartsev, V., Lecci, R., Cretí, S., Masina, S., Coppini, G., & Pinardi, N. (2020). Mediterranean Sea Physical Reanalysis (CMEMS MED-Currents) (Version 1) [Data set]. Copernicus Monitoring Environment Marine Service (CMEMS). https://doi.org/10.25423/CMCC/MEDSEA_MULTIYEAR_PHY_006_004_E3R1

Colella, S., Garnesson, P., Netting, J., Calton, B.,Cesarini, C. (2020a) Global ocean chlorophyll, PP and PFT (Copernicus-GlobeColour) from satellite observations: monthly and dayly interpolated (reprocessed from 1997) , 6.0 edn., Copernicus Monitoring Environment Marine Service (CMEMS)

Colella, S., Garnesson, P., Netting, J., Calton, B.,Cesarini, C. (2020b) Global ocean chlorophyll, PP and PFT (Copernicus-GlobeColour) from satellite observations: monthly and dayly interpolated (near real time) , 6.0 edn., Copernicus Monitoring Environment Marine Service (CMEMS)

FIGURE

Figure S3.1: Density plot for abiotic and biotic factors in presence (magneta line) and absence (blue line) points.
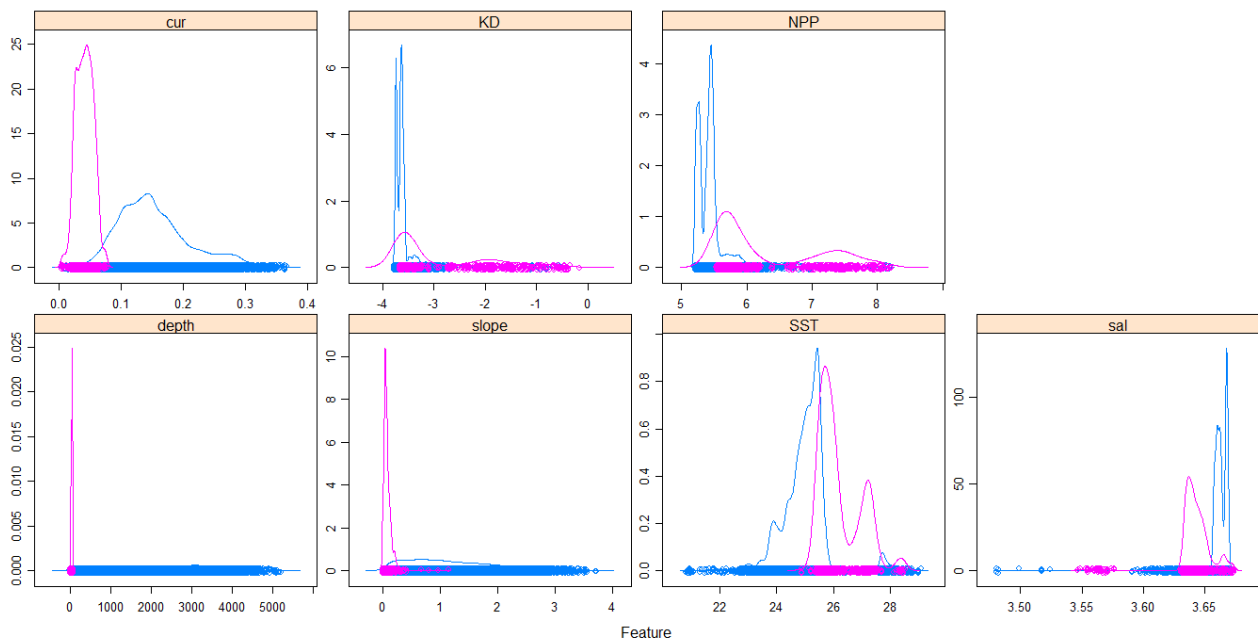

Figure S3.2:. Visualization of the correlation matrix between variables. On top, the absolute value of the Pearson's correlation coefficient. On bottom, the bivariate scatterplots, with a fitted line in red. Along the diagonal, histograms of each variable distribution.
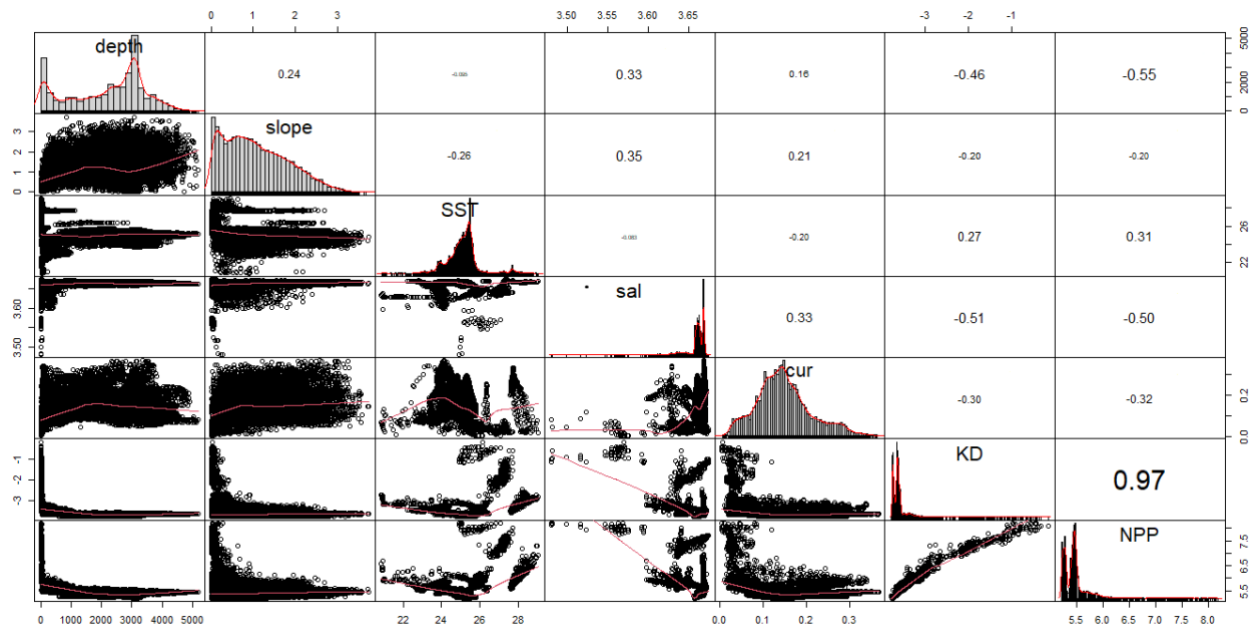
Table S3.1. *Ten best models sorted by AIC value, from the lowest to the highest. "D" stands for dropped factors. Only for the two lowest AIC models p-values are reported (see results). Significant p-value are reported in bold.*

Model ID	Intrcept	cur	depth	NPP	sal	slope	SST	df	AIC	Delta AIC
64	48,50	-64,57	-0,080	-2,336	-9,759	-14,99	0,440	7	729,3	-
p-value	**0.009**	**< 2e-16**	**< 2e-16**	**< 2e-16**	0.072	**< 2e-16**	**0.0001**	-	-	-
56	15,140	-64,12	-0,078	-2,114	D	-15,04	0,301	6	730,5	-1.2
p-value	**1.21e-10**	**< 2e-16**	**< 2e-16**	**< 2e-16**	-	**< 2e-16**	**0.0002**	-	-	-
24	21,51	-61,77	-0,080	-1,888	D	-14,87	D	5	742,6	-13.3
32	2,71	-62,05	-0,078	-1,823	5,047	-14,95	D	6	743,0	-13.7
60	-70,14	-59,36	-0,050	D	22,770	-15,07	-0,165	6	822,3	-93
28	-57,11	-59,89	-0,047	D	17,970	-14,99	D	5	823,8	-94.5
20	8,13	-58,55	-0,046	D	D	-14,62	D	4	839,6	-110.3
52	6,79	-58,75	-0,045	D	D	-14,63	0,050	5	841,1	
62	51,00	-78,06	D	-0,933	-17,390	-15,48	0,951	6	984,7	
54	-6,561	-78,43	D	-0,513	D	-15,56	0,6332	5	990,7	

Table S3.2. *Ten best GAM models sorted by AIC value, from the lowest to the highest. "D" stands for dropped factors. Only for the two lowest AIC models p-values are reported (see results). Significant p-value are reported in bold. K indicates the number of knots for the smoothing function*

Model ID	Intercept	cur.std	depth.std	f(npp,k = 3)	f(sal,k = 3)	f(temp,k =4)	slope.std	df	AIC	Delta AIC
1	-196.31	-3.04	99.59	+	+	+	-26.13	10	589.03	0.00
	< 2e-16	**1.31e-13**	**< 2e-16**	**0.0001**	0.097	**3.73e-07**	**< 2e-16**			
2	-193.26	-2.91	97.43	+	D	+	-25.81	8	589.43	0.40
	2e-16	**8.55e-14**	**< 2e-16**	**4.66e-07**	-	**< 2e-16**	**< 2e-16**	-	-	-
3	-181.49	-2.62	92.45	D	+	+	-25.27	8	604.31	15.28
4	-152.27	-2.59	74.67	D	D	+	-22.64	6	630.45	41.42
5	-202.34	D	107.04	D	+	+	-23.40		691.88	102.85
6	-208.04	D	110.06	+	+	+	-23.72	8	692.56	103.53
7	-206.09	D	108.29	+	D	+	-23.98	7	694.92	105.89
8	-185.10	D	91.57	D	D	+	-21.31	5	719.99	130.97
9	-148.73	-2.56	82.14	+	D	+	D	7	739.66	150.63
10	-148.90	-2.57	82.30	+	+	+	D	8	741.34	152.31

Table S3.3: *AIC values calculated from best GLM model (M56) and best GAM model (M2) from 10 random 80% partitions from the orgiginal datset*

Subset N°	AIC.GLM	AIC.GAM
1	600.8348	491.5686
2	609.3434	492.587
3	567.3804	485.5917
4	591.923	464.8835
5	589.8239	479.2483
6	562.4493	461.7697
7	586.2519	471.5343
8	584.5709	454.9432
9	586.5484	460.3647
10	589.0389	452.5178

Table S3.4: Average and standard deviation of accuracy, type I and II errors calculated for the 20% validation subsets

		Percentage of accuracy	Percentage of Type I error rate	Percentage of Type II error rate
GLM (M56)	Mean value out of 10 subsets	99.20	0.43	6.76
	Standard Deviation	0	0.14	1.78
GAM (M2)	Mean value out of 10 subsets	99.20	0.32	6.63
	Mean value out of 10 subsets	0	0.07	2.09

ANNEX III: Supplementary Material Chapter 5
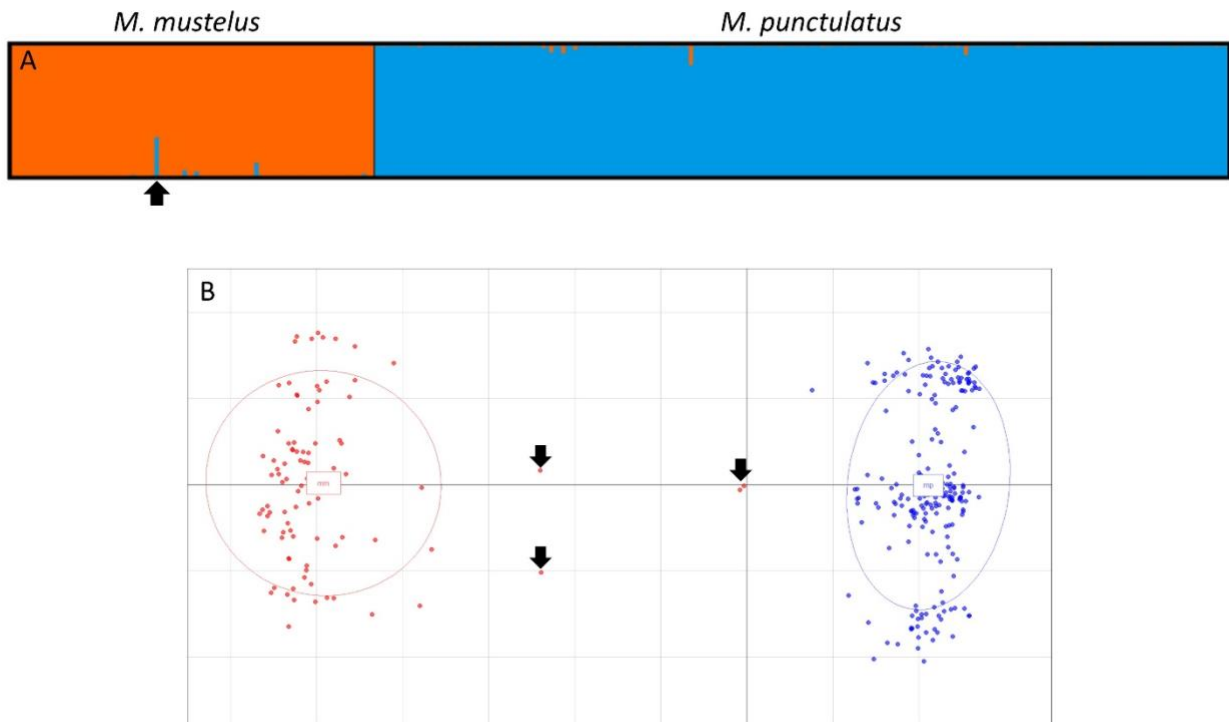
FIGURE

Figure S1: Exploratory analysis by STRUCTURE (A) and PCA analysis (B) to find hybrid or samples with high missing loci based on microsatellite data. Black arrows indicate the samples to be discarded before downstream analysis. One hybrid was found both STRUCTURE and PCA. Whereas 3 samples were identified to have too many missing loci.
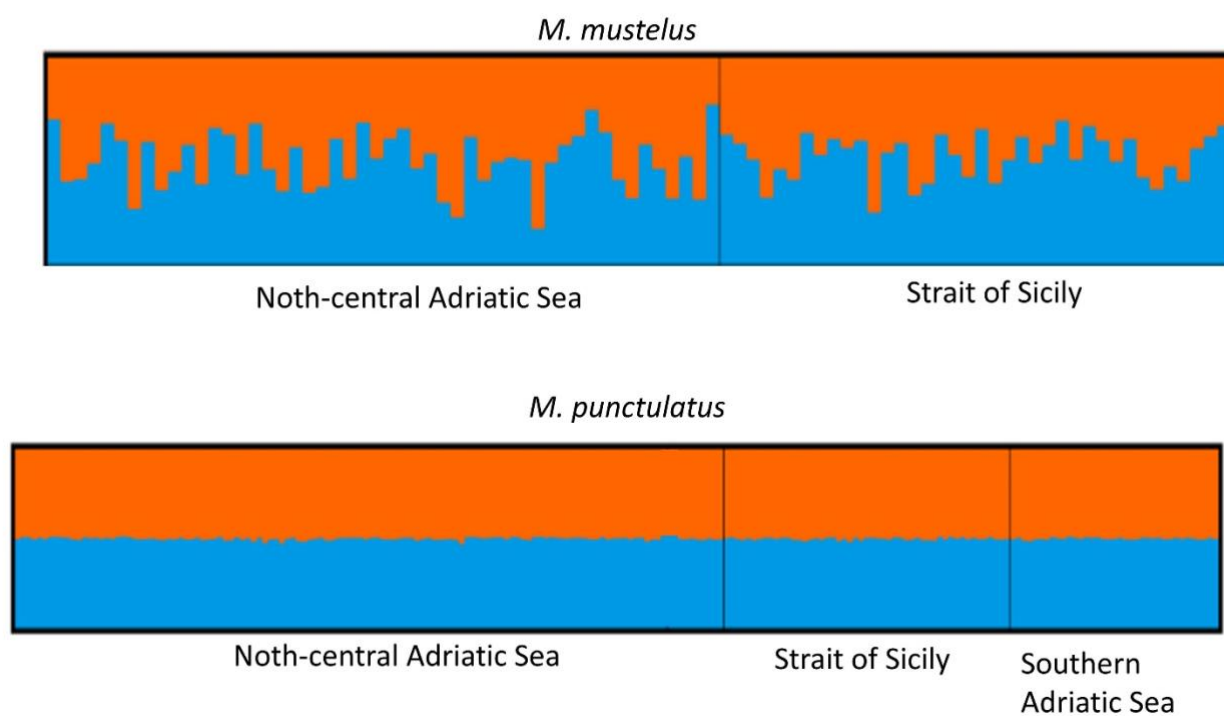
Figure S2: STRUCTURE analysis without prior information on sample origin. The two graphs report for K = 2 (the assumed number of populations)

TABLES

Table S1: Microsatellite panel for both species. Underlined locus name is to specify that was species specific. Asterisk in the Multiplex grouping indicates that the microsatellite has not been amplified in multiplex

Locus	Repeat	Fluorophores	Size	Primer sequence (5' – 3')	Multiplex grouping	Reference
Mmu2	(AC)6	FAM	150-180	F-TTGTCTGCAGGAAACACAGC R-GCATCGTGTGAAATGGGAAT	1	Maduna *et al.,*2017
Mmu3	(TC)7	NED	230-250	F-ATACACGGACCGACTCGAAC R-TAATGCCGAGATCAGGAACC	1	Maduna *et al.,*2017
Mmu4	(TG)7	VIC	58-180	F-TCCATCCAGCGTTAAAGGAC R-GCACCAGAGCTTCCCATTTA	1	Maduna *et al.,*2017

Mmu5	(CTC)6	FAM	268-274	F-ACCACTCCCTGCAGCACTAC R-AGGAGATGCTTTGGCACTTG	2	Maduna *et al.,*2017
Mmu7	(GCT)5	NED	203-217	F-TCCCTCATTTGCTTCAGGAG R-CGACATGAAACGCAGAAAGA	2	Maduna *et al.,*2017
Mmu8	(CAG)5(TGT)5	VIC	417-440	F-AGTAAGGCGCGCTATGATTG R-TAGAAGTCATCGCCCTCCAC	1	Maduna *et al.,*2017
Mmu9	(GAAT)5	NED	172-173	F-ACGGTTCTGAGCAATCGTCT R-TGCGATATTCGTCAGGTGAA	2	Maduna *et al.,*2017
Mmu10	(CATA)5	VIC	267-302	F-AATCCTGAGCACCAGGACAC R-TGTGTGAATTCCCCAGATGA	2	Maduna *et al.,*2017
Mmu11	(CAA)5	PET	203-209	F-ATCTTGTTAACCGCCGACAG R-CGCCATGTTGATCGAAGTAA	*	Maduna *et al.,*2017
Mmu12	(GAT)6	PET	210-213	F-GAGCAGCCAAGCATTAGTCC R-CGGCTTCAGAAATTGGAATC	2	Maduna *et al.,*2017
Mmu13	(GCA)5	NED	85-109	F-TCATTCCTCACACCCACTCA R-GATCCAGGAGCGAAGAACA	1	Maduna *et al.,*2017
Mmu14	(AGC)6	FAM	160-180	F-ACCGCTTGCTTCTGTTGAGT R-TCGCACAGACTGATTGAAGG	2	Maduna *et al.,*2017
Gg22	(GT)*n*	VIC	209–249	F: TCCTGGGATGGCAACTTCG R: AGGCCACCCAACTATCCTG	3	Chabot and Nigenda, 2011
MaD2X	(AG)11	FAM	179–185	F- ACCTGGCCCAAGAACTCTC R-ACTGGTGATGTGTGGACCC	3	Boomer and Stow, 2010
MaND5	(CT)11 (CA)3	FAM	197–208	F-TGGGAGGCCAATGGATCAG R- CGTTTCTGGGTGGTGCTTC	3	Boomer and Stow, 2010
McaB5	(GT)11	VIC	186–209	F- TAATCGACACGCAGTCATCG	3	Giresi *et al.,* 2012

				R-AAGCTCCAATTCTCACTGTGC		
McaB35	(TG)8	NED	206–221	F-AGTGCGTGCCAGTGTATGAG R-GTTCTGCATGGGACGTGAC	**3**	Giresi *et al.*, 2012
Mh9	(GA)9	NED	325–336	F- CAACCATCTTTACTACACTG R-GATGGACCTCACATTTAACAC	**3**	Byrne and Avise, 2012
Mh25	(CT)*n*	FAM	141–154	F- TGCAATAACCGTTCTGCGTC R-TCACACCCGCAGTTAGATCC	**3**	Chabot, 2012
<u>**Mh29**</u>	(CT)*n*	NED	172–185	F- ATCAGCCCAGATTGTCCGC R-AGACATTCCGCCTTCCAGC	**3**	Chabot, 2012

Table S2: *Presence and absence of null alleles by Micro-checker results at 10^5 runs (Confidence interval > 95%). Species-specific loci (Mmu9, Gg22, MaND5, MH29) were removed before running the analysis. Samples a-lised within species (M.mustelus and M.punctulatus) and within sampling origin site northern Adriatic Sea(N-Adri), central Adriatic Sea (C-Adri),Southern Adriatic Sea (S-Adri) and Strait of Sicily (Sic).*

	mm-Adri	mm-Sic	mp-N-Adri	mp-C-Adri	mp-Sic	mp-S-Adri
Mmu3	x					
Mmu4	x				x	
Mmu5	x					
Mmu7						
Mmu8						
Mmu10		x			x	
Mmu11						
Mmu12						
Mmu13						
Mmu14		x				x
MaD2X						
MaB35		x				
Mh9						
Mh25	x					

Table S3: *Adjusted p-value of Hardy Weinberg Exact Tests, estimated by Markov chain method and adjusted by Benjamini-Hochberg (B-H) correction. (Markov chain parameters: Dememorization: 10000; Batches: 500; Iterations per batch: 10000). Adjusted p-valued are reported according to M. mustelus (mm) and M. punctulatus (mp) and site such as northern Adriatic Sea(N-Adri), central Adriatic Sea (C-Adri), Southern Adriatic Sea (S-Adri) and Strait of Sicily (Sic). For M. mustelus Adriatic subbasins were not considered. Statistically significant values are reported in bold.*

Loci	mm-adri	mm-Sic	mm-N-Adri	mp-N-Adri	mp-C-Adri	mp-S-Adri	mp-Sic
Mmu3	**0.0088**	0.33176	0.94392	0.94392	-	1	0.727788
Mmu4	0.0672	1	-	-	-	-	**0.0363**
Mmu5	**0.042**	-	-	-	-	-	-
Mmu7	1	1	-	-	-	-	-
Mmu8	1	0.359667	0.0954	0.0954	-	1	-
Mmu9	-	-	-	-	-	-	-
Mmu10	0.42848	0.082333	1	1	-	-	**0.0022**
Mmu11	1	1	-	-	-	-	-
Mmu12	-	-	0.94392	0.94392	1	0.660167	0.727788
Mmu13	0.070629	-	1	1	0.6932	-	-
Mmu14	0.042	**0.0143**	0.94392	0.94392	-	0.05	1
Gg22	0.4128	1	-	-	-	-	-
MaD2X	0.627345	0.9737	0.94392	0.94392	1	0.660167	0.781367
MaND5	0.4	-	0.94392	0.94392	0.7672	1	0.727788
McaB5	0.070629	0.640529	-	-	-	-	-
McaB35	0.912133	0.2353	**0.0492**	**0.0492**	0.7672	0.660167	1
Mh9	1	0.9737	0.94392	0.94392	1	1	0.727788
Mh25	**0.0032**	0.082333	0.1428	0.1428	0.7672	0.370667	**0.0033**
Mh29	-	-	0.94392	0.94392	0.6932	0.370667	0.5599

Table S4: Haplotype frequency in sampled individuals across the three locations in M. mustelus. The only identified hybrid was reported only in this table in bold

	Haplotype Number	1	2	3	4	5	10	Total
M. mustelus	Northern-central Adriatic Sea			31	14	2	3	50
	Strait of Sicily	**1**	8	23	3	1		36
	Percentage of occurrence	1%	9%	62%	20%	4%	4%	86

Table S5: Haplotype frequency in samples individuals across the three locations in M. punctulatus

	Haplotype Number	1	6	7	8	9	11	12	13	Total
M. punctulatus	Northern-central Adriatic Sea	99		20			1	1	1	123
	Southern Adriatic Sea	33		1		2				36
	Strait of Sicily	40	6	1	1					48
	Percentage of occurrence	83%	3%	11%	0.5%	1%	0.5%	0.5%	0.5%	207

Reference

Byrne R. J. and Avise J.C. (2012) Genetic mating system of the brown smoothhound shark (*Mustelus henlei*), including a literature review of multiple paternity in other elasmobranch species. *Marine Biology* 159: 749–756.

Boomer J. and Stow J. (2010) Rapid isolation of the first set of polymorphic microsatellite loci from the Australian gummy shark, *Mustelus antarcticus* and their utility across divergent shark taxa. *Conservation Genetic Resources* 2: 393–395.

Chabot C. L. and Nigenda S. (2011) Characterization of 13 microsatellite loci for the tope shark, *Galeorhinus galeus*, discovered with next-generation sequencing and their utility for eastern Pacific smooth-hound sharks (*Mustelus*). *Conservation Genetic Resources* 3: 553–555.

Chabot C. L. (2012) Characterization of 11 microsatellite loci for the brown smooth-hound shark, *Mustelus henlei* (Triakidae), discovered with next-generation sequencing. *Conservation Genetic Resources* 4: 23–25.

Giresi M., Renshaw M. A., Portnoy D. S. and Gold J.R. (2012) Isolation and characterization of microsatellite markers for the dusky smoothhound shark, *Mustelus canis*. *Conservation Genetic Resources* 4: 101–104.

These 170 pages and the last advanced version was about to be lost because of the classic Murphy's law. Thanks to Mr. Damiano that recovered the semi-final version of this thesis from my beloved laptop that three days before the deadline crashed and its hardware broke down.

Finally, thanks to Federica Stranci who was next to me, supporting me and calming myself down in the last months of this PhD journey.