

UDRUGA ZA PRIRODU, OKOLIŠ I ODRŽIVI RAZVOJ

sunce

Mapping of marine habitats on the island of Molat - preliminary report

July, 2021.



Mapping of marine habitats on the island of Molat - preliminary report

CLIENT

WWF Adria
Zelinska ul. 2, HR – 10 000, Zagreb

SERVICE PROVIDER

Udruga za prirodu, okoliš i održivi razvoj Sunce
Obala hrvatskog narodnog preporoda 7/III, HR – 21 000 Split

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DIVING GUIDE

Petronije Tasić

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1. INTRODUCTION

Mapping of marine habitats on the south side of the island of Molat, the Association Sunce conducted for WWF Adria as part of the service: "Empowering the legacy of MAVA Mediterranean Partnership: Scaling up co-managed and financially sustainable No-Take Zones / Marine Protected Areas (MAVA NTZ).

The conducted mapping is a necessary precondition for the establishment of monitoring and future adoption of management measures for habitat types and species, especially important for the conservation of habitat types of importance at EU level (such as *Posidonia oceanica*) and the protection of endangered species (such as *Squatina squatina*). The mapping area of the island of Molat included the southern side of the island, which includes four areas within the Natura 2000 ecological network.

HR3000065 Bonaster - Molat Island is located on the southwest side of the island and covers an area of 101.4681 ha, and it is an area of reefs.

HR3000419 J. Molat-Dugi-Kornat-Žirje-Zlarin-Murter-Pašman-Ugljan-Rivanj-Sestrunj-Molat is an area of the south side of the island which covers 85276.74 ha and it is an area with reefs and submerged or partially submerged sea caves.

HR3000064 Brgulje Bay - Molat Island, is an area that covers the entire Brgulje Bay, 512.4503 ha located on the south side of the island. It is an area covered with meadows of *P. oceanica* and large shallow inlets and bays.

HR3000066 SE part of the island of Molat is an area located on the southeast side of the island and covers an area of 571.9119 ha. The area is covered with meadows of *P. oceanica* and reefs (Figure 1).



Slika 1 Natura 2000 network habitats in the southern part of the island of Molat (Source: www.bioportal.hr)

The flowering plant *P. oceanica* is a protected species, endemic to the Mediterranean Sea. It is widespread throughout the Adriatic, from the south, where it forms large meadows, to the western coast of Istria, where there are individual smaller meadows, generally in poor condition (Berković et al., 2018).

Posidonia meadows are important for marine life due to:

- 1) High primary production,
- 2) many organisms (even those that are economically important) use it as a feeding, breeding, or nursery areas. There is an abundance of food for both herbivores and carnivores, as well as for those organisms that feed by filtration (Petricioli, 2011).

The Angelshark (*S. squatina*) is distributed in the north-eastern Atlantic Ocean, from southern Norway and Sweden in the north to the Canary Islands and Mauritania in the south, and throughout the Mediterranean. Today, in the eastern part of the Adriatic, it can be found in the middle and southern part, but also in some northern localities (between the islands of Pag, Lošinj and Dugi otok). It occurs on sandy and sandy-muddy bottoms and in seagrass meadows of *P. oceanica*, as well as on hard bottoms. It prefers coastal waters, so it is common in the shallows. It is also buried in the soft substrate of the bottom all the way to the eyes. It is more active at night when it swims briskly and rises above the bottom, where it rests during the day (Jardas, 2007).

2. METHODS

During the field research, the protocols used by the Sunce Association for many years to map habitats and species throughout the Adriatic were used. Divers dive in pairs, along a line transect, keeping the diving buoy directly (as far as possible under the given conditions) above them. Attached to the diving buoy is a watertight tank containing a GPS device, which records the diver's position during dives at set intervals. Transects were conducted to include as much depth range as possible and as large an area as possible. In order to obtain as accurate data as possible, the transect line begins perpendicular to the shoreline, towards depth, then rotates parallel to the shoreline (following the boundary of two different habitat types, usually the lower edge of *P. oceanica* habitat, if present) and ends with an ascent as perpendicular to the shoreline as possible. During the dive, divers use plates (marked with the appropriate mark) and keep records if they spot invasive and endangered species, and record habitats with a brief description (degraded, continuous, healthy, damaged ...) and the depth at which the habitat begins and ends. Cameras and GoPro cameras are used to document species and habitats. Additionally, data on the distribution of the upper edge of marine habitats were collected by drone aerial photography. During this habitat mapping, special attention was paid to sandy areas within meadows with the aim of finding *S. squattina*. Also, due to the activity of this species, shallow sandy bays were inspected during the night. If necessary, divers also sketch the diving area. Data collection was performed by fieldwork in the period from 29.05.2021. to 03.06.2021.

3. DIVING LOG

On 28th of May six divers dove so each diving pair made two dives a day. With a total of six dives, the following area was mapped:

- Islet Tovarnik - Daniel Kanski, Patrik Krstinić. The dive was made from the north side of the islet, towards the south using an underwater scooter;
- Islet Tovarnik East - Zrinka Jakl, Fedra Dokoza;
- Islet Tovarnik south - Anamarija Paradinović, Ante Gugić;
- Cape Glavica - Anamarija Paradinović, Fedra Dokoza;
- Vapojni Bay - Zrinka Jakl, Ante Gugić;
- Islet Tovarnik towards Cape Glavica - Daniel Kanski, Patrik Krstinić. The dive was made using an underwater scooter.

On 29th of May eight divers dove so each diving pair made two dives a day. With a total of eight dives, the following area was mapped:

- Rt Cape Golubinka - Anamarija Paradinović, Agata Kovačev;
- Rt Cape Žorljevica - Anamarija Paradinović, Hrvoje Čepnija, Zrinka Jakl;
- Za Zagračina bay - Hrvoje Čepnija, Zrinka Jakl;
- Sa Sabus Bay - Fedra Dokoza, Agata Kovačev;
- Islet Vrtlac towards Tun - Vladan Strigo. The dive was made using an underwater scooter;
- Cape of Jakinska Bay - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Islet Vrtlac - Daniel Kanski, Patrik Krstinić. The dive was made using an underwater scooter;
- Cape Žaplo towards Lipišnjak Bay - Daniel Kanski, Patrik Krstinić. The dive was made using an underwater scooter.

On 30th of May eight divers dove so that a total of five dives were made to map the following area:

- Pod Podgarbe bay towards Studena bay - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Brgulje bay, west side - Agata Kovačev, Zrinka Jakl, Ante Gugić;
- Studena bay towards Brgulje - Mosor Prvan, Danijel Kanski. The dive was made using an underwater scooter;

- Brgulje Bay, west side - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Islet of Brgulje, east side - David Jiménez Alvarado, Agata Kovačev.

On 31st of May six divers dove per day, while four divers, according to protocol from the Canary Islands, made a night dive. Area mapped:

- Cape Gračine to the bay Zagračina - Agata Kovačev, Hrvoje Čepnja;
- Cape Konopljička - Anamarija Paradinović, Agata Kovačev, Hrvoje Čepnja;
- Konopljika Bay - David Jiménez Alvarado;
- Cape Golubinka - David Jiménez Alvarado, Anamarija Paradinović;
- Veliki Zaganj Bay - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Cape Stopanj - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Brgulje Bay, North side - David Jiménez Alvarado, Hrvoje Čepnja, Mosor Prvan, Patrik Krstinić.

On 1st of June a total of four dives were made during the day. According to protocol from the Canary Islands, a night dive and survey of the area was also made. Area mapped:

- Cape Kamenjev - Matea Špika, Anamarija Paradinović;
- Central area of Cape Bonaster - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Central area of Cape Bonaster towards Cape Bonaster - Vladan Strigo, Fedra Dokoza. The dive was made using an underwater scooter;
- Strmi bok bay - Matea Špika, Anamarija Paradinović;
- Jazi bay - Vladan Strigo, David Jiménez Alvarado, Mosor Prvan, Patrik Krstinić, Danijel Kanski, Eva MEyers.

On 2nd of June a total of one dive was made. A night dive and survey of the area was also made. Area mapped:

- Rivanj channel - Matea Špika, David Jiménez Alvarado, Vladan Strigo, Mosor Prvan, Patrik Krstinić, Danijel Kanski, Fedra Dokoza, Anamarija Paradinović, Petronije Tasić;
- Konopljika bay - Vladan Strigo, David Jiménez Alvarado, Mosor Prvan, Patrik Krstinić, Danijel Kanski, Eva MEyers.

On 3rd of June a total of one dive was made. Area mapped:

- Islet Bivošćak north - Matea Špika, Anamarija Paradinović;
- The islet of Bivošćak - Vladan Strigo. The dive was made using an underwater scooter.

After the dive, divers draw a diving profile (marked with an assigned mark), recording habitats and species of special interest. If there were significant differences in habitats during dives and ascents, separate profiles are drawn. Data from the GPS device is transferred to a computer, under the dive code, and their spatial location is checked in a spatial analysis program (QGIS 3.10.5) using satellite and orthophoto images (Figure 2).



Slika 2 Mapped area of the island of Molat (Source: Sunce Association)

Data from diving plates are transcribed into a database, which, among other things, contains: a description of the location, depths at which habitats begin and end, and a list of recorded species. In order to capture as much space as possible in the shortest possible time, an underwater scooter was used.

Further data processing will include the production of a habitat map based on data obtained from the field.

The study area is dominated by *Posidonia* meadows that stretch on sedimentary bottoms from 6 to 30 meters deep, and in the narrow coastal belt on a hard surface 10 m deep and shallower are well-developed communities of infralittoral algae, mostly associations with species of the genus *Cystoseira*.

4. CONCLUSION

The area of the Molat island is characterized by a mosaic of mostly shallow areas covered with *Posidonia* meadows, sandy bottoms and rocky bottoms with infralittoral algae. In the area of the Brgulje islet, one dead specimen of *P. nobilis* was recorded. Such habitats favour the reproduction and development of Elasmobranchs, including Angelsharks (*Squatina squatina*). Unfortunately, the presence of Angelshark was not confirmed during the research, but several

specimens of other Elasmobranch species (*Torpedo marmorata*, and species from the Myliobatidae family) were recorded. Angelshark is a species that has only one part of its life cycle related to shallow coastal areas, is active at night and spends most of its time hidden in sediment. It is very difficult to detect this species during diving, so this research method may not be relevant to confirm its presence in an area.

In the western part of the island there are areas with infralittoral algae and meadows of *Posidonia* and a rocky area at the top of Cape Bonaster. This area is also marked on the Navionics map as a good diving position. An interesting area for recreational diving is also located in the eastern part near Cape Stopanj, here the settlements of gorgonians *E. cavolini* have been recorded, which are located in an unusually shallow area, at a depth of 7 m. At Cape Stopanj, the *Posidonia* meadow is also interesting, as it is very well developed here and under the strong influence of sea currents.

To the south, the shallower area of the island of Molat, Maknare Bay, is covered with dense, well-developed *Posidonia* meadows. The lower edge of the meadows is determined by bathymetric maps and represents the maximum potential area of their distribution. Due to the extremely strong sea currents in this area, the exact boundaries of this habitat according to depth can only be determined using acoustic methods or underwater drones with GPS locators and powerful propulsion. This area is also specific for dense settlements of brown algae of the genus *Cystoseira* in the coastal area, up to 5 m deep. Between the islands of Trata and Vrtlac at a depth of about 28 m, an interesting area was recorded where different habitats alternate, *Posidonia* meadows, rocks with infralittoral and circalittoral communities, algae and gorgonia settlements, filters and scyphilic species at the base of the rocks. This diverse habitat, due to strong currents and distance from the coast, is less explored. Given the data that have been collected, it is clear that these are interesting habitats worth further research. The Rivan Channel has long been known as a very special area with strong currents. In the complex mosaic of rocks and sedimentary bottom, different habitat types alternate at almost the same depth - coralligenous biocenosis, *Posidonia* meadows, biocenosis of infralittoral algae and biocenosis of sediments in the infralittoral and circalittoral. These are certainly areas for which the use of underwater scooters, sonar, drones and similar technologies is recommended for more detailed mapping and research.

Although a very small amount of fish was recorded in the whole area, different types of Elasmobranchs were recorded during the dive in the southwest side of the island of Molat, the extreme south and the extreme southeast, which is a rare case and indicates the suitability of this area for these species. In addition to mapping the area, one of the goals of this study was

to find the species *S. squatina*. Although Angelsharks have not been recorded, a good indication of the possible presence is the higher number of recorded Elasmobranch species.

Sklat

Pravila ponašanja prilikom susreta sa sklatom



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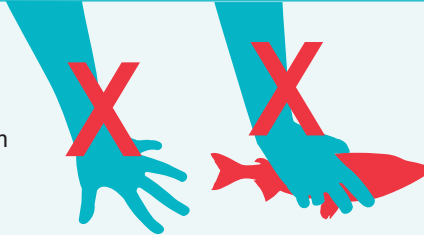


Ako je sklat zakopan u pijesku:

- držite minimalnu udaljenost od 1,5 m između vas i sklata



- ne dirajte ga
- nemojte ga pokušavati hraniti
- nemojte uklanjati pijesak ili sediment koji ga prekriva



Ako sklat pliva:

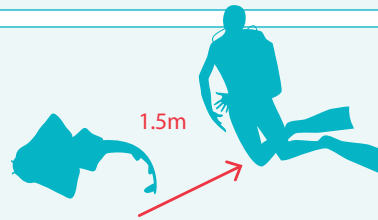
- ne plivajte za njim



- nemojte mu blokirati smjer plivanja

Ako ste u grupi ljudi:

- nemojte se okupljati oko sklata



- priđite mu straga, polako kleknite na dno držeći minimalnu udaljenost od 1,5 m, te se pobrinite da se nitko ne nalazi blizu glave

Kako su sklatovi zaštićeni u Hrvatskoj?

Sklatovi su u Republici Hrvatskoj uvršteni među strogo zaštićene vrste. Prema Zakonu o zaštiti prirode **zabranjeni su svi oblici namjernog hvatanja ili ubijanja sklatova te namjernog uznemiravanja**, posebno u vrijeme razmnožavanja i podizanja mladih. Također je zabranjeno oštećivanje ili uništavanje područja njihova razmnožavanja ili odmaranja, kao i držanje, prijevoz i prodaja, živih ili mrtvih jedinki.

Prijavi svoje opažanje!

Ako ste dovoljno sretni da vidite sklata tijekom ronjenja, prijavite svoje viđenje na telefon: **092/212 5646** ili pošaljite e-poštu na pkrstinic@wwfadria.org Ovi su podaci ključni za bolje razumijevanje i očuvanje ove kritično ugrožene vrste.

Kako pronaći sklata?

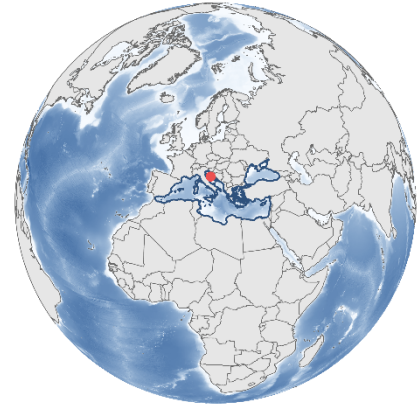
Sklatovi se zakopavaju u pijesak ili mulj kako bi iznenadili svoj plijen ili se sakrili od predatora. To znači da su vrlo dobro kamuflirani i izuzetno ih je teško uočiti pod morem. Prilikom traženja sklatova obratite pažnju na sljedeće:

Spirakuli – često jedini dio tijela sklata koji strši iz pijeska kada je životinja potpuno zakopana. Kroz ove cjevčice koje se na površini otvaraju malim otvorima u obliku polumjeseca sklatovi usisavaju more do škrge, što im omogućuje nesmetano i nepomično disanje dok miruju zakopani u sedimentu.

Obris tijela – ako je morska struja otplavila sediment s površine tijela životinje, možete vidjeti blijedi obris tijela u pijesku.

Peraje na repu – morskom psu je teško pokriti repnu i leđnu peraju sedimentom, pa one ponekad ostanu izložene.

Obavezno provjerite područja pijeska ili mulja u blizini stjenovitih grebena – sklatovi love iz zasjede, pa često vrebaju na mjestima gdje se plijen zadržava (ribe, sipe, lignje).



Blue lines indicate the area meeting the ISRA Criteria; dashed lines indicate the suggested buffer for use in the development of appropriate place-based conservation measures

WESTERN VIRSKO MORE ISRA

Mediterranean and Black Seas Region

SUMMARY

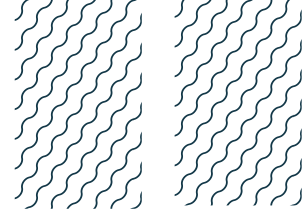
Western Virsko More is a small coastal area in Croatia in the eastern mid-Adriatic Sea. The area includes parts of the coastline of 11 large islands, and 48 smaller islets and rocks, with Olib and Molat Islands being the largest islands in the area. Shallower areas consist of sandy and rocky substrates, while deeper areas (to 85 m depth) comprise muddy-sandy or muddy bottoms. Distinctive habitats include large shallow bays, channel areas, and open water. The area includes 16 Natura 2000 Special Areas of Conservation (SAC). Within the area there are: **threatened species** and **reproductive areas** (Angelshark *Squatina squatina*).

CROATIA

0-85 metres

499 km²





CRITERIA

Criterion A - Vulnerability; Sub-criterion C1 - Reproductive Areas

DESCRIPTION OF HABITAT

Western Virsko More is a small coastal area in Croatia in the eastern mid-Adriatic. The area includes parts of the coastline of 11 large islands, and 48 smaller islets and rocks. Olib and Molat Islands are the largest islands in the area. Water depths range from shallow inshore waters to maximum depths of 85 m. In these deeper areas, the sea bottom is predominantly mud-sand and/or mud formed by heavy sedimentation in the water column. In the shallower areas closer to the islands and in the bays, the sedimentation is lower and therefore the seabed consists mainly of sandy sediments with larger grain sizes. The intertidal zone of this area, with the exception of the beaches of deep bays, consists of rocky bottoms (Dokoza et al. 2021, 2012b).

The area includes distinctive habitats, such as large shallow bays, channel areas, and open water. The Maknare part of the area is called seven gates by local inhabitants due to the seven passages between the islands of Molat, Sestrunj, Rivanj, and Tun Veli, as well as three smaller islets in the northern part of the area. Channel areas are characterised by extremely strong currents that change direction twice a day. Rivanj Channel has the most varied seabed morphology. It consists of boulders up to several metres in diameter scattered between large-grained sand dunes up to several metres high. The deepest point in the channel is 36 m. Due to the strong currents, sedimentation is usually high.

The area's 16 Natura 2000 Special Areas of Conservation (SAC) include especially important areas for the conservation of habitat types at the European Union level. These include Neptune Grass (*Posidonia oceanica*) meadows and biocenosis of: (1) well sorted fine sands; (2) superficial muddy sands in sheltered waters; (3) the coastal detritic bottom; (4) coralligenous habitats; and (5) infralittoral algae.

This Important Shark and Ray Area is benthic and is delineated from inshore waters (0 m) to 85 m based on the bathymetry of the area.

ISRA CRITERIA

CRITERION A - VULNERABILITY

The one Qualifying Species within the area is considered threatened with extinction according to the IUCN Red List of Threatened Species™. The Angelshark is assessed as Critically Endangered (Morey et al. 2019; Soldo & Lipej 2022).

SUB-CRITERION C1 – REPRODUCTIVE AREAS

Western Virsko More is an important reproductive area for one species of shark. The area has been known as a site of occurrence and reproduction for Angelsharks since the 19th century (Soldo 2021). The species is now rare anywhere in the Adriatic Sea, but recent catch records and surveys have confirmed that the area is a hotspot for the species in the Adriatic Sea. From 50 records recorded throughout the Croatian Adriatic in the period from 2010–2020, 19 records (38%) are located in the area. Furthermore, local fishers report the catch-and-release of Angelsharks from the area (A. Soldo et al. unpubl. data 2023).

Angelshark occurrence data in the area were gathered from the Angel Shark Sightings Map and a Local Ecological Knowledge (LEK) study conducted in Croatia by WWF Adria in 2020 (Pike et al. 2020). Both adult and juvenile Angelshark records were reported. Through the LEK survey, in boreal spring 2020, two fishers reported separate cases of pregnant females aborting pups in gillnets. These were a 15 kg female bearing 20 pups and a 20 kg female bearing 10 pups (Ugarković et al. 2020). Furthermore, reported catches include two neonates in 2020 (25 & 31 cm total length [TL]; size-birth is 24–30 cm TL, Ebert & Dando 2021) (Ugarković et al. 2020).

Marine habitat mapping carried out by the Association Sunce in 2021 identified habitats that correspond with Angelshark breeding and nursery areas. The area is characterised by a mosaic of mostly shallow areas covered with *Posidonia* meadows, sandy bottoms, and rocky bottoms with infralittoral algae. Such habitats favour the reproduction and development of Angelsharks (Dokoza et al. 2021a, 2021b).

Environmental DNA (eDNA) surveys conducted in 2022 confirmed that the area is a hotspot for Angelsharks in the Adriatic (Vrečko & Markulin 2022). Three replicate samples were collected from eight locations within the area. Laboratory analysis detected traces of Angelshark DNA in samples from three of the eight locations, with a high confirmation probability (all three replicates positive) at one location (Vrečko & Markulin 2022).

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Patrik Krstinic (WWF Adria; Angel Shark Project: Croatia), Alen Soldo (Department of Marine Studies, University of Split), Simone Niedermueller (WWF Mediterranean; Angel Shark Project: Croatia), Eva K.M. Meyers (Leibniz Institute for the Analysis of Biodiversity Change; Angel Shark

Project: Croatia), and Peter M. Kyne (IUCN SSC Shark Specialist Group – ISRA Project) contributed and consolidated information included in this factsheet. We thank all participants of the 2023 ISRA Region 3 – Mediterranean and Black Seas workshop for their contributions to this process.

This factsheet has undergone review by the ISRA Independent Review Panel prior to its publication.

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Suggested citation

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DRAFT



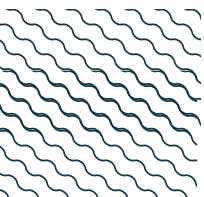
QUALIFYING SPECIES

| Scientific Name | Common Name | IUCN Red List Category | Global Depth Range (m) | ISRA Criteria/Sub-criteria Met | | | | | | | | | |
|--------------------------|-------------|------------------------|------------------------|--------------------------------|---|----|----|----|----|----|----|----|--|
| | | | | A | B | C1 | C2 | C3 | C4 | C5 | D1 | D2 | |
| SHARKS | | | | | | | | | | | | | |
| <i>Squatina squatina</i> | Angelshark | CR | 0-150 | X | | X | | | | | | | |

SUPPORTING SPECIES

| Scientific Name | Common Name | IUCN Red List Category |
|-------------------------------|--------------------------|------------------------|
| SHARKS | | |
| <i>Mustelus mustelus</i> | Common Smoothhound | EN |
| <i>Mustelus punctulatus</i> | Blackspotted Smoothhound | VU |
| <i>Scyliorhinus canicula</i> | Smallspotted Catshark | LC |
| <i>Scyliorhinus stellaris</i> | Nursehound | VU |
| RAYS | | |
| <i>Dasyatis pastinaca</i> | Common Stingray | VU |
| <i>Myliobatis aquila</i> | Common Eagle Ray | CR |
| <i>Raja miraletus</i> | Brown Skate | LC |
| <i>Torpedo marmorata</i> | Marbled Torpedo Ray | VU |

IUCN Red List of Threatened Species Categories are available by searching species names at www.iucnredlist.org. Abbreviations refer to: CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern; DD, Data Deficient.





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ANGEL SHARK LEK CASE STUDY CROATIA

Ugarković, P., Dragičević, B., Janči, T.

Introduction

The Mediterranean is considered a hotspot for biodiversity and approximately 7 % of the world's marine biodiversity is found in the Mediterranean Sea. This number is substantial if we consider that this water body covers only 0.82 % of the world's ocean area. Beside other animal groups and their respective species, Mediterranean Sea is a home to more than 80 species of sharks and rays. Due to intense anthropogenic pressures, including unsustainable exploitation and habitat loss, more than half of the species of sharks and rays are threatened which makes Mediterranean waters the most dangerous place for sharks and rays globally. In contrast to teleost fishes, elasmobranchs have a life history characterised by slow growth, long life, late maturity, and low fecundity, which increases risk of human induced mortality at a population wide scale. Among this group of fishes, angelsharks are considered one of the most endangered globally. The fact that angelsharks inhabit shallow coastal habitats, coupled with their specific life history (i.e. late maturity, reproduction in alternate years, low fecundity) makes them particularly vulnerable to the combined impact of habitat loss and mortality from incidental captures. Three species of angelsharks live in the Mediterranean Sea, namely *Squatina squatina*, *Squatina aculeate* and *Squatina oculata*. All three species are estimated to have declined by at least 80% over the past three generations (~45 years) and are now considered Critically Endangered by IUCN.

The Adriatic Sea sits between Italy and the Balkans, covering a surface area of around 138,600 km². The basin is split into northern, central, and southern Adriatic, with depth increasing from a north to south gradient to a maximum of over 1200 m. The continental shelf covering the north and central Adriatic is the most extensive in the Mediterranean and occurs between depths of 10 and 200 m, making it an ideal zone for coastal species such as the angelshark. Historically, angelsharks were target of commercial fisheries in the Adriatic Sea and, according to old literary sources, represented a reliable fishery resource in coastal areas. Fishing nets used for catching of angelsharks were named "squaenere" or "sklatare", etymology of which is related to the words "squaena" and "sklat" which means angelshark in Italy and Croatia, respectively. This fact suggest that the angelsharks were a regular catch in both western and eastern part of the Adriatic Sea. To this day, older fishermen remember times when "sklatara" nets were used and name of the nets is still present in the memory of older Croatian coastal population.

Two out of three critically endangered species of angelsharks of the Mediterranean Sea have been recorded in the Adriatic Sea, namely smoothback angelshark *S. oculata* and common angelshark *S. squatina*. Although, the records are very scarce it is considered that *S. oculata* has been extirpated from the Adriatic Sea due to overfishing. Landings of *S. squatina* declined dramatically after 1960s, causing them to become considered "economically extinct". During scientific expedition "HVAR", individuals of *S. squatina* were found throughout eastern Adriatic in the period between 1948 and 1958. Literature reports on catches of *S. squatina* in second half of twentieth century, when eastern Adriatic coast is considered, are very scarce. However, in the recent years, owing to more interest in the species, some records have been reported demonstrating that *S. squatina* is still present in the area.

Objectives

Objective of this investigation was to collect historical data as well as recent records on angelsharks catches and sightings in Croatian part of Adriatic Sea and compile them in order to build the basis for further work on protection of this critically endangered species. Through analysis of occurrences we will gain insight into its habitat preference, seasonality and fishery interaction. In December 2019, a regional Mediterranean action plan for angelsharks has been published with WWF MMI as one of the collaborators in the development. In order to inform the development of sub-regional action plans and concrete management measures, WWF MMI will evaluate the current level of areal protection of the Sharks and in particular.

Historical records and personal contacts

Records of *S. squatina* from the eastern Adriatic Sea were extracted or obtained through several means. We have searched the scientific literature indicated through previous studies (Fortibonio 2014; Holcer 2017) and researched additional scientific records and sources. For example, historical newspapers with publicly available archives, namely "Slobodna Dalmacija", "Novo Doba" and "Morsko ribarstvo" were searched for keywords "Squatina", "sklat", "sklatare" but without any results.

The data on some of the recent records have already been collected through personal contacts with fishermen prior to this study and several records have been brought to our attention directly by the fishermen through contacts on the facebook page „Podvodni.hr“, administrated by one of the authors (P.U.). Additional records were collected through data mining on social networks and groups dealing with recreational and professional fishery. Also, many people have been referred to by others and these were contacted directly (snowball sampling); fishermen, marine biology researchers and students, sport fishing and diving enthusiasts, fish market managers, distributors and traders and locals who could know or investigate for any information about angelsharks in their local community, which broaden our survey and covered most of the Croatian coast, including some information from Montenegrine shores.

Main questions asked were:

- Can you recognise angelshark?
- Have you seen or heard about any record of angelshark?
- Can you provide information about the catch (size, fishing gear, depth, date of the catch, contact of the fisherman, photos...)
- Can you think of somebody who would know more about this topic?

LEKFishResCRO data

Data from the surveys administered through the project LEKFishResCRO led by the Institute of oceanography and fisheries collected in the period of 2017-2018 were also considered. The data on 2 historic and 2 recent *S. squatina* occurrences were extracted from a more comprehensive survey which included 55 professional fishermen from all major Adriatic sectors (southern, central and north Adriatic). Within that questionnaire, fishermen were specifically asked about sightings or incidental

catches of *S. squatina* in the recent years.

Online survey data

Majority of recent records were collected through online survey (created and hosted by Google Forms platform) administered in the period from June to July of 2020 on the most popular Facebook groups about recreational and professional fishing in Croatia. The survey was additionally disseminated through relevant Internet portals dealing with fishery and general marine topics. The aim of the survey was to access fishermen's knowledge on observations and incidental catches of *Squatina squatina* along the Croatian part of the Adriatic coast.



TRAŽIMO SKLATA

Poštovani,

pred vama se nalazi on-line anketni upitnik čija je svrha prikupljanje informacija o slučajnim ulovima ili uočavanjima sklata. Sklat sivac je hrvatski naziv za vrstu morskog psa čiji je znanstveni naziv *Squatina squatina*. U prošlosti je ova vrsta bila široko rasprostranjena u Sredozemnom moru no uslijed intenzivnog ribolova populacije sklatova su značajno smanjenje na cijelom području njegova rasprostranjena te je prema IUCN-ovoj klasifikaciji ova vrsta ocijenjena kao KRITIČNO UGROŽENA. Specifičan način života, dugotrajan reproduktivni ciklus te mali broj potomaka ovu vrstu čine izuzetno osjetljivom na ribolovni pritisak te je njen dugoročni opstanak neizvjestan.

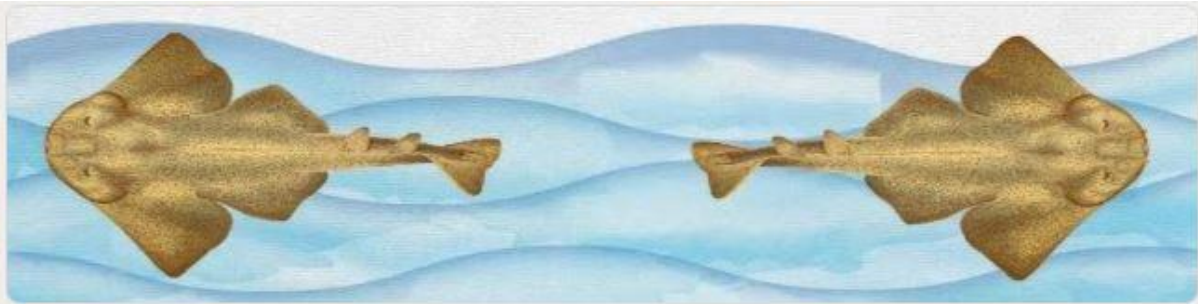
U Jadranskom moru ova vrsta je sve do sredine prošlog stoljeća bila relativno uobičajena. Danas je situacija drugačija te je sklat nestao na velikom dijelu područja gdje je prije obitavao, a prema dostupnim podacima, manja populacija još uvijek je prisutna na širem području Zadarskog i Šibenskog arhipelaga.

Kako bismo dobili što detaljniji uvid u njegovu trenutnu rasprostranjenost, potrebne su nam informacije o njegovoj pojavnosti te se nadamo da nam u tome možete pomoći ispunjavajući ovaj upitnik.

Unaprijed Hvala!

Photo 1- Front page of the online survey

Survey included general questions aimed at a person's fishery status (professional, recreational), general data (age, experience), fishery related data (fishing gears, area of fishing activity) and details on sightings of *S. squatina*.



TRAŽIMO SKLATA

Profesionalni ribolov

Koje ribolovne alate najčešće koristite? (Označite najviše 3 alata ili skupine alata koja često koristite)

- Mreže stajaćice
- Obalne mreže potegače
- Povlačni ribolovni alati (koća)
- Parangal
- Okružujući ribolovni alati (plivarice)
- Vrše za rakove ili ribu
- Other: _____

Photo 2 – Question about fishing gears used by the participants

Structure of the questionnaire allowed participants to report their own sightings or details on incidental catches as well as historical sightings and anecdotal evidence. For each sighting or incidental catch, participants were asked to provide additional data such as date, location, size, depth and fishing gear in regard to each catch.

Podatci o ulovima ili uočavanjima sklata (manje od 5)

Molimo Vas da nam za svaku jedinku upišete podatke koji su označeni u zagradama. Ukoliko se ne sjećate svih detalja, upišite one kojih se sjećate.

Jedinka 1 (sezona i godina; područje; veličina jedinke; alat ulova; dubina)

Your answer

Jedinka 2 (sezona i godina; područje; veličina jedinke; alat ulova; dubina)

Your answer

Jedinka 3 (sezona i godina; područje; veličina jedinke; alat ulova; dubina)

Your answer

Jedinka 4 (sezona i godina; područje; veličina jedinke; alat ulova; dubina)

Photo 3- Providing the data such as date, location, size, depth and fishing gear in regard to each catch.

The survey included a drawing and a quality photo of *S. squatina* in its natural habitat to prevent confusion with similar species. The survey also included a section where participants could leave their contact information and any additional data regarding *S. squatina* (links, photos and similar).

Zahvaljujemo se na ispunjenoj anketi! Za završetak molimo Vas pritisnite
"Podnesi/Submit"

Anketa je služila isključivo u svrhe izraživanja rasprostranjenosti i učestalosti pojavljivanja sklata. Ako do sada niste znali, sklat se nalazi na listi STROGO ZAŠTIĆENIH VRSTA ŽIVOTINJA u RH te su ciljani ribolov i prodaja ove vrste zakonom zabranjeni. Ukoliko imate priliku, molimo vas da poštedite ovu ribu, vratite je u more i ohrabrite ostale da je puštaju.

S obzirom da imate nama važna saznanja o ovoj vrsti, ukoliko ste voljni ostaviti nam Vaš kontakt, molimo Vas da nam ostavite vašu e-mail adresu ili broj mobitela. Unaprijed Hvala!

Your answer

Ukoliko imate bilo kakav komentar, možete ga ostaviti ovdje.

Your answer

Photo 4- A place to leave contact information and any additional data.

Taking into account the protection status of the species and sensitivity of personal data provided by some participants, data are not publicly available in its raw form.

In all, online survey was well received. The general public showed a desire to help to find the animal. After reading the introductory post, many people provided positive comments about the need for protection of angelsharks. In total, 368 participants filled the questionnaire out of which 44 person reported personal catches or sighting of angelsharks. Eleven participants reported the encounter with angelshark on more than one occasion. Only 13 participants of the survey did not personally catch the specimen but had useful information about its occurrence. Many people just left the comment, e.g.: "I did not see angelshark since the 70's." or "One fisherman told me he used to catch it with gillnets". Those kind of information can be used generally to underline angelsharks dramatic decline but cannot be considered useful since there was not a single information about the specimen.

Results

Based on the data on historical records, LEKFishResCRO data, our contacts and the online survey, the table presents all collected records of angelshark catches and sightings. In chronological order.

| Year | Season | Fishing gear | Location | GPS (Approximate area/location) | Photo | Depth | Size | Sex | Fate |
|------|--------|------------------|------------------------|--|-------|------------|--------|--------|--------------------------------------|
| 2020 | spring | gillnet | Sukošan | 44.023102, 15.302533 | no | n/a | 40 cm | n/a | released |
| 2020 | spring | trawling boat | Southwest of Kornat | 43.771925, 14.902192 | no | 150 meters | 5 kg | n/a | released |
| 2020 | n/a | gillnet | Silba | 44.361947, 14.668427 | no | 60 meters | 20 kg | n/a | n/a |
| 2020 | spring | diving | Sestrunj | 44.182378, 14.945170 | no | 30 meters | 1.5 m | n/a | free and wild |
| 2020 | spring | gillnet | Sestrunj | 44.190184, 14.966224 | yes | 20 meters | 25 cm | n/a | killed |
| 2020 | spring | gillnet | Rivanj | 44.154506, 15.021530 | no | n/a | 13 kg | n/a | n/a |
| 2020 | spring | gillnet | Rivanj | 44.171792, 15.039564 | yes | 20 meters | 15 kg | female | killed (20 juveniles released) |
| 2020 | spring | gillnet | Rivanj | 44.171829, 15.020028 | yes | 20 meters | 31 cm | n/a | killed |
| 2020 | spring | gillnet | Pašman channel | 43.984614, 15.370655 | yes | 7 meters | 17 kg | n/a | n/a |
| 2020 | spring | gillnet | Molat | 44.232690, 14.886879 | yes | 20 meters | 18 kg | female | killed (10 juveniles released) |
| 2020 | spring | gillnet | Filip Jakov | 43.943261, 15.415416 | yes | n/a | 1.55 m | female | killed |
| 2019 | summer | diving | Žut | 43.864014, 15.292581 | no | 10 meters | n/a | n/a | free and wild |
| 2019 | n/a | gillnet | Žirje | 43.676764, 15.600575 | no | n/a | n/a | n/a | n/a |

| | | | | | | | | | |
|-------------|--------|------------------|-------------------------------------|-------------------------|-----|-------------------|--------------------------------|-----|-----------------------|
| 2019 | spring | gillnet | Sestrunj | 44.192368, 14.932044 | no | 22 meters | 7-9 individual juveniles | n/a | n/a |
| 2019 | fall | gillnet | Rava | 44.029840, 15.070339 | no | 25 meters | 5 kg | n/a | n/a |
| 2019 | fall | gillnet | Molat | 44.199637, 14.869419 | no | 10 meters | 15 kg | n/a | n/a |
| 2019 | fall | gillnet | Molat | 44.199637, 14.869419 | no | n/a | 8 kg | n/a | killed |
| 2019 | fall | gillnet | Molat | 44.199637, 14.869419 | no | n/a | 10 kg | n/a | killed |
| 2019 | fall | gillnet | Molat | 44.237256, 14.864868 | yes | n/a | 15 kg | n/a | killed |
| 2019 | spring | trawling boat | Between Rab and Trstenik | 44.666424, 14.580202 | yes | n/a | 25 cm | n/a | sold on fishmarket |
| 2019 | spring | longline | Between Korčula and Pelješac | 42.949618, 17.234609 | no | 80 meters | 4 kg | n/a | released |
| 2018 | n/a | gillnet | Privlaka | 44.246034, 15.118003 | no | n/a | 20 kg | n/a | n/a |
| 2018 | n/a | trawling boat | Mid Adriatic, close to Jabuka | 43.302209, 15.225929 | no | 100-200 meters | n/a | n/a | n/a |
| 2018 | spring | gillnet | Between Silba and Ilovik | 44.440074, 14.619834 | no | n/a | 10 kg | n/a | killed |
| 2017 | n/a | n/a | Velebit channel | 44.634607, 14.829361 | no | n/a | n/a | n/a | n/a |
| 2017 | spring | diving | South west Žirje | 43.676394, 15.598770 | no | 15 meters | n/a | n/a | free and wild |
| 2017 | n/a | diving | Rijeka | 45.285341, 14.517948 | yes | 25 meters | 10 kg? | n/a | free and wild |
| 2017 | n/a | longline | Poje bay, Cres | 44.731391, 14.467187 | no | 30 meters | 1 m | n/a | released |

| | | | | | | | | | |
|-------------|--------|---------------|----------------------------|----------------------|-------|-----------|--------|------|--------------------|
| 2017 | fall | trawling boat | Murtersko more | 43.774195, 15.524964 | yes | 80 meters | 4 kg | n/a | n/a |
| 2017 | n/a | n/a | Molat | 44.222268, 14.870731 | no | n/a | 17 kg | n/a | n/a |
| 2017 | n/a | n/a | East Istra | 44.873308, 14.170745 | no | n/a | 4 kg | n/a | sold on fishmarket |
| 2016 | n/a | gillnet | Šibenik county | 43.702956, 15.737849 | no | n/a | 10 kg | n/a | sold on fishmarket |
| 2016 | winter | gillnet | Rava | 44.029629, 15.074571 | no | 25 meters | 3 kg | n/a | n/a |
| 2016 | winter | trawling boat | Murtersko more | 43.774195, 15.524965 | no | 80 meters | 6 kg | n/a | n/a |
| 2016 | n/a | gillnet | Between Sestrunj and Molat | 44.200163, 14.927834 | yes | n/a | 10 kg | male | killed |
| 2015 | n/a | trawling boat | West Cres | 44.883174, 14.236296 | no | 60 meters | n/a | n/a | n/a |
| 2015 | n/a | n/a | n/a | n/a | yes | n/a | n/a | n/a | n/a |
| 2015 | n/a | gillnet | Murter | 43.774195, 15.524964 | no | n/a | n/a | n/a | n/a |
| 2015 | winter | gillnet | Silba | 44.420001, 14.670852 | maybe | n/a | 8 kg | n/a | killed |
| 2014 | fall | longline | Rava | 44.029629, 15.074571 | no | 20 meters | 5 kg | n/a | n/a |
| 2014 | fall | longline | Rava | 44.029629, 15.074571 | no | 20 meters | 6 kg | n/a | n/a |
| 2014 | n/a | gillnet | Murter? | 43.774195, 15.524964 | no | n/a | n/a | n/a | n/a |
| 2014 | n/a | diving | Mali Drvenik | 43.448841, 16.059075 | n/a | 18 meters | 3-4 kg | n/a | free and wild |
| 2013 | n/a | n/a | Between Lošin and Silba | 44.440074, 14.619834 | maybe | n/a | n/a | n/a | n/a |

| | | | | | | | | | |
|-------------|--------|------------------|----------------------------------|-------------------------|-----|---------------------|----------------------|--------|---------------|
| 2013 | n/a | gillnet | Iž channel | 44.044044, 15.122150 | no | n/a | n/a | n/a | n/a |
| 2013 | n/a | gillnet | Between Molat and Sestrunj | 44.202313, 14.921912 | yes | n/a | 10 kg | male | killed |
| 2011 | n/a | gillnet | Malinska | 45.131529, 14.498455 | no | n/a | n/a | n/a | killed |
| 2010 | summer | diving | Umag | 45.436183, 13.508410 | no | 20 meters | 15 cm | n/a | free and wild |
| 2010 | n/a | n/a | Island Trstenik (Pag) | 44.666424, 14.580202 | no | n/a | n/a | n/a | free and wild |
| 2010 | n/a | trawling boat | Biševo | 42.984987, 16.043967 | no | n/a | 10 kg | n/a | killed |
| 2009 | spring | harpoon | Olib | 44.357459, 14.820609 | no | 10 meters | 25 kg | n/a | killed |
| 2009 | fall | gillnet | Olib | 44.401995, 14.762638 | yes | n/a | 20 kg | n/a | killed |
| 2009 | n/a | gillnet | Murtersko more | 43.774195, 15.524966 | no | 25 meters | n/a | n/a | n/a |
| 2008 | n/a | gillnet | Split | 43.492845, 16.424021 | no | n/a | n/a | n/a | n/a |
| 2008 | n/a | longline | Krapanj | 43.669772, 15.910543 | no | n/a | n/a | n/a | n/a |
| 2007 | summer | gillnet | Vis | 43.047348, 16.271746 | no | n/a | 15 kg | n/a | killed |
| 2007 | n/a | diving | Susak | 44.500264, 14.308284 | no | n/a | 7-8 kg | n/a | free and wild |
| 2007 | n/a | trawling boat | Murtersko more | 43.774195, 15.524967 | no | 85 meters | n/a | n/a | n/a |
| 2006 | n/a | trawling boat | Blitvenica | 43.601031, 15.554722 | no | 100 - 200 meters | 10 kg + 1 newborn | female | both released |
| 2004 | fall | gillnet | South Pelješac | 42.949618, 17.234609 | no | 60 meters | 10 kg | n/a | released |

| | | | | | | | | | |
|-------------|--------|---------------|-----------------------------|----------------------|----|------------|---------|--------|---------------|
| 2004 | fall | trawling boat | Between Premuda and Grujica | 44.386860, 14.574945 | no | 50 meters | 15 kg | n/a | n/a |
| 2003 | spring | gillnet | Pašman channel | 44.024912, 15.293272 | no | 10 meters | 30 kg | n/a | n/a |
| 2002 | n/a | n/a | Pelješac channel | 43.084371, 17.146508 | no | n/a | n/a | n/a | n/a |
| 2002 | n/a | n/a | Maslenica channel | 44.316830, 15.250162 | no | n/a | n/a | n/a | n/a |
| 2001 | n/a | gillnet | Zadar channel | 44.143063, 15.150168 | no | 30 meters | n/a | n/a | taxidermist |
| 2001 | n/a | gillnet | South Pelješac | 42.949618, 17.234610 | no | 60 meters | 15 kg | n/a | killed |
| 2000 | n/a | diving | Unije | 44.612882, 14.265534 | no | n/a | 5 kg | n/a | free and wild |
| 2000 | spring | gillnet | Ugljan | 44.096428, 15.123066 | no | 10 meters | 1 kg | n/a | n/a |
| 2000 | n/a | n/a | Silba | 44.369844, 14.697483 | no | n/a | n/a | n/a | n/a |
| 2000 | n/a | n/a | Olib | 44.357459, 14.820609 | no | 16 meters | 20 kg | female | n/a |
| 2000 | n/a | longline | Kanica | 43.496437, 15.996959 | no | n/a | n/a | n/a | n/a |
| 2000 | n/a | trawling boat | Between Vis and Svetac | 43.031094, 15.923581 | no | 100 meters | n/a | n/a | n/a |
| 1997 | n/a | gillnet | South Pelješac | 42.949618, 17.234611 | no | 60 meters | n/a | n/a | n/a |
| 1997 | n/a | diving | Reef west of Susak | 44.480955, 14.350036 | no | n/a | 8-10 kg | n/a | free and wild |
| 1996 | n/a | trawling boat | South Palagruža | 42.291615, 16.260721 | no | 130 meters | 45 kg | n/a | n/a |
| 1996 | n/a | longline | Pašman channel | 44.024912, 15.293273 | no | 13 meters | 14 kg | n/a | n/a |

| | | | | | | | | | |
|-------------|--------|--------------------|---------------------------|-------------------------|----|-----------|----------|--------|--------------------|
| 1995 | n/a | gillnet | Zmajan | 43.707350, 15.740321 | no | n/a | n/a | n/a | n/a |
| 1995 | n/a | gillnet | South Čiovo | 43.474950, 16.292549 | no | n/a | 15-20 kg | n/a | n/a |
| 1995 | summer | gillnet | Pelješac channel | 43.084371, 17.146509 | no | n/a | n/a | n/a | n/a |
| 1994 | n/a | trawling boat | Vis channel | 43.223706, 16.105344 | no | 80 meters | 14 kg | female | juveniles released |
| 1994 | n/a | gillnet | Virsko more | 44.293178, 14.962942 | no | 40 meters | 4 kg | n/a | n/a |
| 1991 | n/a | trawling boat | Between Vis and Biševo | 42.984987, 16.043967 | no | n/a | 4 kg | n/a | n |
| 1990 | n/a | gillnet | Vrgada | 43.848541, 15.494633 | no | n/a | velik | n/a | n/a |
| 1990 | n/a | gillnet | Vis east | 43.051295, 16.280975 | no | 20 meters | 30 kg | n/a | n/a |
| 1990 | n/a | gillnet | Vis east | 43.051295, 16.280976 | no | 20 meters | 30 kg | n/a | n/a |
| 1990 | n/a | gillnet | Vis east | 43.051295, 16.280977 | no | 21 meters | 30 kg | n/a | n/a |
| 1990 | n/a | n/a | Rovinj | 44.147872, 15.058218 | no | n/a | 4-5 kg | n/a | n/a |
| 1990 | n/a | n/a | Prvić | 43.730168, 15.786979 | no | n/a | 15 kg | n/a | n/a |
| 1990 | n/a | trawling boat | Between Vis and Biševo | 42.984987, 16.043968 | no | n/a | 4 kg | n/a | n |
| 1989 | n/a | gillnet | Vis east | 43.051295, 16.280978 | no | 20 meters | 30 kg | n/a | n/a |
| 1987 | n/a | hook, kančanica | Silba | 44.369844, 14.697482 | no | n/a | n/a | n/a | killed |
| 1985 | n/a | n/a | Omišalj | 45.279082, 14.484341 | no | n/a | n/a | n/a | n/a |

| | | | | | | | | | |
|-------------|--------|-------------------------|--------------------|----------------------|----|----------------|--------------------------|-----|-------------|
| 1985 | n/a | trawling boat - gillnet | Various locations | 43.004245, 17.399465 | no | n/a | n/a | n/a | many killed |
| 1985 | n/a | gillnet | Neretva chanell | 43.004245, 17.399465 | no | n/a | 45 kg | n/a | n/a |
| 1985 | n/a | longline | Konavle | 42.477733, 18.370627 | no | 100 meters | n/a | n/a | n/a |
| 1980 | n/a | gillnet | Pag | 44.475723, 14.950953 | no | 5 meters | 30+ kg | n/a | killed |
| 1980 | n/a | gillnet | Iž | 44.044044, 15.122150 | no | 80 meters | 10-15 kg | n/a | n/a |
| 1973 | n/a | gillnet | Pelješac channel | 43.084371, 17.146510 | no | 50 meters | n/a | n/a | n/a |
| 1973 | n/a | gillnet | Pelješac channel | 43.084371, 17.146511 | no | 51 meters | n/a | n/a | n/a |
| 1948 | summer | trawling boat - HVAR | North of Svetac | 43.087602, 15.849010 | no | 115 meters | 0.2 kg | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | South of Šolta | 43.301389, 16.216667 | no | 95 meters | 9 kg (2 individuals) | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | Biševo | 43.087602, 15.849010 | no | 95 meters | 0.5 kg | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | Southeast Lastovo | 42.655473, 17.074691 | no | 150 meters | 13.1 kg | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | South of Dubrovnik | 42.412254, 17.995433 | no | 154-276 meters | 5.13 kg (2 individuals) | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | South of Lopud | 42.519831, 17.837606 | no | 125-204 meters | 8 kg | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | South of Konavle | 42.185106, 18.407025 | no | 305-377 meters | 15.77 kg (3 individuals) | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | Montenegro | 41.627943, 18.828998 | no | 155-196 meters | 0.25 kg | n/a | n/a |
| 1948 | spring | trawling boat - HVAR | South Albania | 40.696407, 19.026446 | no | 148-172 meters | 0.15 kg | n/a | n/a |

Overall, 39 records of angelsharks were found in the period of the last 5 years while total of 50 records were found in the span from 2010. to 2020. This number of sightings is quite extraordinary given the fact that recent publications indicated near disappearance of the species and emphasized low number of records in the last decades. Such number of angelshark records in the recent period could be attributed to a fresh memory of contacted people, more people engaged in social media and communication that made them more aware of this animal, and the fact that the generations of fishermen changes over time. Although we contacted also the older fisherman, nevertheless, there were more answers from “younger” people. In our online survey, 66% of people who wrote in the questionnaire their age, were under 40 years of age and reported an encounter with angelshark in the last 10 years, while only 2 records are reported by people older than 60. Considering angelshark records from the last century, only 5% were provided by people younger than 40 years of age.

Many answers provided information from past with only general data about specimens since older people did not remember many details and considered angelshark to be just an ordinary species in that period. In fact, an older trawling fishermen who was fishing back in the 1980’s told us they used to kill angelshark on purpose and throw it back in the sea as they considered it as a useless animal.

Seasonally, in the last 10 years, people who could remember more details, found 15 records in spring, 2 in summer, 3 in winter, and 8 in fall. This results suggest it is more commonly caught in spring and fall, and this information might be useful for future studies and angelshark search.

Regarding the fishing methods provided by participants, a total of 27 records in the last 10 years were caught using bottom set gillnets, 4 using longline and 6 using bottom trawl. As can be seen, bottom set gillnets represent by far the most efficient fishing gear for catching angelsharks.

Approximate size was given to most of the records, as noted in the table. Participants in the survey stated either weight or length. Some of them did weight the angelshark but not all. In the cases where precise size was not known participants gave their best guess based on their subjective approach. Keeping a track of the accuracy of given data was not possible in many answers of the online survey. Taking that in the consideration the data could have a high probability of an error, give or take few kg for non-juvenile specimens.

We found that 10.5 % of the records were juveniles of less than 30 cm or less than 0.5 kg, 69.7 % were between 31 and 100 cm in length or 0.6 and 18 kg in weight, and 19.7 % were adults of a length greater than 100 cm or weight of 19 kg. Some records contained qualitative information where a female angel shark had aborted pups during the fishing procedure suggesting it was caught in its breeding and/or nursery area.

All the data from this study were also provided to **London zoological society** and based on the given locations, using GPS data with 3 possible ranges; specific, middle and broad, ZLS have created maps on occurrences of angelsharks in Adriatic Sea. As can be seen from these maps, majority of records in the last 10 years occurred in the area of north Adriatic with greatest number of records in Zadar archipelago. Our records will be further analysed and a more comprehensive study based on these

results will be made in accordance to WWF objectives.

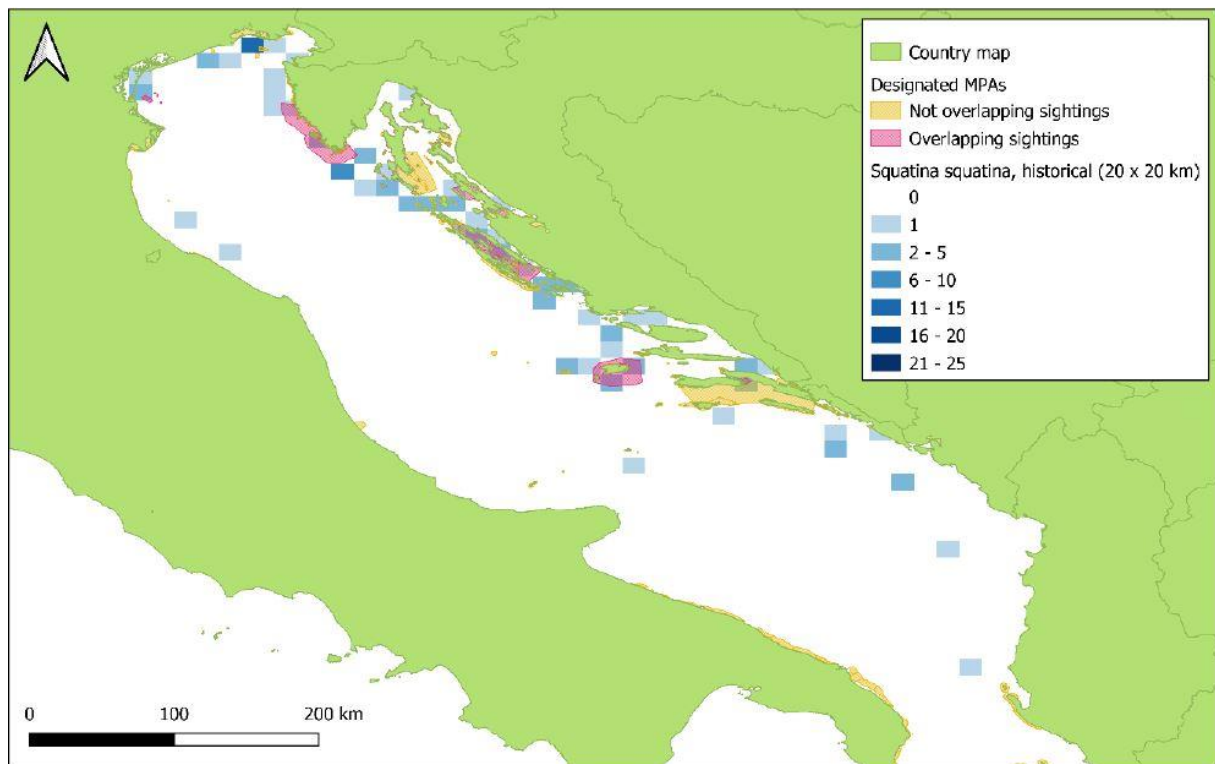


Figure 1- Historical (1947 - 2009 inclusive) records of *S. squatina* at 20*20 km resolution in relation to designated MPAs in Adriatic sea.

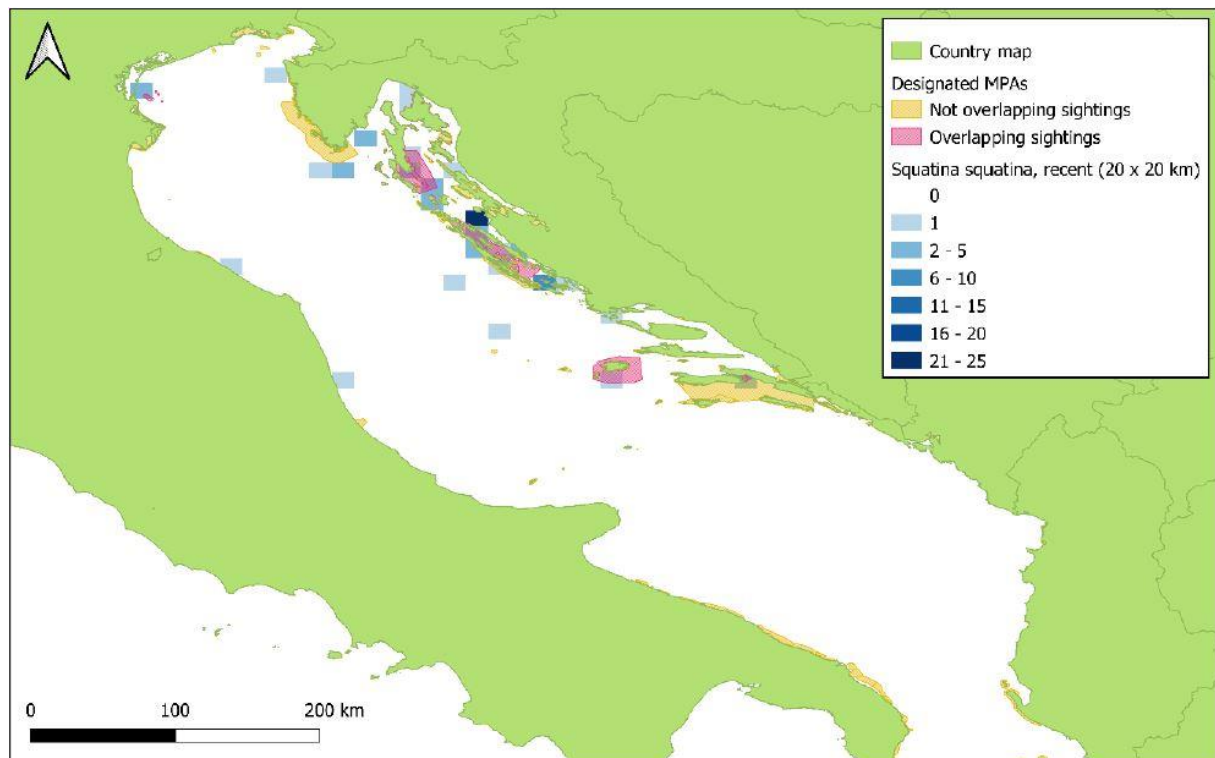


Figure 2- Recent (2010 - 2020) records of *S. squatina* at 20*20 km resolution in relation to designated MPAs in Adriatic sea.

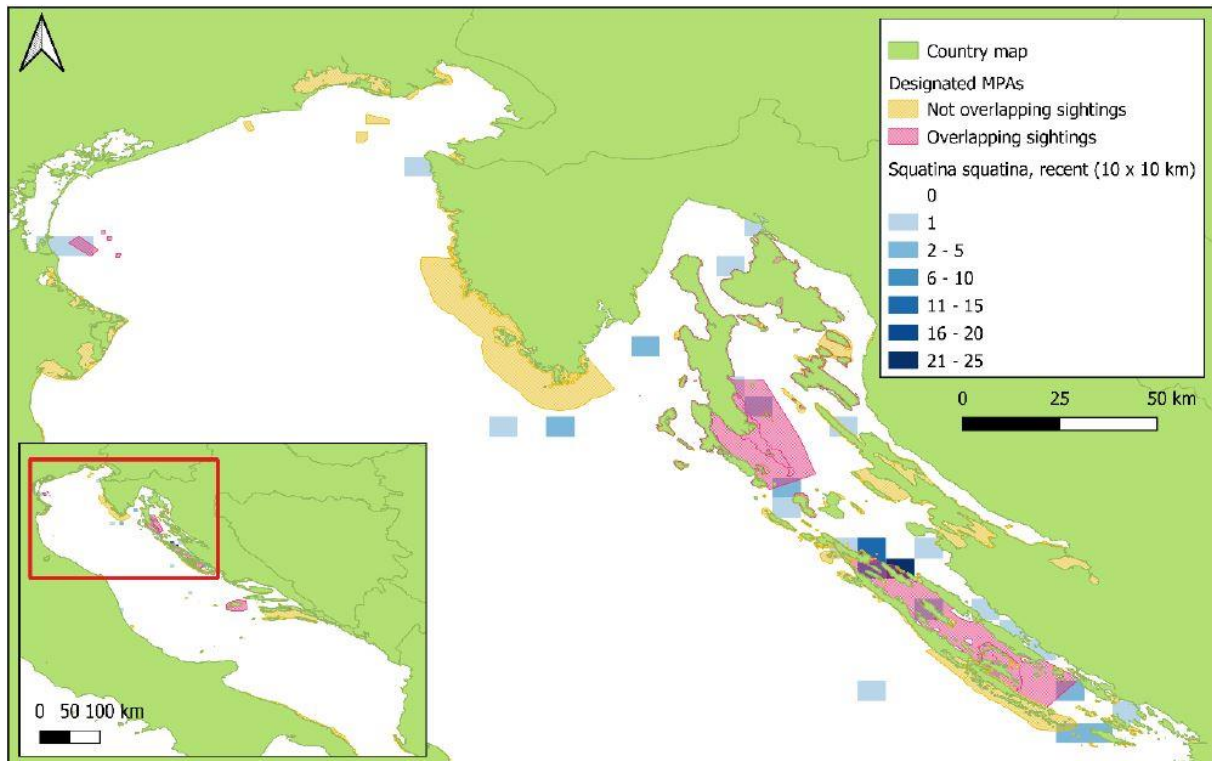


Figure 3- North Adriatic recent (2010 - 2020) records in 10 * 10 km resolution in relation to designated MPAs in Adriatic sea.

During our study, we have collected photos of angelsharks (as noted in the column in our table). While the fisherman felt safe showing the photos to us on the spot, due to strict law it was not expected that someone would give such an image to our use. It is easily perceived that the photo of a protected animal given to somebody would be risky. Under the Croatian law, the protected animal, if captured accidentally, has to be reported to the environment agency and in this case not many people do so. The images we collected are photos with given permission to be used in our study. As far as we know, not a single photo or video was taken underwater, during diving or snorkelling.



Photo 5 - Angelshark from Molat island 2013.

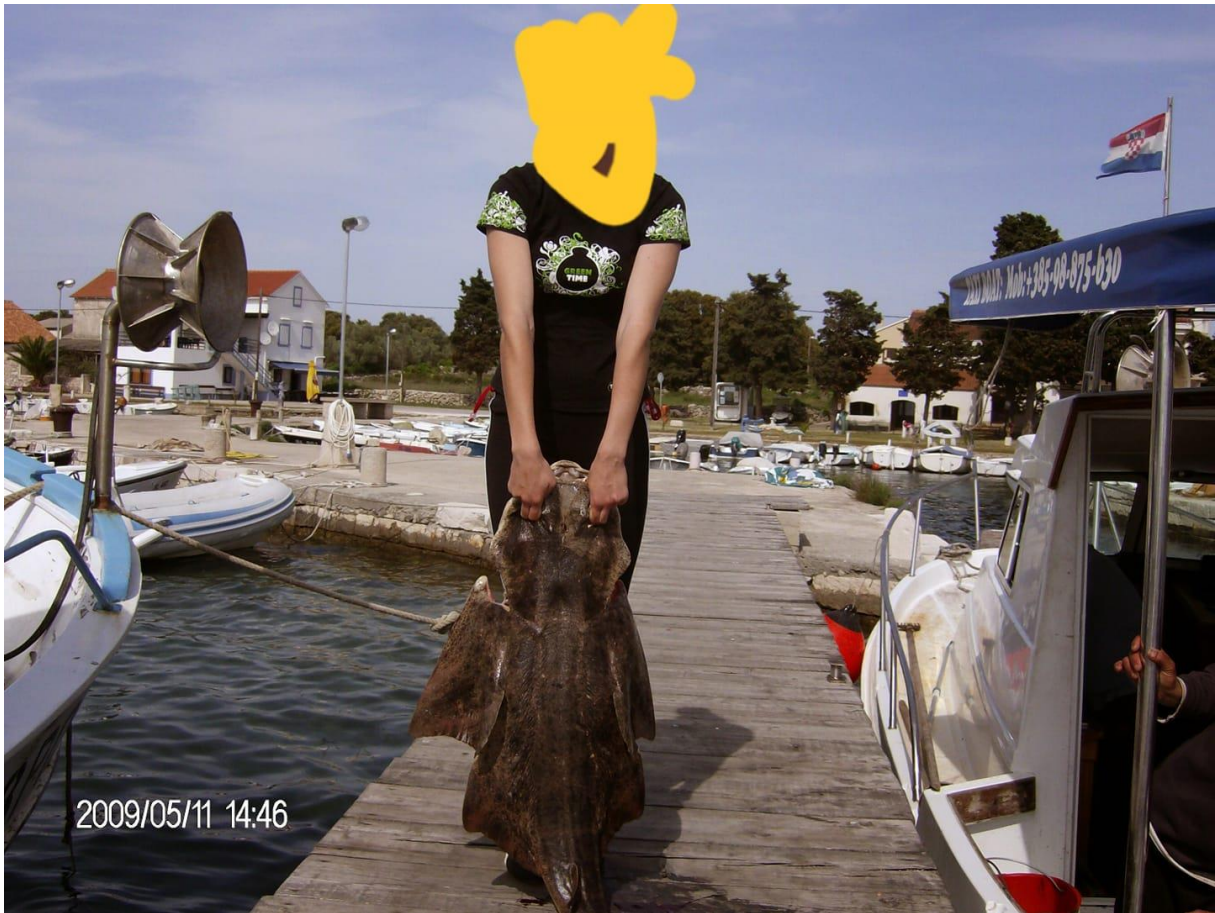


Photo 6 - Olib island



Photo 7- Specimen from Murter sea, captured in fall 2018.



Photo 8- Juvenile captured by trawling boat near Silba island 2018, taken to taxidermy.



Photo 9- A specimen captured on Molat 2013.



Photo 10 – Photo of the specimen appeared on facebook 2020.

Conclusions:

- One major hotspot in Eastern Adriatic has been located on the basis of records of *S. squatina*. It is the region northwest from Zadar, with island Molat at its center.
- Majority of records have been located in the area of the north side of Island Molat and this is considered as the area with the greatest abundance of *S. squatina*. However, several records have also been located around nearby islands and islets, from island Silba to island Ugljan.
- Taking into account findings of several individuals with pups and several juvenile specimens in the hotspot zone, it is reasonable to suggest that this area could potentially serve as a nursery area for *S. squatina*.
- Results from our study suggest that the angelsharks have been severely depleted from the most part of its historical range, specifically from southern and middle Adriatic.
- Few records have also been found outside of the suggested hotspot indicating possibility of wider, but impoverished area of its presence. Such area is that of “Murter channel” (the region south of Murter island, 70 km far from Island Molat) as some of the caught specimens in recent period were recorded in that zone, or recorded in the channels between Molat and Murter Sea. Future research, especially on its migration patterns should shed more light on the extent of its distribution in other areas and establish which areas could be defined as areas of persistence or transitional areas.
- Based on our general knowledge and information obtained from fishermen, two hypothesis can be suggested in relation to its presence in proposed hotspot area:
 1. strong currents surrounding hotspot make fishing less efficient
 2. lesser number of fishermen, who at the same time have broader range of excellent fishing locations south of Molat (not north where angelshark hotspot is proposed by our study), compared with the number of fisherman from whole east Adriatic.
- 3. trawling is forbidden in the major part of the proposed hotspot area
- Future studies combining *in situ* observations and LEK and fishery surveys should provide new insights into life history and spatio-temporal dynamics of the species
- Our data suggests bottom set gillnets are the most used fishing gear for capturing angelsharks in the Adriatic Sea. Hence, focus of conservation efforts should be directed toward reduction of fishing effort by this type of nets in the hotspot area. Additionally, in order to mitigate fisheries impact on the species, education of fishermen, which are mostly unaware of its status, should also be considered. This should specifically include fishermen which operate in the hotspot areas between Silba and Ugljan, and at the north side of Molat archipelago which could serve as the nursery ground for the species.

ANALYSIS REPORT

Species-specific detection of *Squatina squatina* in environmental samples

Prepared:

Sabina Vrečko

BIA Separations CRO MBL

20.09.2022

Date

Sabina Vrečko



Signature

Lucija Markulin

Labena d.o.o. Croatia

22.09.22.

Date



Signature

Checked:

Teja Petra Muha

BIA Separations CRO MBL

20.09.2022

Date

Teja Petra Muha



Signature

Approved:

Uršula Prosenc Zmrzljak

BIA Separations CRO MBL

20.09.2022

Date

Uršula Prosenc Zmrzljak



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1. INTRODUCTION

Aim of this analysis was to detect presence of the species *Squatina squatina* (hrv. sklat sivac) in the environmental samples collected in the Adriatic Sea.

2. SAMPLES

Received: 05.09.2022.

Submitted by: Patrik Krstinić (WWF Adria)

Accepted by: Lucija Markulin (Labena d.o.o. Croatia)

A. Samples description

Received filters had 0.5-2 L of sea water filtered through them. Filter material was mixed cellulose ester with pore size 0.45 µm and diameter 50 mm (Whatman, cat. no. 10406872, lot. no. A29481435). Filters were stored in 1.5 mL tube in the Longmire buffer. Transport was done at the room temperature and samples were stored on +4-8 °C until DNA extraction.

B. Samples list

We have received 32 filters for 24 samples (**Table 1**). Samples were collected in triplicates at eight locations. For some replicates sea water was filtered through multiple filters.

Table 1 List of filters submitted by WWF Adria.

| No. | Sample name | No. of filters | Sampling location |
|-----|-------------|----------------|---------------------|
| 1 | BO1 | 1 | Bovišćak |
| 2 | BO2 | 1 | |
| 3 | BO3 | 1 | |
| 4 | Lj1 | 1 | Luka Jazi |
| 5 | Lj2 | 1 | |
| 6 | Lj3 | 1 | |
| 7 | SR1 | 1 | Solinski rt (Istok) |
| 8 | SR2 | 1 | |
| 9 | SR3 | 1 | |
| 10 | BR1 | 4 | Brgulje |
| 11 | BR2 | 4 | |
| 12 | BR3 | 2 | |
| 13 | KO1 | 2 | Uvala Konopljika |
| 14 | KO2 | 1 | |
| 15 | KO3 | 1 | |
| 16 | USN1 | 1 | Uvala Sabuša |
| 17 | USN2 | 1 | |
| 18 | USN3 | 1 | |
| 19 | UV1 | 1 | Uvala Vapojni |
| 20 | UV2 | 1 | |
| 21 | UV3 | 1 | |
| 22 | RI1 | 1 | Rivanjski kanal |
| 23 | RI2 | 1 | |
| 24 | RI3 | 1 | |

3. ASSAY VALIDATION

A. Primer design and *in silico* primer validation

In order to detect targeted species two primer sets were designed and tested *in silico* (**Table 2**). Both primer sets (primer set 1 and primer set 2) target mitochondrial *COI* gene and were designed in a way that they match 100% only with target species *Squatina squatina*. After laboratory optimisation the primer set 1 was selected for the analysis of environmental samples.

Table 2 Details on the two primer sets designed for species-specific *Squatina squatina* assay.

| <i>Squatina squatina</i> primer set 1* | | |
|--|--|----------------|
| | Forward primer | Reverse primer |
| Length (bp) | 21 | 24 |
| GC (%) | 48 | 42 |
| T _m (°C) | 59.9 | 60.6 |
| Amplicon length (bp) | 153 | |
| Target region | cytochrome oxidase subunit 1 (<i>COI</i>) gene (mtDNA) | |
| <i>in silico</i> PCR † | <i>Squatina squatina</i> (with 100% match) | |
| <i>Squatina squatina</i> primer set 2 | | |
| | Forward primer | Reverse primer |
| Length (bp) | 23 | 26 |
| GC (%) | 48 | 35 |
| T _m (°C) | 62.7 | 57.7 |
| Amplicon length (bp) | 137 | |
| Target region | cytochrome oxidase subunit 1 (<i>COI</i>) gene (mtDNA) | |
| <i>in silico</i> PCR † | <i>Squatina squatina</i> (with 100% match) | |

* Primer set selected for analysis of environmental samples.

† Tested using Primer-BLAST (Ye et al., 2012) against database “nr” with Organism option empty and rest of the parameters set as default.

B. Experimental validation

I. Determining optimal annealing temperature

The annealing temperature is one of the key parameters in qPCR and has large effect on assay quality (Bustin et al., 2009). The optimal annealing temperature was determined experimentally using temperature gradient between 55 °C and 65 °C at eight temperature points. The qPCR reactions were run on C1000 Touch Thermal Cycler CFX386 (BioRad) using SsoAdvanced Universal SybrGreen Supermix (cat. no. 1725271, lot. no. 644398826), forward and reverse primer concentrations of 600 nM and *Squatina squatina* DNA quantity 0.6 ng. Each qPCR reaction was done in triplicate. Amplification plot is presented in **Figure 1**. Annealing temperature of 60 °C was selected for further analysis.

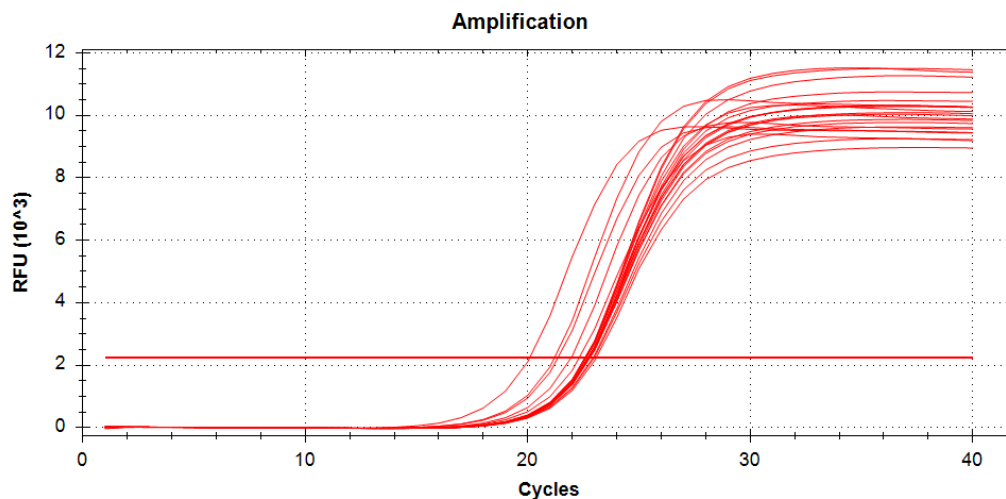


Figure 1 Amplification plot of functionality analysis of *Squatina squatina* assay 1 performed on the qPCR. Each reaction contained 0.6 ng of target DNA. Eight annealing temperatures were tested ranging between 55-65 °C.

II. Linear dynamic range

In order to determine linear dynamic range of the assay *i.e.* range over which reaction is linear (Bustin et al., 2009) series of 2-fold dilutions of positive control DNA isolated from *Squatina squatina* tissue were prepared. The qPCR reactions were run on C1000 Touch Thermal Cycler CFX386 (BioRad) using SsoAdvanced Universal SybrGreen Supermix (cat. no. 1725271, lot. no. 64439826), forward and reverse primer concentration of 600 nM and 2-fold dilutions of *Squatina squatina* DNA (5.00, 2.50, 1.25, 0.63, 0.31, 0.16, 0.08 and 0.04 ng). Each qPCR reaction was done in triplicate. The linearity regression curve analysis proved high correlation coefficient ($R^2= 0.9997$) with high primer set amplification efficiency ($E= 97.59\%$) (**Figure 2**).

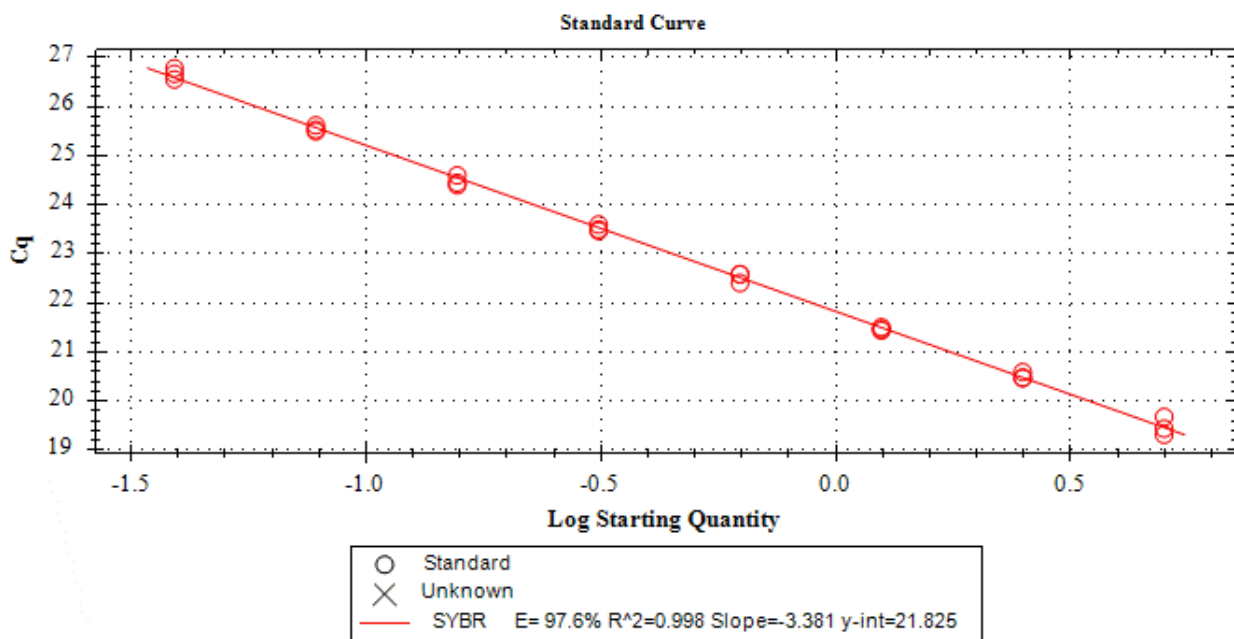


Figure 2 *Squatina squatina* assay 1 linearity range analysis targeting range of DNA quantities of *Squatina squatina*.

III. Limit of detection (LOD)

The limit of detection (LOD) is defined as the lowest concentration at which 95% of positive sample can be detected (Bustin et al., 2009). The LOD was determined from a standard curve. Standard curve was prepared in duplicates using 2-fold dilution series of positive control *Squatina squatina* DNA quantity (0.00500. 0.00250. 0.00125. 0.00063. 0.00031 and 0.00016 ng). The ddPCR reactions were done using 2x QX200™ ddPCR™ EvaGreen® Supermix BioRad (cat. no. 1864033, lot. no. 64460425) and QX200™ Droplet Generation Oil for EvaGreen BioRad (cat. no. 1864006, lot. no. 64460425) with forward and reverse primer concentration of 600 nM. Droplets were prepared on QX200 Droplet Generator, PCR reactions were run on C1000 Touch Thermal Cycler (BioRad) and droplets were read using QX200 Droplet Reader (BioRad).

The LOD was determined as the lowest DNA concentration where in both replicates at least two positive droplets were detected. The LOD was determined at 0.00031 ng/μL, and this is proposed sensitivity limit for *Squatina squatina* assay 1 (**Figure 3, Figure 4**).

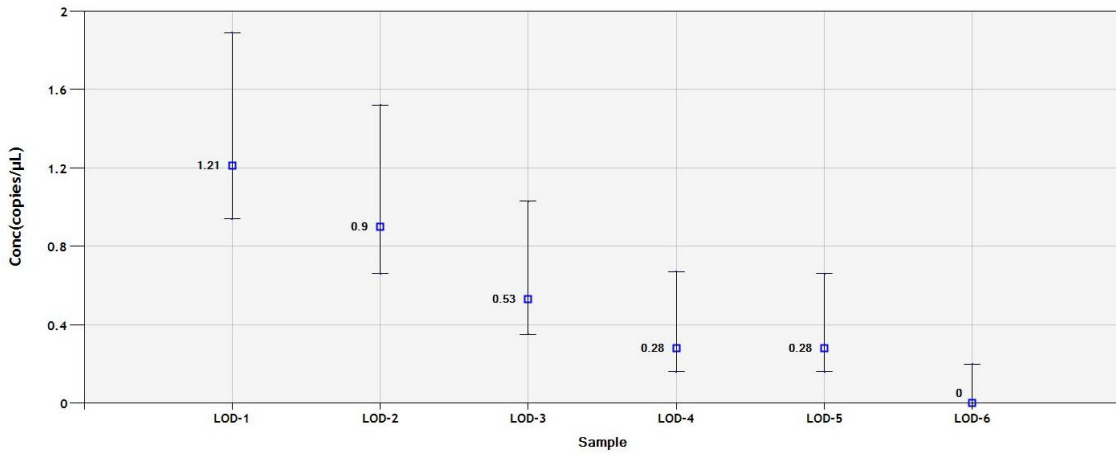


Figure 3 LOD on ddPCR was determined at LOD-5 (0.00031 ng/μL), with at least two positive droplets detected.

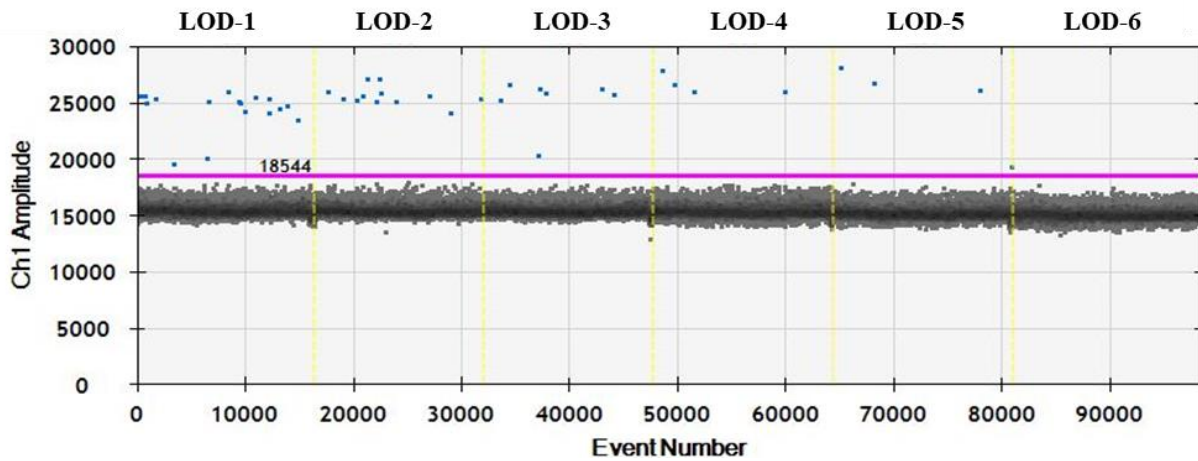


Figure 4 1D amplitude plot showing results of LOD analysis on ddPCR with the positive droplets showing amplification of target DNA (blue dots) and negative droplets without amplification of the target DNA (grey dots). DNA quantities in wells LOD-1 to LOD-6 are: 0.00500, 0.00250, 0.00125, 0.00063, 0.00031 and 0.00016 ng.

IV. Cross-amplification test

To confirm absence of cross-amplification primers were tested against pooled DNAs of fish species available in our laboratory. No cross-amplification was detected with the tested DNAs (**Figure 5**).

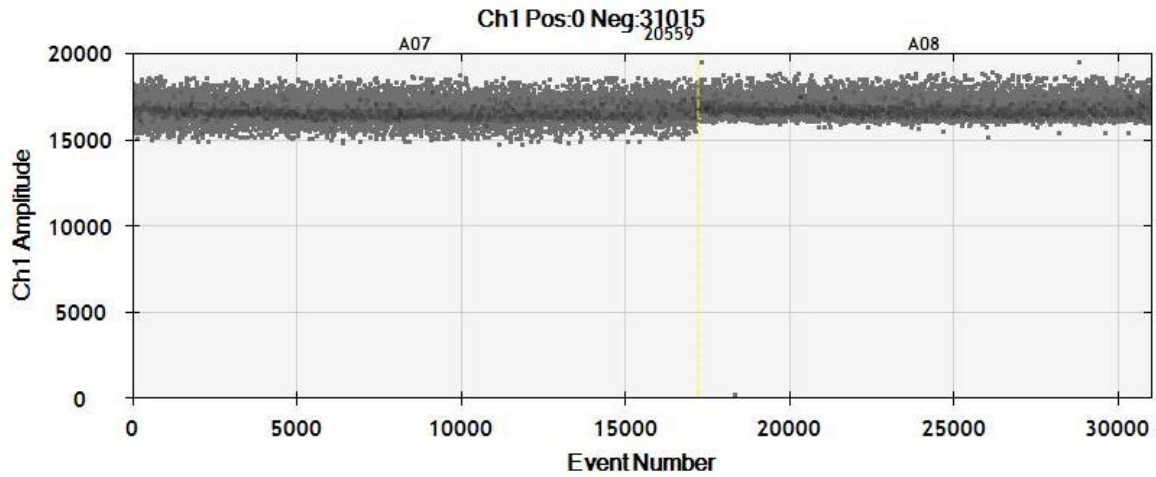


Figure 5 ddPCR cross-amplification test performed in duplicate using a mock sample of DNAs isolated from 12 fish species. No amplification (no positive droplets) was detected in cross-species assessment test using *Squatina squatina* assay 1.

4. eDNA EXTRACTION

For eDNA extraction filters were removed from Longmire buffer and dried under the flow hood. Extraction of the DNA from the filters was done using DNeasy PowerWater Kit (Qiagen, cat. no. 14900-100-NF, lot. no. 172014375) following manufacturer's instructions (Qiagen, 2020).

A. Quantification of DNA

Following the extraction of the DNA from filters DNA quantity was measured with NanoPhotometer N60. Results are presented in **Table 3**. DNAs from same replicate isolated from multiple filters were pooled together before measuring DNA concentration. Pooled samples are indicated as such in **Table 3**.

Table 3 Measured DNA concentrations isolated from each sample filter. Filters were prepared by WWF Adria from sea water collected at eight different locations in triplicate. For some locations more than one filter was prepared per replicate which is designated in filter column.

| Sample | Location | c (ng/μL) |
|------------|---------------------|-----------|
| BO1 | Bovišćak | 9.950 |
| BO2 | | 5.650 |
| BO3 | | 7.200 |
| Lj1 | Luka Jazi | 8.100 |
| Lj2 | | 5.800 |
| Lj3 | | 6.950 |
| SR1 | Solinski rt (Istok) | 8.450 |
| SR2 | | 5.500 |
| SR3 | | 5.800 |
| BR1-pooled | Brgulje | 2.850 |
| BR2-pooled | | 2.350 |
| BR3-pooled | | 2.850 |
| KO1-pooled | Uvala Konopljika | 2.300 |
| KO2 | | 4.350 |
| KO3 | | 2.800 |
| USN1 | Uvala Sabuša | 5.100 |
| USN2 | | 4.750 |
| USN3 | | 4.550 |
| UV1 | Uvala Vapojni | 3.050 |
| UV2 | | 4.200 |
| UV3 | | 5.150 |
| RI1 | Rivanjski kanal | 3.350 |
| RI2 | | 6.700 |
| RI3 | | 2.350 |

5. ANALYSIS OF eDNA SAMPLES

A. ddPCR methodology

Droplet digital PCR (ddPCR) method accurately quantifies targets of interests based on copy number determination. ddPCR works on an end-point measurement approach. ddPCR divides sample to a large number of partitions, in which one (or few) or none of the intact target sequences are identified. Singleplex reactions were used in *Squatina squatina* assay 1, in a total reaction volume of 20 μ l. QX200 droplet generator (Bio-Rad, Pleasanton, CA) was used for the generation of droplets, with samples applied to 8-well cartridges, to which 70 μ l of oil for probes (Bio-Rad) was added. A 40 μ l of droplets in each sample were transferred to a 96-well plate and amplified in a C1000 PCR cycler (Bio-Rad, Pleasanton, CA). QX200 droplet reader (Bio-Rad, Pleasanton, CA) was used for obtaining the data. Analysis was performed using QuantaSoft software (BioRad, Pleasanton, CA). The separation of positive droplets with targets from negative droplets was induced by applying a fluorescence threshold manually (1D amplitude). The samples were dropped out of the analysis in case of a low droplet events (< 12000). Positive control and no template control (NTC) were added to the analysis. Presence of *Squatina squatina* DNA in the location was determined when copies were identified in at least two out of three location replicates.

B. Results

At a given 0.6 ng/ μ L of a positive control *S. squatina* DNA, a 15960 copies per/20 μ L of the reaction were detected. NTC sample had no amplification. Threshold level was set manually at 19000-20000. *S. squatina* DNA was detected in low concentrations in five out of twenty-four sampling replicates, in all three replicates of the sample from Uvala Konopljika (KO1-3), and in one replicate of the samples from Uvala Vapojni (UV1) and Rivanjski kanal (Ri3) (**Table 4, Figure 6**). The detection of the target species *Squatina squatina* is confirmed only for Uvala Konopljika in low concentrations at the level of the LOD. The confirmation of *S. squatina* eDNA in such a low quantity potentially identifies species presence at the proximity of the Uvala Konopljika. Additionally, in parallel tested positive control and NTC were positive and negative, respectively.

Stringency level to assign certain sample (location) positive for the presence of the target species *Squatina squatina* was set at the minimum of two out of three replicates with positive signal from the monitored location. Given that eDNA assays most often detect DNA in environmental samples at very low levels more than one positive replicate is required to claim that a monitored species is present at certain location to increase confidence in the results. Therefore, presence of *Squatina squatina* is assigned only for the sample from Uvala Konopljika with all three positive replicates while samples from Uvala Vapojni and Rivanjski kanal with only one positive replicate in this analysis remain negative for the presence of the target species. Further analysis

with higher number of replicates and larger volume of sea water that are usually used in marine eDNA research would be needed to confirm positive signal at those locations.

Table 4 Summary of the results. Presence or absence of amplification of *Squatina squatina* DNA in each sample and number of copies detected per 20 μ L in each sample replicate.

| No. | Sample name | Sampling location | Result (copy number/20 μ L) | Confirmation of <i>S. squatina</i> detected by their eDNA |
|-----|--------------|---------------------|---------------------------------|---|
| 1 | BO1 | Bovišćak | 0 | Absent |
| 2 | BO2 | | 0 | |
| 3 | BO3 | | 0 | |
| 4 | Lj1 | Luka Jazi | 0 | Absent |
| 5 | Lj2 | | 0 | |
| 6 | Lj3 | | 0 | |
| 7 | SR1 | Solinski rt (Istok) | 0 | Absent |
| 8 | SR2 | | 0 | |
| 9 | SR3 | | 0 | |
| 10 | BR1 (pooled) | Brgulje | 0 | Absent |
| 11 | BR2 (pooled) | | 0 | |
| 12 | BR3 (pooled) | | 0 | |
| 13 | KO1(pooled) | Uvala Konopljika | 2.8 | Present |
| 14 | KO2 | | 1.4 | |
| 15 | KO3 | | 3 | |
| 16 | USN1 | Uvala Sabuša | 0 | Absent |
| 17 | USN2 | | 0 | |
| 18 | USN3 | | 0 | |
| 19 | UV1 | Uvala Vapojni | 3 | Absent |
| 20 | UV2 | | 0 | |
| 21 | UV3 | | 0 | |
| 22 | RI1 | Rivanjski kanal | 0 | Absent |
| 23 | RI2 | | 0 | |
| 24 | RI3 | | 1.6 | |

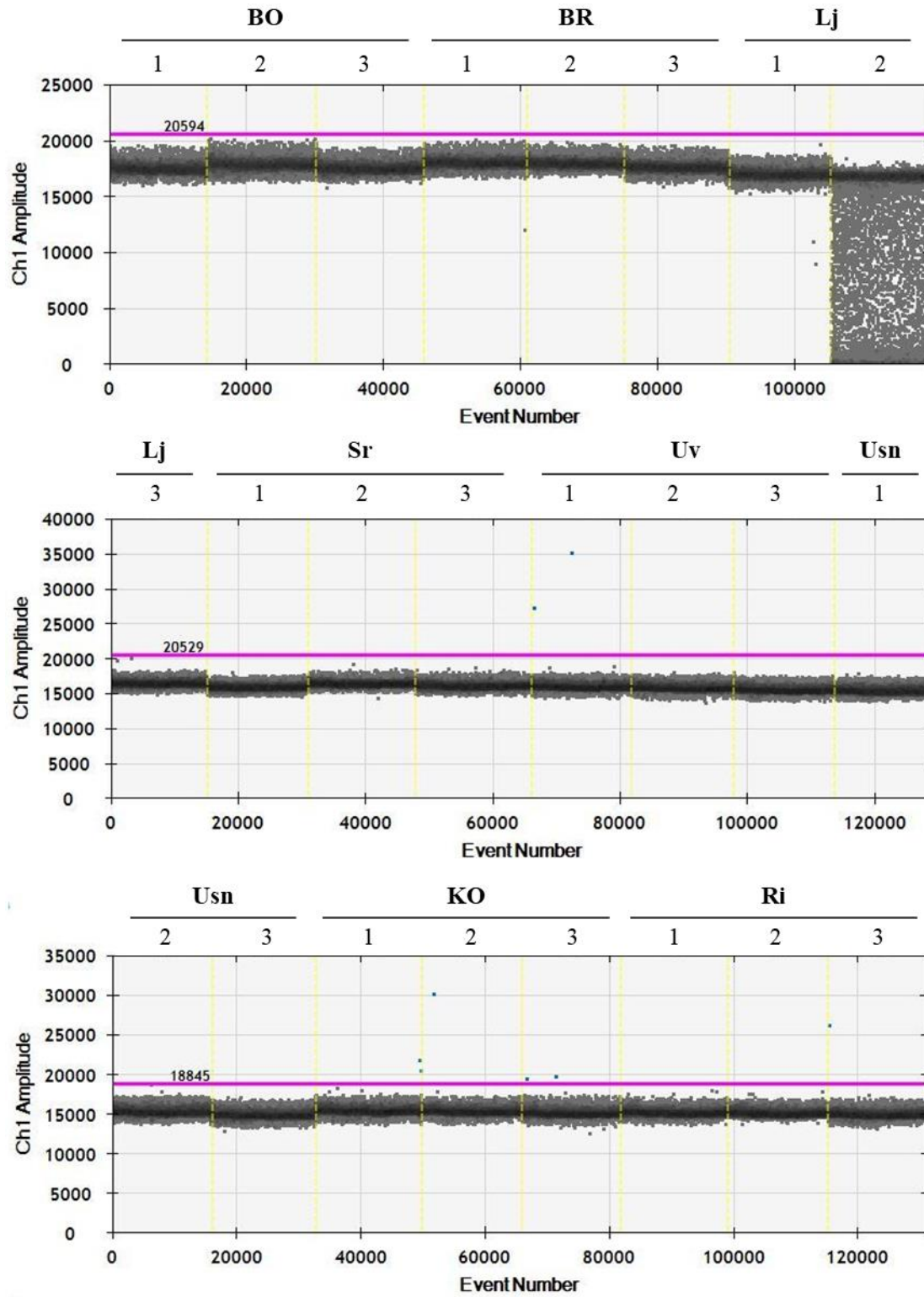


Figure 6 Individual copies of *Squatina squatina* eDNA detected in different samples by ddPCR using *S. squatina* assay 1.

6. REFERENCES

- Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*(4), 611–622. <https://doi.org/10.1373/clinchem.2008.112797>
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Deliverable 5: Final Draft Report

Contract Number: 464/2020/NL201770/A.1 & 9E075820

Project title: “MPA Push Angel Sharks in the Med FY20” (Project no. NL201770) & “MAVA Fisheries/BlueFinTuna” (Project no. 9E075820)

Date: 11 Sep 2020

Confidentiality: Please do not share outside of partners listed in the signed contract

Saving the last Angel Sharks of the Mediterranean Sea: X-ray report on spatial protection for Angel Sharks with a focus on the Adriatic Sea.

Developed by the Zoological Society of London on behalf of the Angel Shark Project; contracted by WWF Mediterranean

Citation: Pike, C^{1,2}., Barker, J^{1,2}., Dragicevic, B³., Ugarkovic, P³., Kristinic, P⁴., Kanski, D⁴., Meyers, E^{2,5}., Jiménez Alvarado, D^{2,6}., Gomei, M⁷., Niedermüller, S⁷. 2020. Saving the last Angel Sharks of the Mediterranean Sea: X-ray report on spatial protection, with a focus on the Adriatic Sea. WWF Mediterranean

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4: WWF Adria, Croatia

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6: Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain

7: WWF Mediterranean, Rome, Italy

Executive Summary

Three angel shark species are found in the Mediterranean Sea, *Squatina squatina* (Angelshark), *S. aculeata* (sawback Angelshark), and *S. oculata* (smoothback Angelshark) all of which are classified as Critically Endangered on the International Union for the Conservation of Nature’s (IUCN) Red List of Threatened Species. A Regional Action Plan for Angel Sharks in the Mediterranean (MedRAP) was published in 2019 to focus efforts on coordinated conservation action for these species, with specific Sub-Regional Action Plans (SubRAPs) to be developed to facilitate this. This study aimed to identify possible angel shark hotspots; evaluate the overlap of angel sharks with designated Marine Protected Areas (MPAs) and Fisheries Restricted Areas (FRAs); provide recommendations to improve angel shark protection; and act as a baseline to develop the Adriatic SubRAP. Although some outputs span the Mediterranean Sea, this study and recommendations focus on the Adriatic Sea.

Angel shark occurrence data were gathered from the [Angel Shark Sightings Map](#) and a Local Ecological Knowledge (LEK) case study conducted in Croatia by WWF Adria. These data were analysed in the context of the existing network of MPAs and FRAs designated in the Mediterranean Sea. 27.8 % of angel shark records in the Mediterranean Sea were found to occur within a designated MPA, and 97 % of these were *S. squatina*. None of the MPAs had a management plan or an implemented management plan, and the majority were designated as Natura 2000 sites.

In the Adriatic Sea, only *S. squatina* records were reported, adding to the documented uncertainty of whether *S. oculata* remains extant in this part of its historic range. The LEK case study provided a substantial number of new *S. squatina* records, with the majority caught and landed. Recent records (2010–2020 inclusive) were mainly distributed in the northern Adriatic Sea, with a potential hotspot

identified in the Molat Island archipelago. Distribution of *S. squatina* records supported published literature on habitat preference, with 62 % of sightings located shallower than 50m depth on soft sediments. Presence of juvenile *S. squatina* and anecdotal evidence of females aborting pups when accidentally caught, suggest a potential *S. squatina* nursery area located in the Molat Island archipelago.

Six recommendations to improve understanding and conservation of angel sharks in the Adriatic Sea were developed:

1. Work with the Government of the Republic of Croatia and Natura 2000 management officials to improve understanding of angel shark presence in the region, and where appropriate, account for this in management plans.
2. Further angel shark research at possible hotspots to strengthen baseline knowledge of angel sharks in the region.
3. Train fishers in best-practice guidance to safely release angel sharks and work with communities around Molat Island archipelago.
4. Develop a sub-regional action plan for the Adriatic Sea.
5. Develop angel shark LEK case study template to replicate research in other countries.
6. If Angelshark hotspots are confirmed with further study, identify mechanisms to develop MPAs to protect these habitats (see Recommendation 2 & 3).

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Background

Biodiversity in the Mediterranean Sea:

The Mediterranean Sea is a biodiversity hotspot, of significant cultural and economic importance (Bianchi & Morri 2000, Myers *et al.* 2000, Abdulla *et al.* 2009, Claudet & Fraschetti 2010, Coll *et al.* 2010, 2012). Approximately 7 % of the world's marine biodiversity is found in the Mediterranean Sea, which is substantial for a water body covering just 0.82 % of the global marine area (Bianchi & Morri 2000, Coll *et al.* 2010, Micheli *et al.* 2013). The Mediterranean Sea is under intense pressure from various human impacts, including unsustainable exploitation and habitat loss, and threats have accelerated and diversified with a growing human population (Claudet & Fraschetti 2010, Coll *et al.* 2010, 2012, Lotze *et al.* 2011).

Elasmobranchs in the Mediterranean:

Elasmobranchs (sharks, skates and rays) are particularly susceptible to overfishing and habitat degradation, and there is evidence of severe declines in large predatory sharks throughout the Mediterranean Sea (Ferretti *et al.* 2008, Dulvy *et al.* 2014). Many elasmobranchs have a life history characterised by slow growth, long life, late maturity, and low fecundity, which increases risk of human induced mortality at a population wide scale (Stevens *et al.* 2000, Ferretti *et al.* 2008). Sharks are often top predators, and reduction within a community can cause cascading trophic effects, altering ecosystem structure and function (Stevens *et al.* 2000, Ferretti *et al.* 2008, Lotze *et al.* 2011).

Angel Sharks in the Mediterranean Sea:

There are at least 22 species of angel sharks in the family Squatinidae, identified as the third most threatened family of elasmobranchs in the world (Dulvy *et al.* 2014, Kyne *et al.* 2019). Three angel shark species are found in the Mediterranean Sea, *Squatina squatina* (Angelshark), *S. aculeata* (sawback Angelshark), and *S. oculata* (smoothback Angelshark); all are classified as Critically Endangered on the International Union for the Conservation of Nature's (IUCN) Red List of Threatened Species (Morey *et al.* 2019a,b,c). It is estimated that the ranges of these species have declined between 48 % to 58 % in the last century (Lawson *et al.* 2020). The fact that angel sharks inhabit shallow coastal areas, coupled with their extended life history, make them particularly vulnerable to the combined impact of habitat loss and mortality from incidental capture (Barker *et al.* 2016, Gordon *et al.* 2017, Giovos *et al.* 2019, Lawson *et al.* 2020).

Angel sharks are protected under various legislations within the Mediterranean Sea. Including; the EU Common Fisheries Policy Council Regulation (EC) 43/2009 and Council Regulation (EC) 43/2014, on the Barcelona Convention Annex II of the Protocol concerning Specially Protected Areas and Biological Diversity (SPA/BD Protocol), and more specifically in Spanish waters through the Spanish List of Species Under Special Protection in the Mediterranean (LESPRE) Orden AAA/75/2012 (Fortibuoni *et al.* 2016, Gordon *et al.* 2017). *S. squatina* is listed under CMS Appendix I and II, as well as on Annex I of the CMS Sharks MOU. In addition, concerted actions for angel sharks have been adopted by the Convention of Migratory Species (CMS) at the 12th Conference of the Parties (CMS 2017). Migratory species listed under CMS benefit from internationally coordinated conservation throughout their migratory range, through agreements between Range States that are parties to CMS (Gordon *et al.* 2019).

A Regional Action Plan for Angel Sharks in the Mediterranean (MedRAP) has been published to focus efforts for coordinated, collaborative conservation action (Gordon *et al.* 2019). Under this framework, Sub-Regional Action Plans (SubRAPs) for the Mediterranean will be developed to better facilitate action in each respective subregion (Gordon *et al.* 2019). Identified threats to angel sharks categorised

within the MedRAP include: Agriculture and aquaculture, biological resource use, climate change, human intrusion and disturbance, invasive species and diseases, pollution, transportation and service corridors, and development (Gordon *et al.* 2019). Within these categories, fishing activity and habitat destruction were identified as the priority threats (Gordon *et al.* 2019).

Marine Protected Areas:

Establishing a representative network of Marine Protected Areas (MPAs) is an essential tool for conservation of marine ecosystems (Abdulla *et al.* 2009). Protecting a range of habitats allows natural systems to continue to function, with reduced disturbance, enabling the ecosystem to provide associated services from which humans benefit (Abdulla *et al.* 2009, Gomei *et al.* 2019). MPAs have the potential to be a useful tool for elasmobranch conservation, if their design and management is informed by scientific understanding of movement, biology, and habitat (Rigby *et al.* 2019). Gomei *et al.* (2019) assessed progress in the Mediterranean Sea towards implementing an effective network of MPAs. Actions were evaluated in the context of MPA targets included in Aichi target 11 set by the Convention on Biological Diversity (CBD) and signed by Mediterranean countries, to protect 10 % of coastal and marine waters by 2020 (Gomei *et al.* 2019). It was found that 9.68 % of the surface of the Mediterranean Sea was covered by designated MPAs (Gomei *et al.* 2019). However, MPAs with a management plan only covered 2.48 % of the surface, and MPAs where actions within the management plan were actually being implemented covered an even smaller surface area, only 1.27 % (Gomei *et al.* 2019). These findings revealed that a large proportion of designated MPAs are not performing effectively, and that countries are failing to ensure proper management and monitoring of designated areas (Gomei *et al.* 2019).

Geographic focus – The Adriatic Sea:

The Adriatic Sea sits between Italy and the Balkans, covering a surface area of around 138,600 km² (Danovaro & Boero 2019). The basin is split into northern, central, and southern Adriatic, with depth increasing from a north to south gradient to a maximum of over 1200 m (Danovaro & Boero 2019). The continental shelf covering the north and central Adriatic is the most extensive of the Mediterranean Sea and occurs between depths of 10 and 200 m, making it ideal for coastal species such as the angel shark, and demersal fisheries (Pinardi *et al.* 2006, Coll *et al.* 2007, Holcer and Lazar 2017, Lotze *et al.* 2011, Fortibuoni *et al.* 2016, Danovaro & Boero 2019). The basin is semi-enclosed, composed of a muddy/sandy substratum, and receives the largest freshwater influx of all the Mediterranean Sea (Pinardi *et al.* 2006, Fortibuoni *et al.* 2016, Danovaro & Boero 2019). Exchange of water between the Adriatic and Ionian Sea occurs through the Strait of Otranto (Pinardi *et al.* 2006, Danovaro & Boero 2019). Threats to the Mediterranean Sea are reflected in the Adriatic, and depletion of large consumers and predators through intensive exploitation has resulted in simplified and therefore more unstable food webs and reduced ecosystem function (Lotze *et al.* 2011). Elasmobranchs have undergone severe declines in the region throughout the twentieth century (Lotze *et al.* 2011).

Both *S. squatina* and *S. oculata* were once common throughout the Adriatic Sea, but have undergone substantial declines, and there is uncertainty as to whether *S. oculata* remains extant in this part of its range (Gordon *et al.* 2017, Holcer & Lazar 2017, Morey *et al.* 2019b, Lawson *et al.* 2020). Presence of angel sharks has been well documented in this region; historically *S. squatina* sustained a large fleet of commercial fishing boats in the Adriatic Sea, which used targeted nets named “squaenere” or “sklatare” to land angel sharks, amongst other fish (Holcer & Lazar 2017). Landings of *S. squatina* declined dramatically after the 1960s, causing them to become considered “economically extinct” (Raicevich & Fortibuoni 2013, Fortibuoni *et al.* 2016). Although scientific surveys occasionally found of

S. squatina during sampling in the Adriatic Sea between 1948 and 1958, they were not present in surveys occurring after this time, suggesting extirpation from the area (Ferretti *et al.* 2013, Fortibuoni *et al.* 2016, Maynou *et al.* 2011). However, more recently, new angel shark records have been reported, demonstrating that at least *S. squatina* was still present (Fortibuoni *et al.* 2016, Holcer & Lazar 2017, Dragicevic & Ugarkovic, unpublished data). It is therefore vital that effective conservation measures are developed to secure the future of this species in the Adriatic.

Project Aims:

The major aim of this study was to identify possible angel shark hotspots in the Adriatic Sea and evaluate the potential effectiveness of designated MPAs^[1] and Fisheries Restricted Areas (FRAs) for these species across the Mediterranean Sea. Angel shark occurrence data were gathered from the Angel Shark Sightings Map and fisher surveys conducted in Croatia by WWF Adria. Gomei *et al.* (2019) was used to evaluate the occurrence of angel shark records in the existing network of MPAs, categorised by their management status. Results will be used as a baseline to help develop the Adriatic SubRAP and provide recommendations on the next steps to improve protection of angel shark habitat.

Methods

Data collection

Angel Shark Sightings Map:

The Angel Shark Sightings Map (ASSM) is an interactive map that allows citizen scientists to report their angel shark sightings: www.angelsharkproject.com/map. Sightings information includes species, sex, depth, size, location and type of record. The ASSM is hosted by the Zoological Society of London (ZSL) on behalf of the Angel Shark Project (a collaboration between Universidad de las Palmas de Gran Canarias (ULPGC), Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) and ZSL) and Angel Shark Conservation Network, led by the following partners: IUCN Shark Specialist Group, Shark Trust, Submon, ULPGC, ZFMK and ZSL. Sightings data in the Mediterranean Sea were downloaded from the ASSM on 18/06/2020; there were a total of 173 sightings with 241 angel shark records.

Local Ecological Knowledge surveys in Croatia:

In 2017 and 2018, the Institute of Oceanography and Fisheries gathered records of *S. squatina* through contacting fishers, fish markets, universities and divers directly and via social media. This broad-scale approach gathered data from both Croatia and Montenegro. In June and July 2020, a Local Ecological Knowledge (LEK) case-study was conducted by WWF Mediterranean, WWF Adria, and the Institute of Oceanography and Fisheries in order to complement these data (Dragičević & Ugarkovic *in prep.*). This involved asking fishers from across the coast of Croatia to complete an online survey to better understand recent interactions with angel sharks, including information on date, location, size, depth, and gear type for each angel shark record. The survey was made available to fishers through various online platforms, relevant to both commercial and recreational fishing sectors, and a total of 368 surveys were collected.

From these data sources, a total of 106 sightings and 147 angel shark records were used in the analysis presented here. These data were given a confidence score in relation to GPS point accuracy: a 'specific' confidence value referred to points within the radius of a 1–2 km area; 'medium' up to 20 km; and 'broad' anything larger than this.

¹ See methods for which MPAs are included in the analysis.

MPAs and FRAs:

Shapefiles of 1,228 MPAs, designated under the following legislation were used in this analysis: nationally designated MPAs, Natura 2000 sites, the marine part of Ramsar sites (wetlands of international importance under the Ramsar Convention), the marine part of UNESCO Biosphere reserves, and internationally designated Specially Protected Areas of Mediterranean Importance (SPAMIs) (Gomei *et al.* 2019). MPAs have a range of protected status, ie. can include fully protected no-take zones, but also areas where monitoring and management is not taking place (“paper parks”) (Gomei *et al.* 2019).

The analysis also includes eight Fisheries Restricted Areas (FRAs). A consensus has not yet been reached on whether to consider FRAs (designated under the General Fisheries Commission for the Mediterranean) as MPAs in the region (Gomei *et al.* 2019). As a result of this, they are considered separately within this report.

GIS shapefiles for MPAs and FRAs in the Mediterranean Sea were provided by WWF Mediterranean to be used in this analysis. Environmental data were gathered from open access sites; modelled substrate was downloaded from EMOD.net (EMODnet 2020), and bathymetry data was downloaded from Bio-ORACLE (Tyberghein *et al.* 2012).

Analysis

Analysis of data was conducted using QGIS 3.10 (QGIS.org 2020). All data were analysed as point records, but results were visualised as grid squares to hide the specific location of records. Grids were constructed for both the Mediterranean Sea (60 x 60 km) and for the Adriatic at varying scales (20 x 20 km, 10 x 10 km, 5 x 5 km). Using the combined data for both the Mediterranean Sea and Adriatic Sea, the ‘count points within polygon’ function was used to identify the number of sightings within each grid square. This method was conducted firstly using number of occurrence points only, giving a value for the number of ‘**sightings**’ and secondly using number of points weighted to the number of angel sharks recorded within the sightings^[2]. This gave a value for the specific number of ‘**angel shark records**’, accounting that there could be multiple angel sharks within one sighting. At the Mediterranean Sea scale, analysis was split by species (*S. squatina*, *S. aculeata*, *S. oculata*), and by time. For temporal analyses, the data were split into ‘recent’ sightings (2010–2020) and ‘historic’ sightings (1947–2009 inclusive). One angel shark generation length (GL), an average of 11 years, was used to delineate the threshold between these categories.

The ‘count point within polygon’ function was used to identify the number of sightings, and number of angel sharks, overlapping designated MPAs or FRAs in the Mediterranean Sea, with a focus on the Adriatic Sea. To better understand the overlap between sightings and differently managed MPAs in the Mediterranean Sea, the designated MPAs were further split into those that had a management plan, and those that had a management plan in force, *i.e.* those MPAs where actions within the management plan are being implemented (“implemented management plan”). For visualisation, the MPAs and FRAs were coloured according to this overlap. Pink indicated that an angel shark record occurred within the boundary of the MPA or FRA (“overlapping sightings”), and yellow indicated that there were no records within the boundary (“not overlapping sightings”).

² Weighting the records by number of angel sharks automatically assumes all the sharks within that record are the same size (length or weight). Errors in the following size analysis therefore had to be manually corrected, for records where multiple sharks were observed in a single sighting, but size was only given related to the mother, and not the pups.

Size analysis was also conducted for the Adriatic Sea, where records were split by size categories for both length and weight. ASSM records have pre-determined length categories (<30 cm, 31–100 cm, >100 cm), whereas specific lengths and weights were given in the LEK case study. These were transformed into the pre-determined length categories using corresponding weight category estimates assigned based on anecdotal information (<0.5 kg, 0.6–18 kg, >19 kg) (Angel Shark Project, unpublished data).

Angel shark occurrence data in the Adriatic Sea was compared against open access environmental data. For modelled substrate type (EMODnet 2020), the 'count points within polygon' function was used to identify potential habitat types being utilised by angel sharks in the Adriatic. For bathymetry (Tyberghein *et al.* 2012), the 'sample raster values' function in QGIS was used to assign each occurrence point a depth value.

Results

Mediterranean Sea

27.8 % (n = 67) of angel shark records in the Mediterranean Sea (n = 241), were found to occur within a designated MPA (Fig. 1). Almost all of these were *S. squatina* (n = 65), with one record of *S. oculata*, one record of an *angel shark* not identified to species level and no records of *S. aculeata*.

18.7 % (n = 20) of historic records (n = 107) and 35.1 % (n = 47) of recent records (n = 134) were found to occur within a designated MPA in the Mediterranean Sea (Fig. 2a and 2b respectively). A large proportion of the MPAs found to overlap recent angel shark records were designated Natura 2000 sites (Table 1). Nine angel shark records also occurred within two MPAs with different designations: Sitsko-žutska otoèna skupina, a nationally designated site within the Adriatic Sea; and the Pelagos Sanctuary for The Conservation of Marine Mammals, protected under the Barcelona Convention spanning waters of France, Italy, and Monaco (Table 1). None of the MPAs overlapping angel shark records were found to have a management plan, or an implemented management plan.

A singular record of *S. squatina* was found to overlap a FRA, this occurred in the boundaries of Jabuka Pit FRA in the Adriatic Sea (Fig. 3, Table 1). Locations of MPAs and FRAs overlapping recent angel shark records in the Mediterranean Sea are highlighted in Fig. 4.

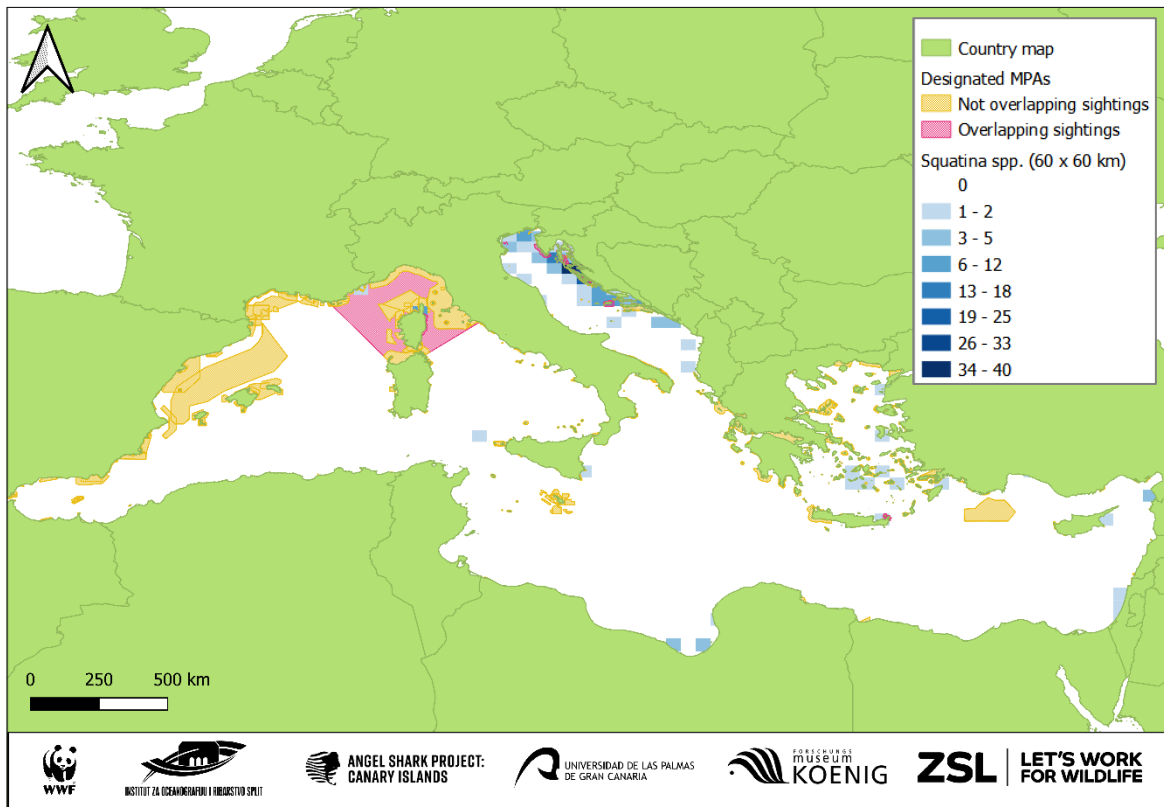


Figure 1 - Angel shark records (*Squatina* spp.) at 60 x 60 km resolution, in relation to **designated Marine Protected Areas (MPAs)** in the Mediterranean Sea .

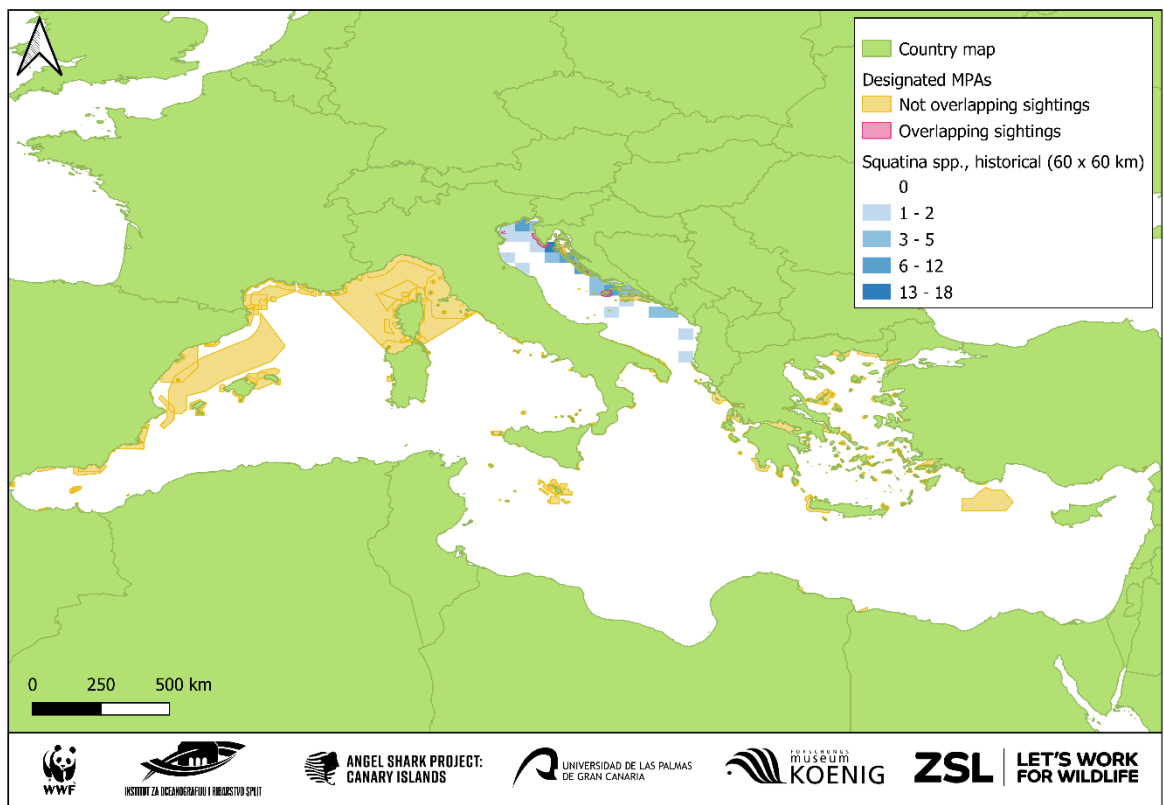


Figure 2a - **Historic (1947–2009 inclusive)** angel shark records (*Squatina* spp.) at 60 x 60 km resolution, in relation to **designated MPAs** in the Mediterranean Sea.

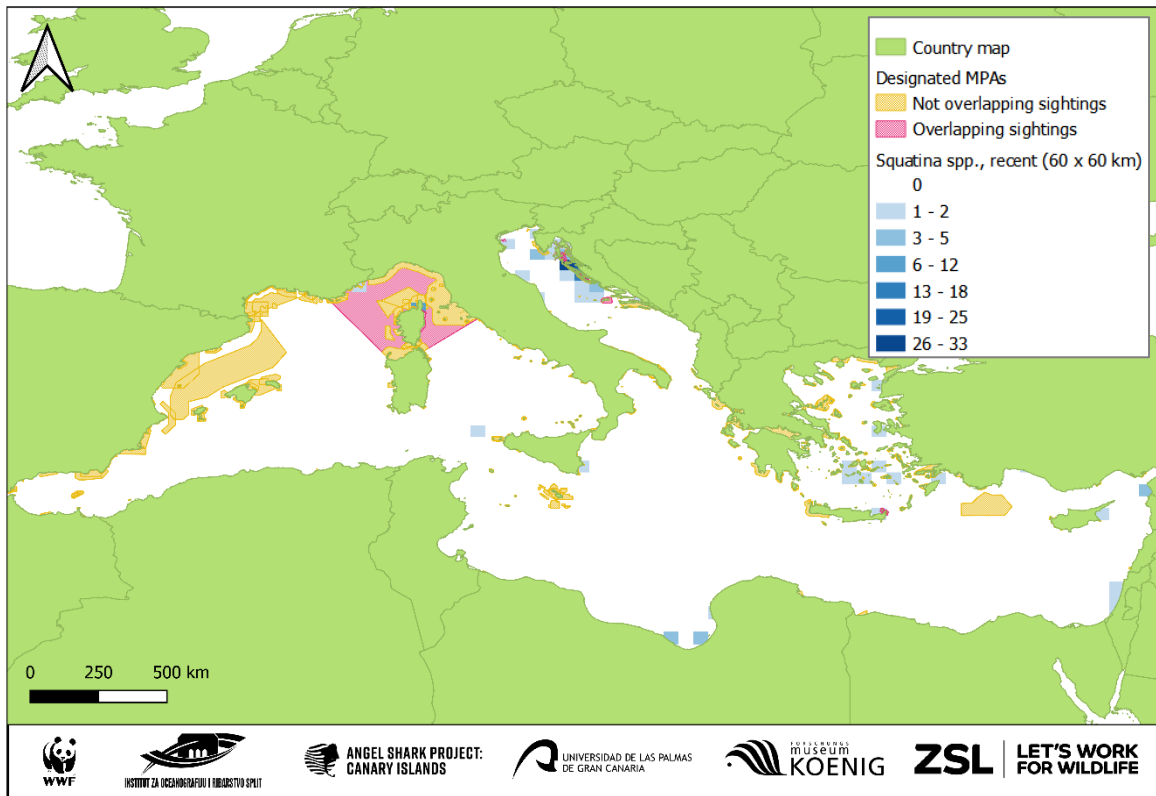


Figure 2b - Recent (2010–2020) angel shark records (*Squatina* spp.) at 60 x 60 km resolution, in relation to designated MPAs in the Mediterranean Sea.

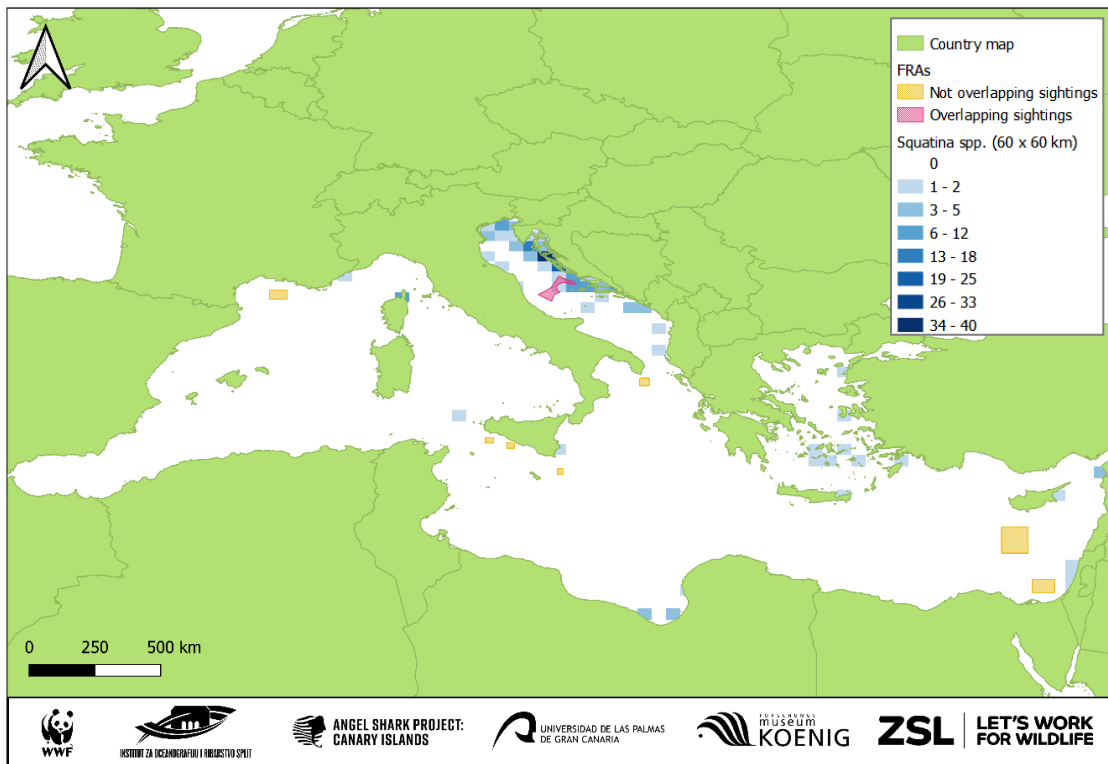


Figure 3 - Angel shark records (*Squatina* spp.) at 60 x 60 km resolution, in relation to Fishery Restricted Areas (FRAs) in the Mediterranean Sea.



Figure 4 – Locations of MPAs and FRAs overlapping recent (2010–2020) angel shark records.

| MPA name | | Designated | Country | Year | MgtPlan ^[3] | MgtImp ^[4] | Sightings ^[5] | Angel sharks ^[6] |
|----------------------|---|---|-----------------------|------|------------------------|-----------------------|--------------------------|-----------------------------|
| Adriatic Sea | J. Molat-Dugi-Kornat-Murter-Pašman-Ugljan-Rivanj-Sestrunj-Molat | Natura 2000 | Croatia | 2013 | N | N | 11 | 17 |
| | Jl dio o. Molata | Natura 2000 | Croatia | 2013 | N | N | 3 | 13 |
| | Srednjedalmatinski otoci i Pelješac | Natura 2000 | Croatia | 2013 | N | N | 2 | 2 |
| | Rivanjski kanal sa Sestricama | Natura 2000 | Croatia | 2013 | N | N | 2 | 2 |
| | Tegnùe di Chioggia | Natura 2000 | Italy | 2010 | N | N | 1 | 1 |
| | Sitsko-žutska otoèna skupina | National designation | Croatia | 1967 | N | N | 1* | 1* |
| | Kvarnerski otoci | Natura 2000 | Croatia | 2013 | N | N | 1* | 1* |
| | Viški akvatorij | Natura 2000 | Croatia | 2013 | N | N | 1 | 1 |
| | Cres - Lošinj | Natura 2000 | Croatia | 2013 | N | N | 1* | 1* |
| | Badija i otoci oko Korčule | Natura 2000 | Croatia | 2013 | N | N | 1 | 1 |
| | Cres - rt Suha - rt Meli | Natura 2000 | Croatia | 2013 | N | N | 1* | 1* |
| | Silba - podmorje | Natura 2000 | Croatia | 2013 | N | N | 1* | 1* |
| Jabuka Pit | FRA | Croatia, Italy | 2018 | n/a | n/a | 1 | 1 | |
| Outside the Adriatic | VOREIOANATOLIKO AKRO KRITIS: DIONYSADES, ELASA KAI CHERSONISOS SIDERO (AKRA MAVRO MOURI - VAI - AKRA PLAKAS) KAI THALASSIA ZONI | Natura 2000 | Greece | 2011 | | | 1 | 1 |
| | DIONYSADES NISOI | Natura 2000 | Greece | 1997 | | | 1 | 1 |
| | Pelagos Sanctuary For The Conservation Of Marine Mammals | Specially Protected Area of Mediterranean Importance (Barcelona Convention) | France, Italy, Monaco | 1999 | N | N | 7 | 8 |
| | Grand herbier de la côte orientale | Natura 2000 | France | 2015 | N | N | 6* | 7* |

* Shows duplicated sightings / angel sharks where some MPAs overlap each other. This has been accounted for within the analysis.

³ "MgtPlan" is whether there is a management plan for an MPA.

⁴ "MgtImp" is whether the management plan is in force, whether actions within the plan are being implemented.

⁵ "Sightings" means the number of sighting provided to ASSM or LEK study.

⁶ "Angel sharks records" is the total number of angel sharks counted within those sighting records, i.e. accounting that there may be multiple sharks observed in a single sighting.

Adriatic Sea

Of the records provided by the LEK study, 80.2 % occurred as a result of fishing interactions, 8.5 % whilst diving and the remaining 11.3 % did not record the source. Of those fishing records, 48.1 % were caught in gillnets, 22.6 % from trawls, 7.5 % from longlines, and 1.8 % from harpoon or hook. 28.7 % (n = 58) of Adriatic *S. squatina* records (n = 202) occurred within a designated MPA (Fig. 5). Of these, 18.7 % (n = 20) of historic *S. squatina* records and 40 % (n = 38) of recent *S. squatina* records were found to occur within a designated MPA (Fig. 6a and 6b respectively). Analysis of historic and recent *S. squatina* records were visualised at northern (Fig. 7a and 7b respectively), central (Fig. 8a and 8b respectively), and southern (Fig. 9a and 9b respectively) Adriatic scale. Historic *S. squatina* records accounted for just over half (n = 107) of all Adriatic records (n = 202), and these records covered a greater area of the Adriatic Sea than those from recent years. This is particularly apparent in the central and southern Adriatic, where there are little to no recent records of *S. squatina* (Fig. 8b & 9b). Hotspots of recent *S. squatina* records in the Adriatic were identified around the northern Croatian Islands, specifically the Molat island archipelago (Fig. 10a and 10b respectively).

Size analysis found only 37.6 % (n = 76) of all Adriatic *S. squatina* records had information relating to the length or weight of the angel shark. Of these records, 10.5 % (n = 8) were juveniles of less than 30 cm or less than 0.5 kg; 69.7 % (n = 53) were between 31 and 100 cm in length or 0.6 and 18 kg in weight; and 19.7 % (n = 15) were adults of a length greater than 100 cm or weight of 19 kg (Fig. 11).

Within the LEK case study data, it was found that some records contained qualitative information where a female angel shark had aborted pups during the fishing procedure. After identification of these records, further size analysis was conducted for juvenile angel sharks, which included these records, in conjunction with sightings of juvenile *S. squatina* described as less than 30 cm in length and less than 0.5 kg in weight (Fig. 12). Analysis indicated a cluster of points (n = 4) around the islands off Zadar, including Molat, Sestrunj, Rivanj, and Tun Veli. Three of the four sightings within this area were given a 'specific' confidence value and the remaining record was given a 'medium' value, suggesting the area is relatively accurate. This hotspot could be important for *S. squatina* juveniles, as a breeding and/or nursery area. Using modelled substrate data (EMODnet 2020a), three of the points were identified to fall on infralittoral sandy mud, and one on infralittoral coarse and mixed sediment.

S. squatina records were analysed in relation to modelled substrate type for the Adriatic Sea (EMODnet 2020). *S. squatina* records mostly occurred on 'sandy mud' substrate type, followed by 'muddy sand', 'sand', 'coarse and mixed sediment', and 'fine mud' (Table 2). Some of the *S. squatina* records from the Adriatic Sea had no substrate type due to being records from fish markets, and others due to the GIS raster layer not covering the area of the point. Most *S. squatina* records occurred in either infralittoral or circalittoral biozones, although three records were within the bathyal zone.

| Substrate | Number of <i>S. squatina</i> records | % |
|---------------------------|--------------------------------------|------|
| Sandy mud | 66 | 32.7 |
| Muddy sand | 40 | 19.8 |
| Sand | 38 | 18.8 |
| Coarse and mixed sediment | 24 | 11.9 |
| Fine mud | 19 | 9.4 |

Sightings of *S. squatina* were also analysed in relation to depth of the sea floor (bathymetry) for the Adriatic Sea (average depth of sea floor, 30 arcsecond spatial resolution) (Tyberghein *et al.* 2012) (Figure 13). *S. squatina* sightings mostly occurred at depths shallower than 50 m (Table 3), with the

deepest sighting recorded at 867 m from a trawling vessel. There were also sightings that occurred at +2 m, even though the points were located in the Adriatic Sea. This may have been a result of tides or an error within the bathymetry layer.

| Table 3 - Sightings of <i>S. squatina</i> in relation to depth of sea floor (bathymetry) for the Adriatic Sea | | |
|--|------------------------------|------|
| Bathymetry (m) | <i>S. squatina</i> sightings | % |
| + 2 | 3 | 2.2 |
| - 0–25 | 48 | 34.5 |
| - 25.1–50 | 35 | 25.2 |
| - 50.1–100 | 33 | 23.7 |
| - 100.1–150 | 7 | 5 |
| - 150.1–200 | 3 | 2.2 |
| - >200 | 7 | 5 |

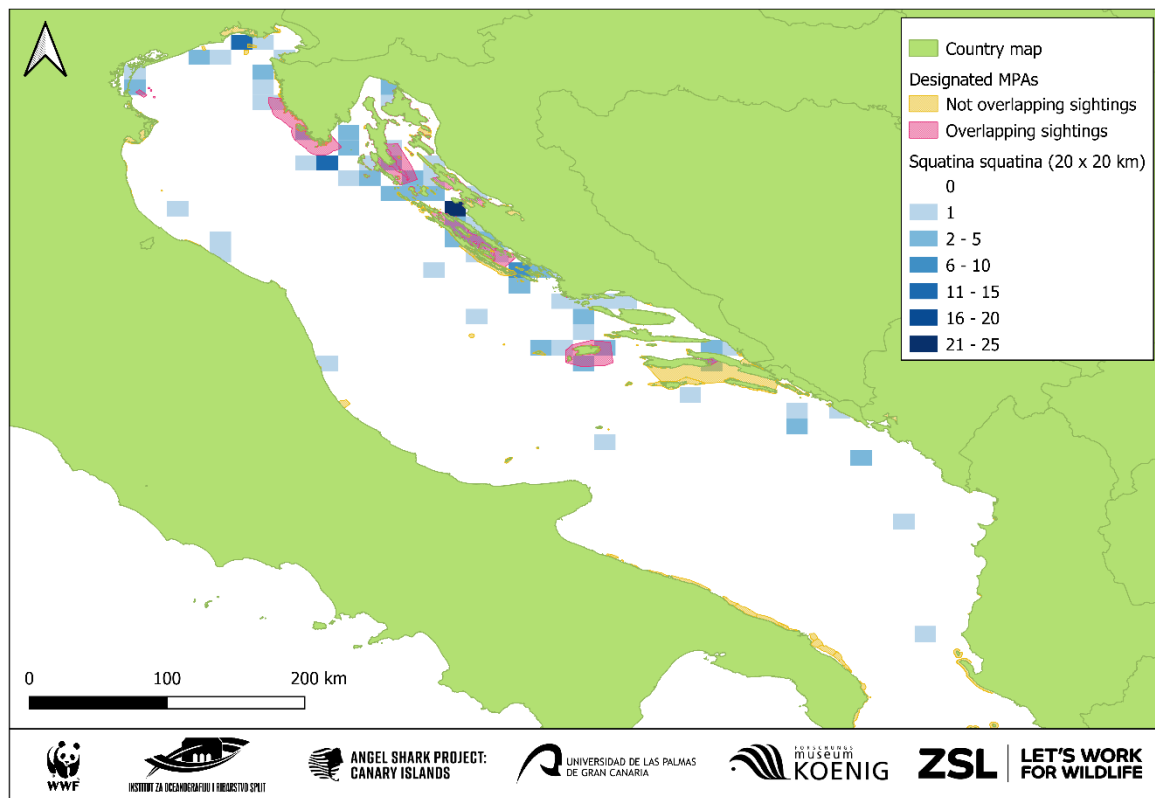


Figure 5 - *S. squatina* records at 20 x 20 km resolution, in relation to designated MPAs in the Adriatic Sea.

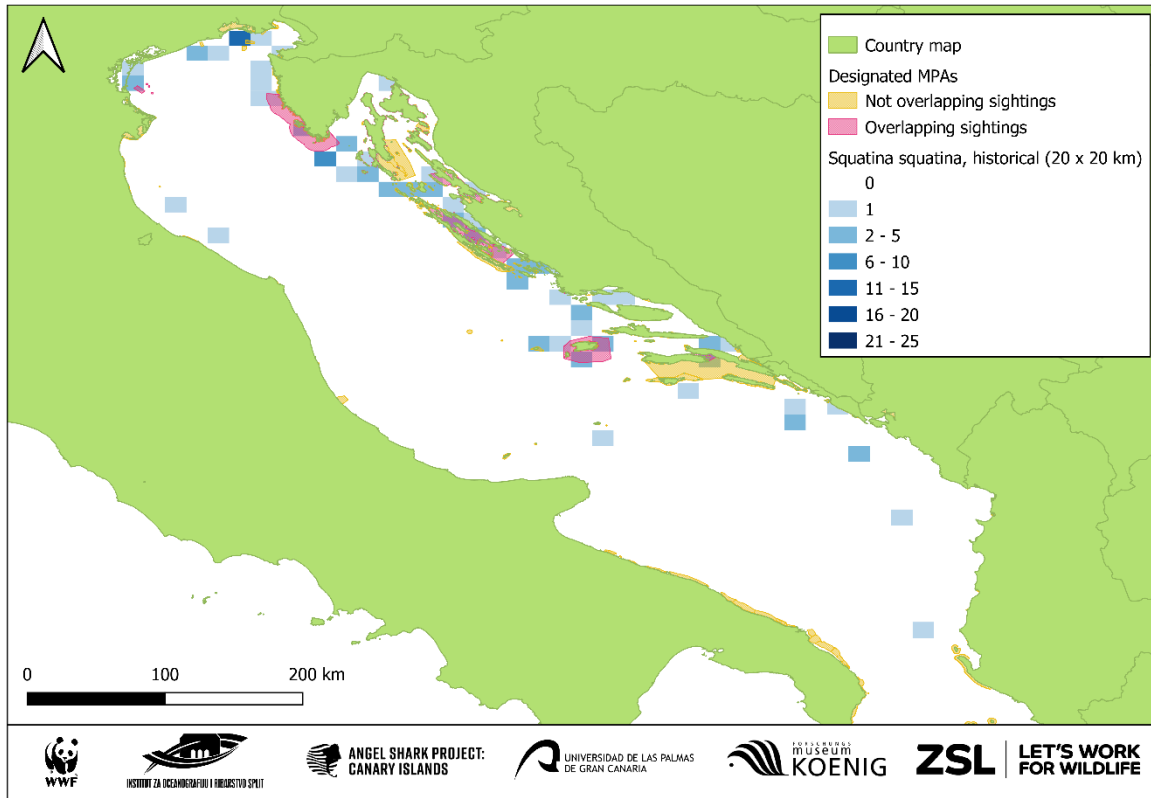


Figure 6a - Historic (1947–2009 inclusive) angel shark records (*S. squatina*) at 20 x 20 km resolution, in relation to designated MPAs in the Adriatic Sea.

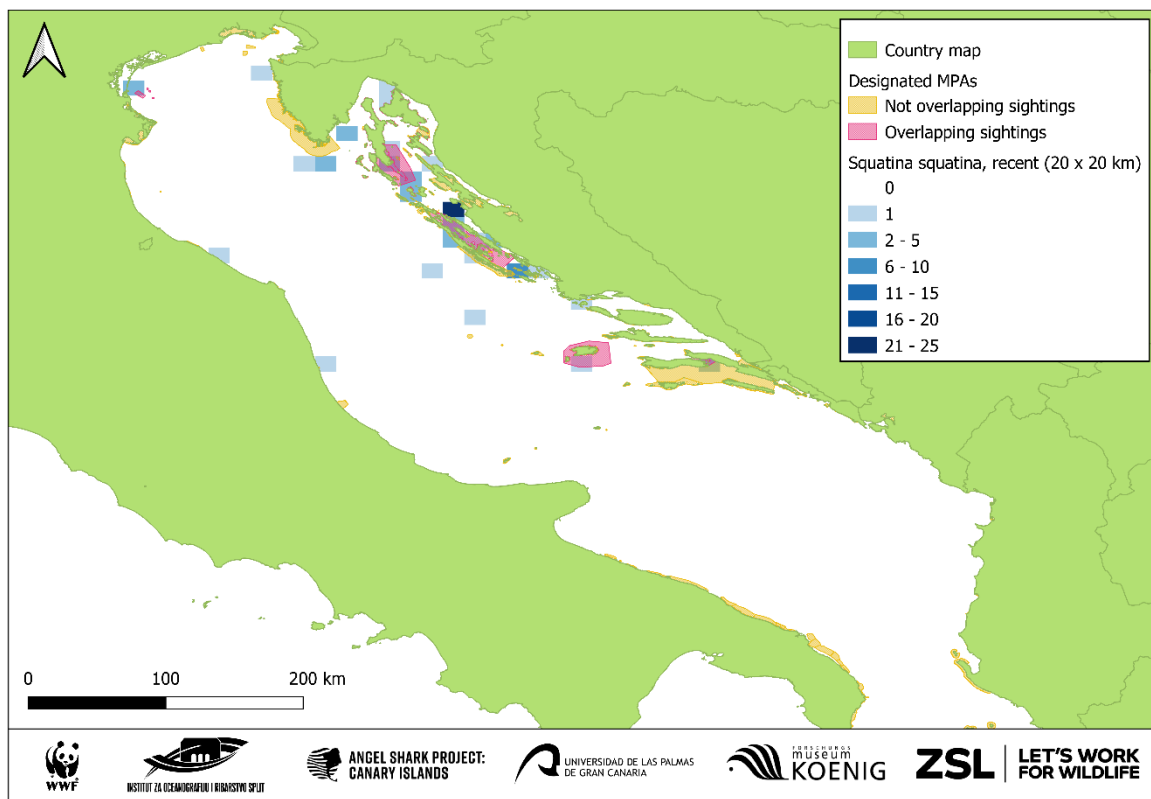


Figure 6b - Recent (2010–2020) angel shark records (*S. squatina*) at 20 x 20 km resolution, in relation to designated MPAs in the Adriatic Sea.

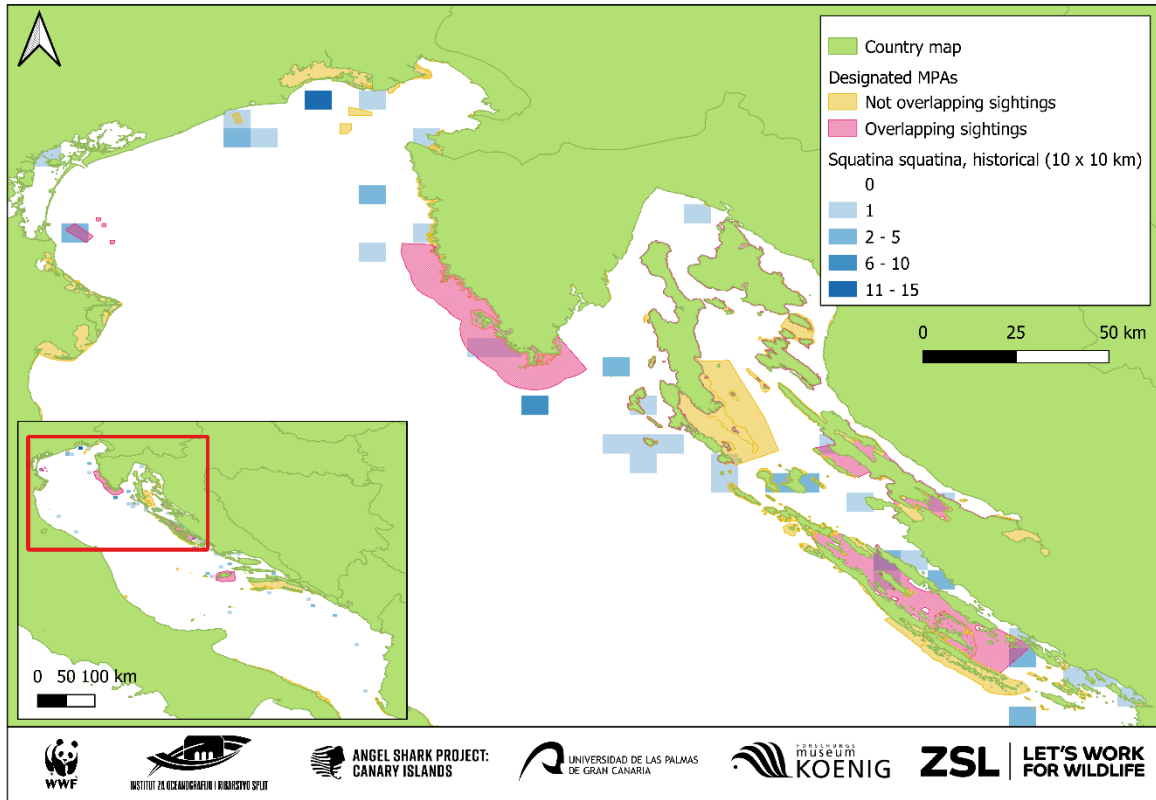


Figure 7a – Northern Adriatic visualisation of **historic** (1947–2009 inclusive) angel shark records (*S. squatina*) at 10 x 10 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

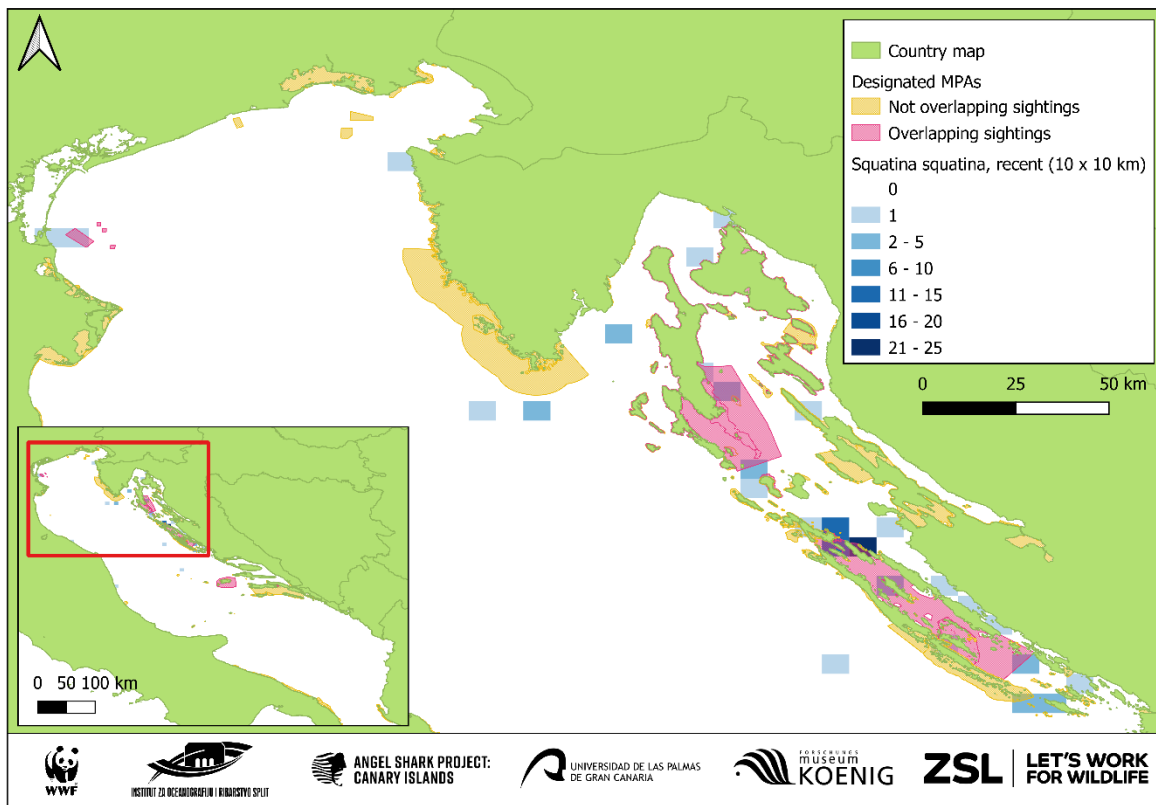


Figure 7b – Northern Adriatic visualisation of **recent** (2010–2020) angel shark records (*S. squatina*) at 10 x 10 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

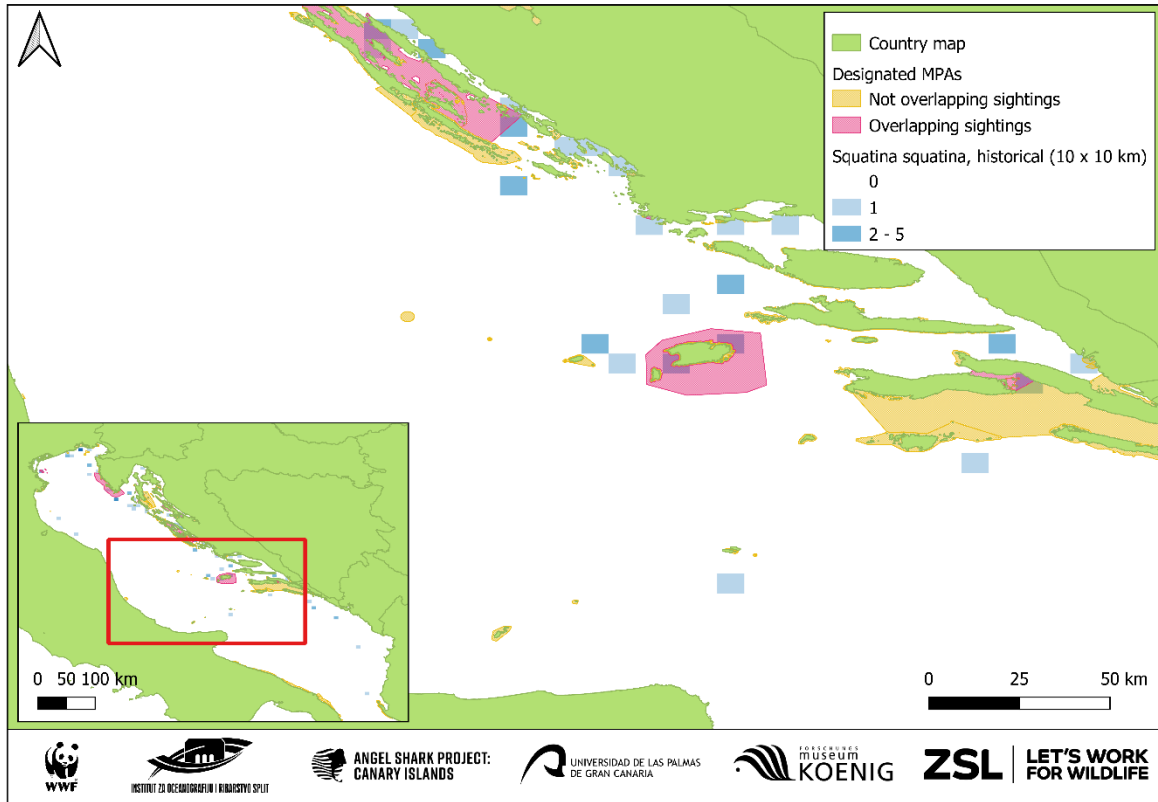


Figure 8a – Central Adriatic visualisation of **historic** (1947–2009 inclusive) angel shark records (*S. squatina*) at 10 x 10 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

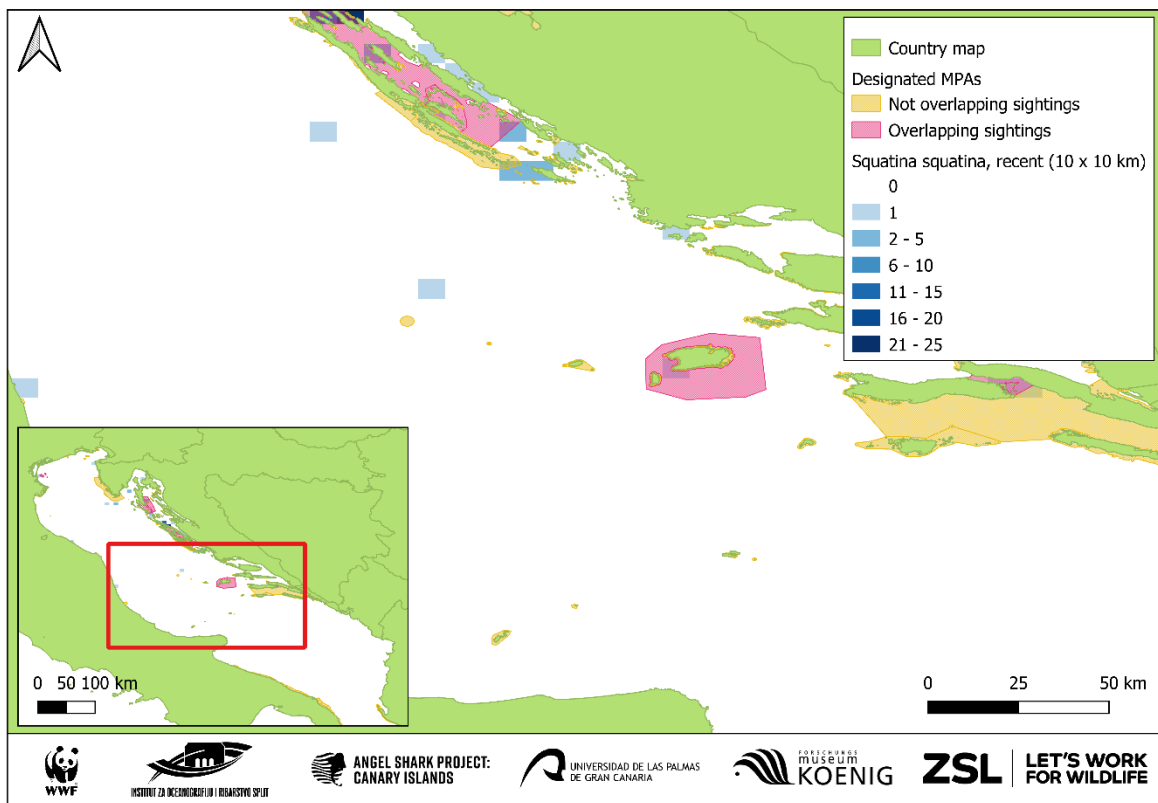


Figure 8b – Central Adriatic visualisation of **recent** (2010–2020) angel shark records (*S. squatina*) at 10 x 10 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

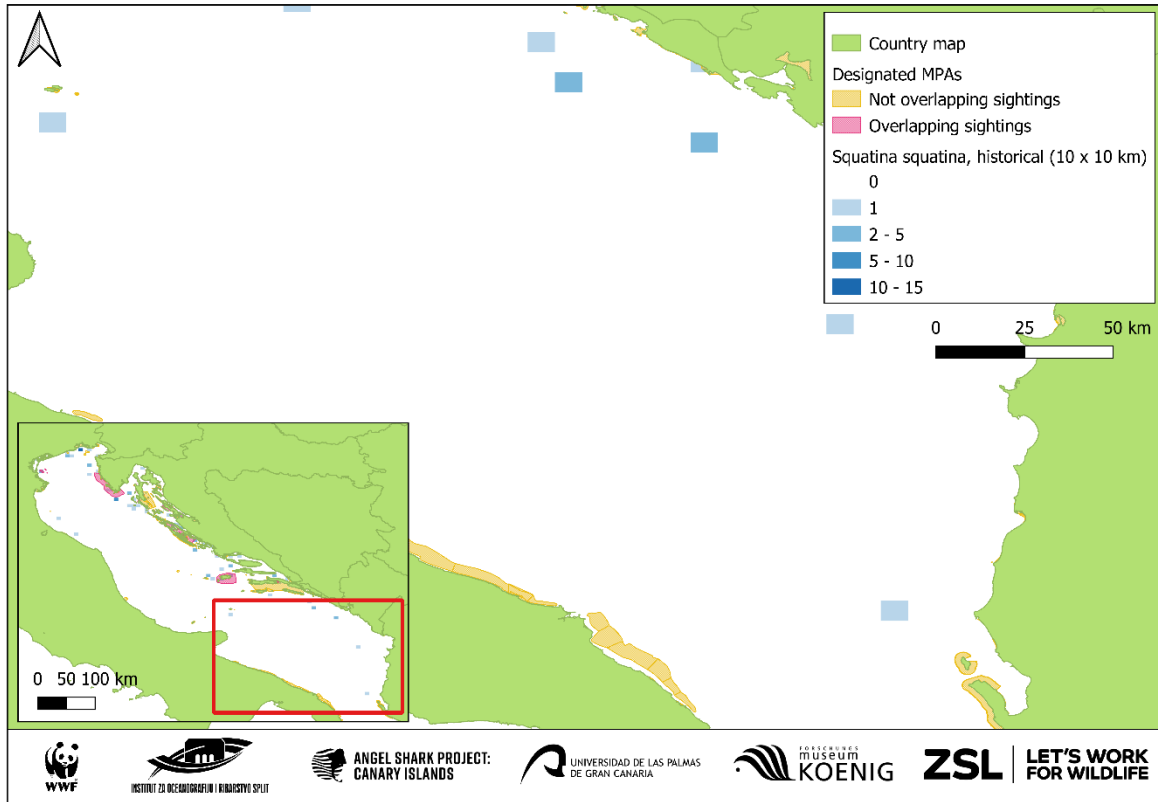


Figure 9a – Southern Adriatic visualisation of **historic** (1947–2009 inclusive) angel shark records (*S. squatina*) at 10 x 10 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

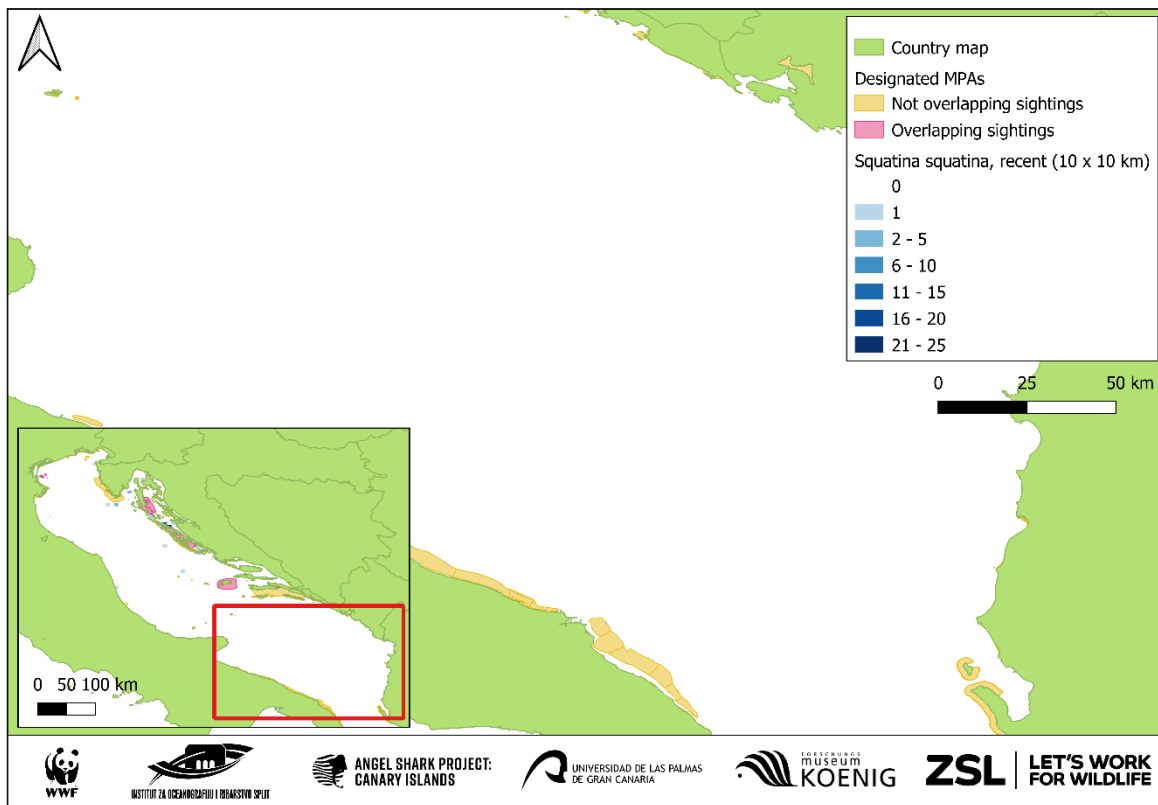


Figure 9b – Southern Adriatic visualisation of **recent** (2010–2020) angel shark records (*S. squatina*) at 10 x 10 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

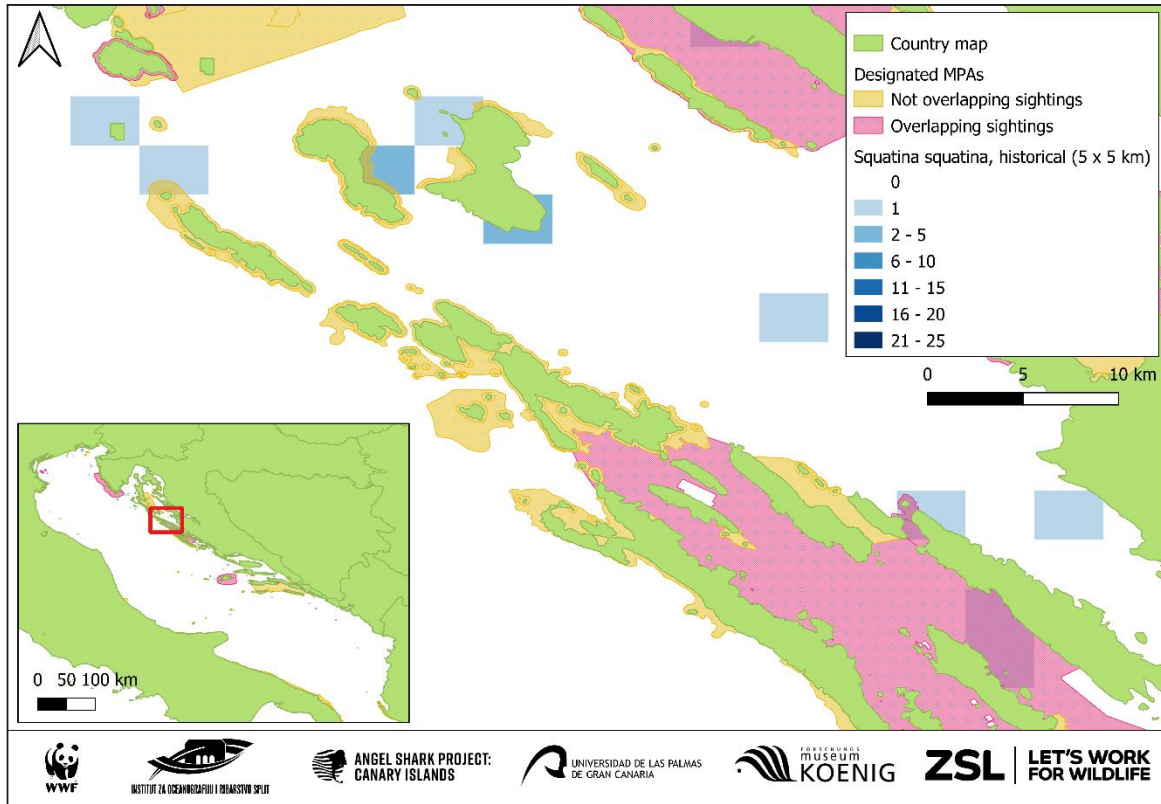


Figure 10a – Visualisation of Molat island, showing **historic** (1947–2009 inclusive) angel shark records (*S. squatina*) at 5 x 5 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

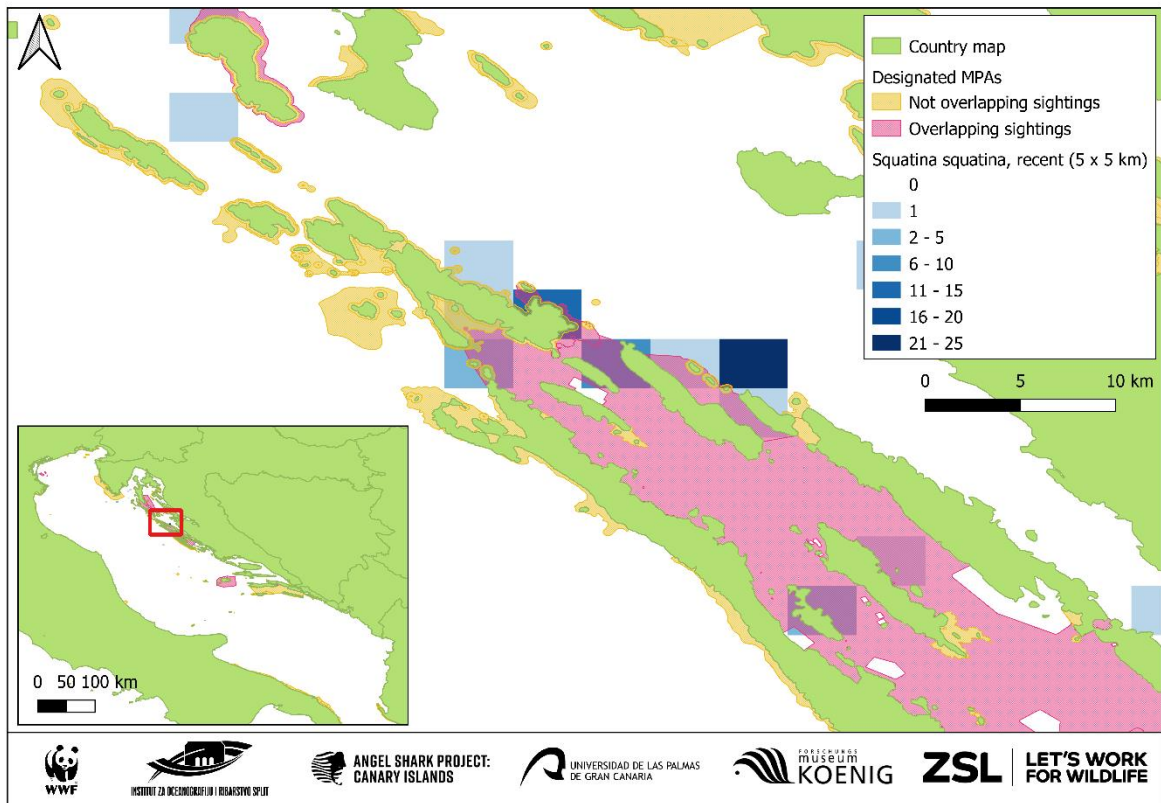


Figure 10b – Visualisation of Molat island, showing **recent** (2010–2020) angel shark records (*S. squatina*) at 5 x 5 km resolution, in relation to **designated MPAs** in the Adriatic Sea.

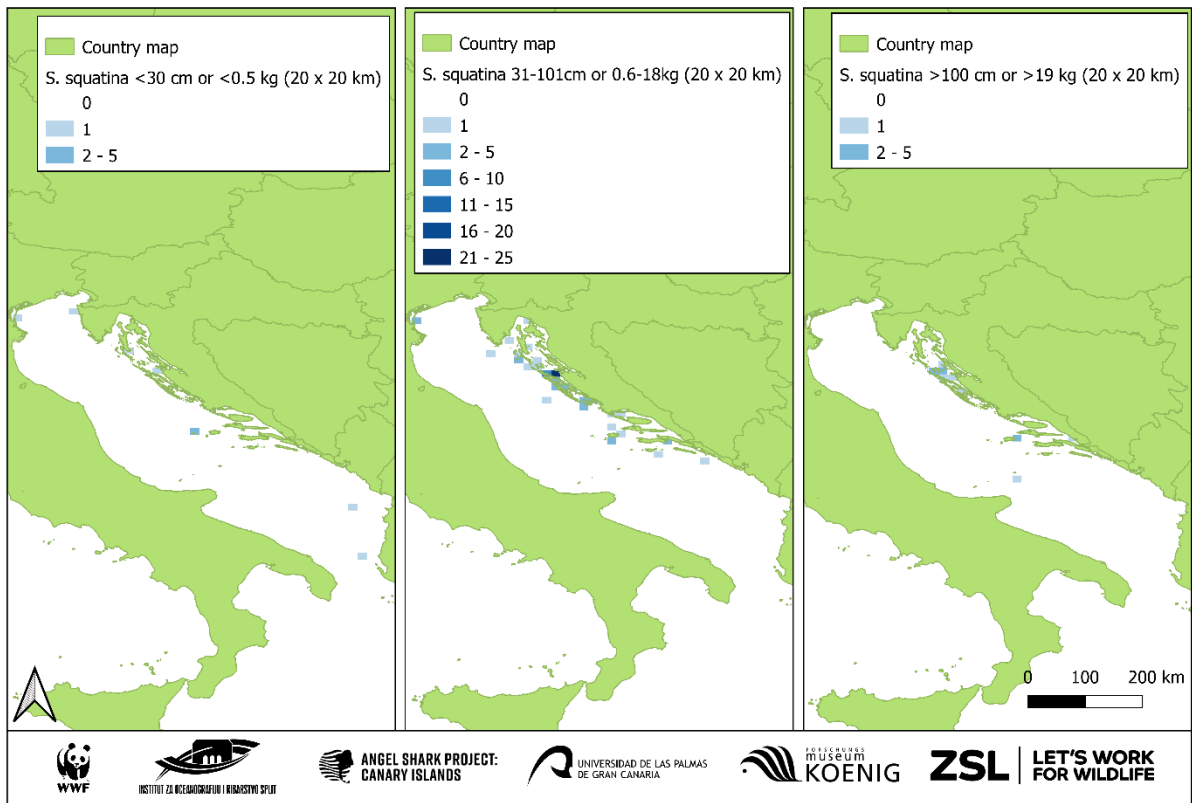


Figure 11 – Size analysis showing *S. squatina* records described as either less than 30 cm in length or less than 0.5 kg in weight, between 31–100 cm or 0.6–18kg, and greater than 100 cm or 19 kg, at 20 x 20 km resolution (where information was available).

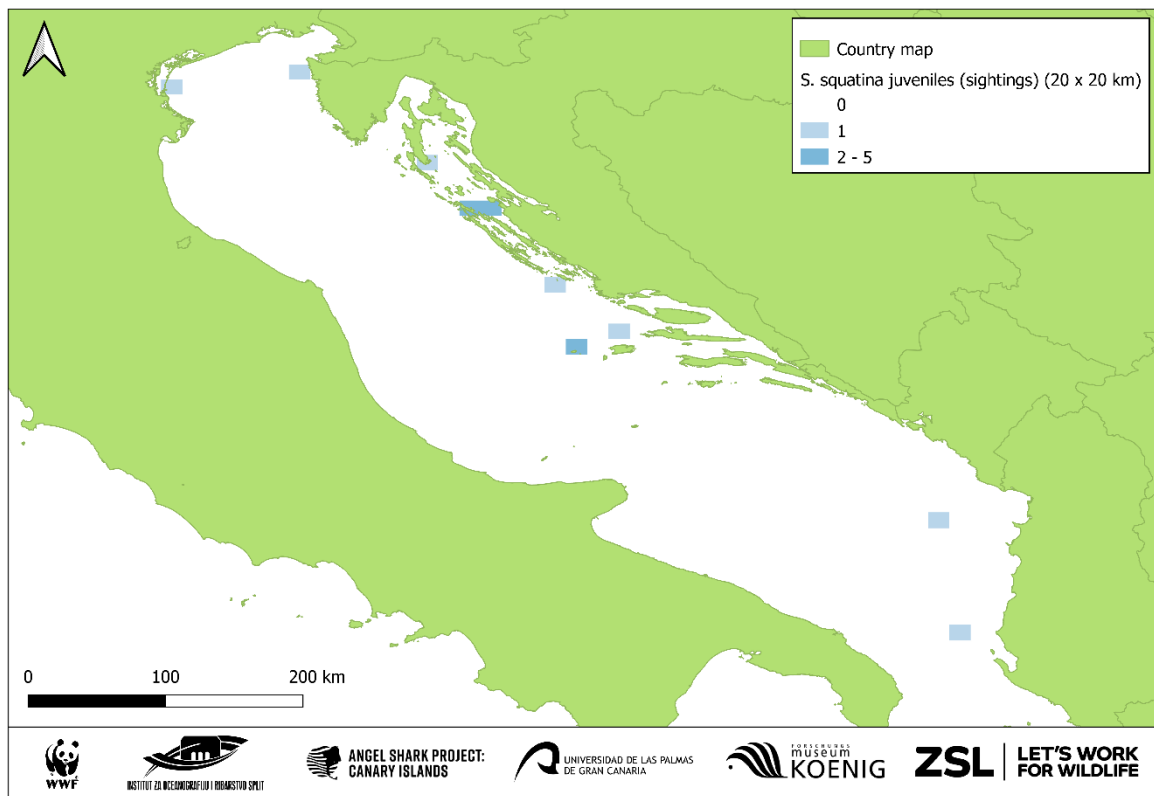


Figure 12 – Size analysis showing sightings of juvenile *S. squatina* described as either less than 30 cm in length, less than 0.5 kg in weight, or records within the LEK study where size was given for an adult *S. squatina*, but there was a note to describe that the sighting also included juveniles of which the size was not recorded, at 20 x 20 km resolution.

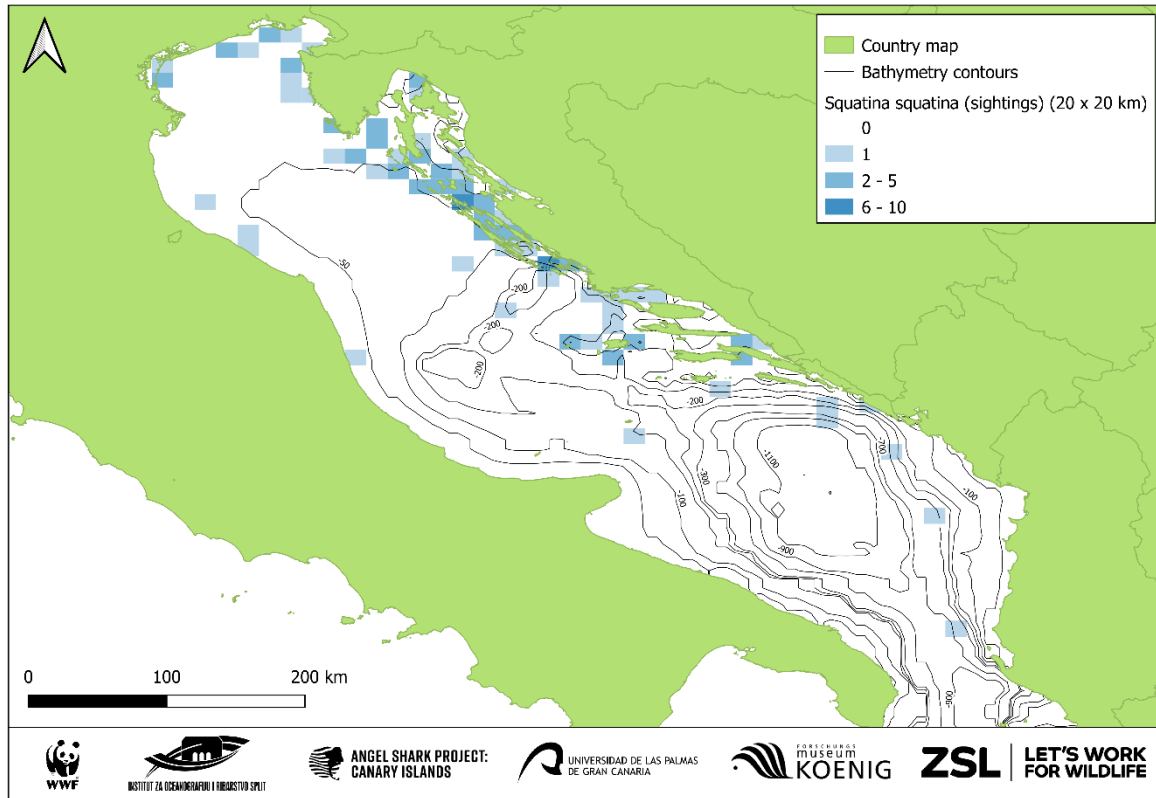


Figure 13 – Sightings of *S. squatina* at 20 x 20 km resolution, in relation to depth of sea floor (bathymetry) for the Adriatic Sea (average depth of sea floor, 30 arcsecond spatial resolution).

Discussion

Mediterranean Sea

Of the three species of angel shark found in the Mediterranean Sea, *S. squatina* was the most common species recorded to the ASSM. *S. squatina* presence was confirmed off the coast of Corsica, Sicily, Libya and Israel, and in the Aegean and Adriatic Seas. Records for *S. oculata* and *S. aculeata* in the Mediterranean Sea were sparse, with records occurring in the Aegean Sea, on the south eastern coast of Sicily and the southern coast of Turkey. This aligns with recently published research investigating the extant range of the three *Squatina* species in the Eastern Atlantic and Mediterranean Sea (Lawson *et al.* 2020) and the IUCN Red List assessments for *S. squatina* (Morey *et al.* 2019a), *S. oculata* (Morey *et al.* 2019b) and *S. aculeata* (Morey *et al.* 2019c). Further research could be targeted at these locations to better understand angel shark distribution and ecology.

Angel shark occurrence in MPAs and FRAs

Recent records of angel sharks were found to show overlap with some MPAs and FRAs in the Mediterranean Sea (Table 1). The most common type of MPA to overlap sightings was designated Natura 2000 sites, none of which were found to have management plan, or implemented management plan. One FRA, Jabuka Pit, overlapped angel shark records.

Natura 2000 sites cover both aquatic and terrestrial ecosystems across all EU countries (European Commission 2020). The network of Natura 2000 sites aim to protect rare species and habitats listed under the EU Birds Directive or EU Habitats Directive respectively (European Commission 2020). No elasmobranchs are included in the species list of these directives; thus Natura 2000 sites cannot be designated specifically for angel sharks. However, angel sharks could benefit from some level of de-

facto protection as habitats listed under the EU Habitats Directive have been shown to be used by this species: “1110 Sandbanks which are slightly covered by sea water all the time” (Akyol *et al.* 2015, Meyers *et al.* 2017, Morey *et al.* 2019b,c); “1130 Estuaries” (Morey *et al.* 2019c), “1140 Mudflats and sandflats not covered by seawater at low tide” (Morey *et al.* 2019b,c); “1150 Coastal lagoons” (Lapinski and Giovos 2019); “1160 Large shallow inlets and bays” (Morey *et al.* 2019c, Meyers *et al.* 2017, Barker *et al.* 2019, Jiménez-Alvarado *et al.* 2020); “1170 Reefs” (Meyers *et al.* 2017). In addition, an angel shark species could feature as a species of conservation interest within the management plan for the Natura 2000 site, if data shows that the species occurs within the designated area.

Within the Adriatic there were four Natura 2000 sites where two or more angel sharks had been sighted. These Natura 2000 sites occur in Croatia; three were listed under the Habitats Directive for reefs, *Posidonia oceanica* beds, sea caves (submerged or partially submerged) and bottlenose dolphins. The remaining site was listed under the Birds Directive. Outside of the Adriatic, there was one Natura 2000 site in France where over two angel sharks were sighted. This site was listed under the Habitats Directive for *P. oceanica* beds, sandbanks (slightly covered by seawater), mudflats and sandflats (not covered by seawater at low tide), and loggerhead turtles. None of these sites have a management plan or implemented management plan.

Less than 10 % of the Mediterranean Sea is covered by MPAs, meaning international targets have not been met by 2020. Within the context of the Post-2020 Global Biodiversity Framework, scientists and global leaders are calling for increasingly ambitious biodiversity targets, to effectively protect at least 30 % of the ocean to maintain and restore biodiversity. Gomei *et al.* (2019) found that MPAs with a management plan only covered 2.48 % of the surface of the Mediterranean Sea, and MPAs where actions within the management plan were actually being implemented only covered only 1.27 %, suggesting current levels of protection are not effective. The results of the current analysis reflect this shortcoming, and highlights the lack of spatial protection for threatened species, such as angel sharks. Findings of this report underline the urgency to expand and designate additional areas.

Adriatic Sea

Records from the LEK case study revealed that 80.2 % of the sightings resulted from interaction with fisheries, most commonly with a gillnet. Other studies show angel sharks are susceptible to gillnets as a result of their demersal nature (Lawson *et al.* 2020). Anecdotal evidence collected during this study found that most fishers did not return their angel shark catch, and instead were landed for consumption. This highlights the urgency of working with the fishing communities to educate in the protected status of angel sharks and train in how to safely release angel sharks encountered. LEK case study and ASSM records found only *S. squatina* to be present in the Adriatic Sea. Fortibuoni *et al.* (2016) suggested *S. squatina* underwent a collapse in the 1970s in the Adriatic Sea, although there were some records remaining from the area between 2000 and 2013. In this study, more historical records in the northern Adriatic occurred around the Italian coast between Venice and Trieste, whereas in recent years the records were clustered around the northern Croatian Islands. In the central and southern Adriatic, there were less recent records in comparison to historical records. The LEK case study was focussed mostly in Croatia, and so a comparable study conducted in other countries bordering the Adriatic may produce new records. This would allow better comparison across the Adriatic Sea to fully understand angel shark distribution.

In Croatian waters, the Molat Island archipelago was identified as a potential hotspot for *S. squatina*. This was apparent when mapping recent records and just juvenile sightings. The currents around Molat Island archipelago are some of the largest in Croatia and surrounding reef ecosystems mean there are fewer opportunities for fishing (D. Kanski *pers. comms.*). Thus, low levels of historic and

current fishing pressure in this area may provide an explanation for the location of this angel shark hotspot. Further research is needed to evaluate this.

Angel sharks bury themselves in soft sediment to camouflage from predators and prey (Compagno 1984). When overlaying angel shark records with modelled habitat data, the most common substrate types were sand and mud-based sediments. Around Molat Island archipelago, three of four juvenile records were on sandy mud substrate type, with one record occurring on coarse and mixed sediment. This supports research in the Canary Islands that show *S. squatina* presence was most commonly associated with sand habitat, close to reefs, although they were also found in reef habitat, and within seagrass beds (Meyers *et al.* 2017, Jiménez Alvarado *et al.* 2020). Angel sharks have also been linked with seagrass in Corsica (Lapinski & Giovos 2019), and three of the four Natura 2000 sites overlapping sightings in the Adriatic were found to have protections for *P. oceanica* beds. This habitat may therefore be important for *S. squatina*, and further study is needed to confirm this. However, in recent years there have been observed large decline in seagrass beds across the Mediterranean Sea, attributed to coastal development and construction, pollution, aquaculture, trawling, and rapid ocean warming which has been shown to trigger shoot mortality (Ruíz *et al.* 2009, Marba & Duarte 2010).

Depth has also been shown to be a factor in angel shark distribution, with shallower areas (< 25m) more commonly occupied by juvenile angel sharks, with adult angel sharks found at greater depths (Meyers *et al.* 2017, Jiménez Alvarado *et al.* 2020). In this study, most sightings occurred at depths shallower than 50 m. Juvenile angel shark presence in shallower areas may be due to protection from predators, greater prey abundance or higher temperatures to increase rate of development (Meyers *et al.* 2017). Molat Island archipelago is composed of many shallow inlets and bays, coupled with soft sediment and reef habitat, which may provide the environmental conditions needed for *S. squatina* to thrive. Further study in the Molat Island archipelago would be beneficial to confirm whether it is a potential nursery area for *S. squatina* in Croatia.

Recommendations

The following recommendations have been developed using the results of this study. They focus on improving understanding and conservation of angel sharks in the Adriatic Sea, specifically in Croatian waters where the most records were collected. As our understanding of angel sharks in the Adriatic Sea and wider Mediterranean Sea grows, these recommendations will need further development.

RECOMMENDATION 1: Work with the Government of the Republic of Croatia and Natura 2000 management officials to improve understanding of angel shark presence in the region, and where appropriate, account for this in management plans.

Firstly, contact officials that manage the specific Natura 2000 areas that overlap angel shark occurrences, to share the results of this study and increase understanding of angel shark ecology. Due to Natura 2000 site designation process, a specific designation for angel sharks would not be possible (see Discussion). However, angel sharks could feature as an additional species of conservation interest within management plans at these Natura 2000 sites, where appropriate.

Secondly, complete a consultation with the respective authorities including the with the Ministry of Economy and Sustainable Development (Government of the Republic of Croatia) to discuss how angel sharks could be included in the development and implementation of management plans, with possible consideration of specific fishing restrictions or seasonal protection of specific habitat. This should focus on the following priority Natura 2000 sites:

- J. Molat-Dugi-Kornat-Murter-Pašman-Ugljan-Rivanj-Sestrunj-Molat
- JI dio o. Molata
- Srednjedalmatinski otoci i Pelješac
- Rivanjski kanal sa Sesticama

In the future, if research confirms that angel sharks depend on specific habitats listed under the EU Habitats Directive during a particular life stage, angel sharks could be added as a “typical species” or “notable species” for this habitat as part of the Natura 2000 framework. Significant additional data are needed for this.

RECOMMENDATION 2: Further angel shark research at possible hotspots to strengthen baseline knowledge of angel sharks in the region.

Resources should be secured to enable dedicated angel shark research in the Adriatic Sea to gather data on distribution, movement, ecology, and abundance to inform future conservation and management. The Angel Shark Project have suggested the following research techniques as a priority for Croatian waters:

- Continue to strengthen citizen science data collection to better understand angel shark distribution in Croatia.
- Conduct a systematic environmental DNA (eDNA) study at possible angel shark hotspots to investigate occurrence throughout the year.
- Complete visual surveys via scuba diving and snorkelling at possible hotspots (where conditions allow) to identify angel sharks and assess habitats present.
- Use results of the above techniques to assess whether an angel shark tagging study or genetic research could be viable in the Adriatic Sea, in order to understand movement and connectivity.

RECOMMENDATION 3: Train fishers in best-practice guidance to safely release angel sharks and work with communities around Molat Island archipelago.

Molat Island archipelago was identified within this study as a potential hotspot for *S. squatina*. Scoping work around this island is necessary to confirm this hypothesis, which should include pilot studies as outlined in Recommendation 2. As a first step, focused work alongside the fishing community should be conducted in this area to:

- Raise awareness of the conservation status and ecology of angel sharks.
- Educate fishers of the protected status of angel sharks, and that if caught, individuals must be released.
- Train fishers in best practise handling to ensure highest chance of survival for accidental angel shark catches.

In addition, a focused threat analysis could be conducted to assess possible threats to angel sharks in this area. It is important to closely involve fishing and local communities in any future work, e.g. angel shark research or designing conservation measures in this area. Both methods would also highlight the importance of sharing angel shark records with the scientific community.

RECOMMENDATION 4: Develop a sub-regional action plan for the Adriatic Sea.

The Mediterranean Regional Action Plan for Angel Sharks (MedRAP) was developed and published in 2019 to focus angel shark conservation efforts (Gordon *et al.* 2019). The MedRAP sets out a road map for the development of sub-regional action plans (SubRAPs) to better facilitate action in each subregion of the Mediterranean, and allow effective delivery of aims set out within the MedRAP (Gordon *et al.* 2019). The SubRAP for the Aegean Sea was the first to be published in August 2020 (Gordon *et al.* 2020).

The objective of the SubRAPs are to gather data and evidence, share information, standardise approaches and allow better cooperation and communication across subregions in the Mediterranean (Gordon *et al.* 2019). The results of this study can be used as a baseline to help develop the Adriatic SubRAP with partners across the Adriatic Sea and should be considered a priority.

RECOMMENDATION 5: Develop angel shark LEK case study template to replicate research in other countries.

The LEK case study conducted in Croatia revealed many new angel shark records, not reported to the ASSM, demonstrating that establishing positive relationships with local coastal communities are vital to better understand distribution. The LEK study used in this analysis was focused on Croatia, therefore it is difficult to make recommendations at a Mediterranean Sea or Adriatic Sea scale until similar studies are conducted in other localities to allow for comparison. Replication of the LEK study across other countries in the Mediterranean Sea, in particular those bordering the Adriatic Sea would significantly improve our understanding of angel shark distribution. Areas identified within the analysis where sightings overlap MPAs could also be used to guide where to implement LEK studies, for example in Corsica, where sightings overlapped the Pelagos Sanctuary. To enable consistent and comparable data to be collected, an angel shark LEK Study template should be created and shared with colleagues across the Mediterranean Sea.

RECOMMENDATION 6: If Angelshark hotspots are confirmed with further study, identify mechanisms to develop MPAs to protect these habitats (see Recommendation 2 & 3).

The Nature Protection Strategy and Action Plan of the Republic of Croatia (2017–2025) will derive strategic goals from the Aichi Biodiversity targets (listed in the Strategic Plan for Biodiversity 2011-2020 of the Convention of Biological Diversity) and the EU 2020 Biodiversity Strategy targets. This includes potential for development of new MPAs in Croatian waters. As a first step, results of this project should be discussed with the Ministry of Economy and Sustainable Development whilst further research is being carried out to confirm presence and use of angel shark hotspots (See Recommendation 2 and 3).

Any future MPAs developed for angel sharks in Croatia should consider:

- Areas where sightings have been reported and further research confirms the importance of this site for angel shark life history.
- Involve local communities in identification, development of management protocols and designation of possible future MPAs.

- Evaluate different management tools that may be necessary to enable best benefit for angel sharks, e.g. consideration of specific fishing restrictions or seasonal protection of specific habitat.
- Ensure the management plan includes angel shark monitoring programmes and evaluation of impact measures.

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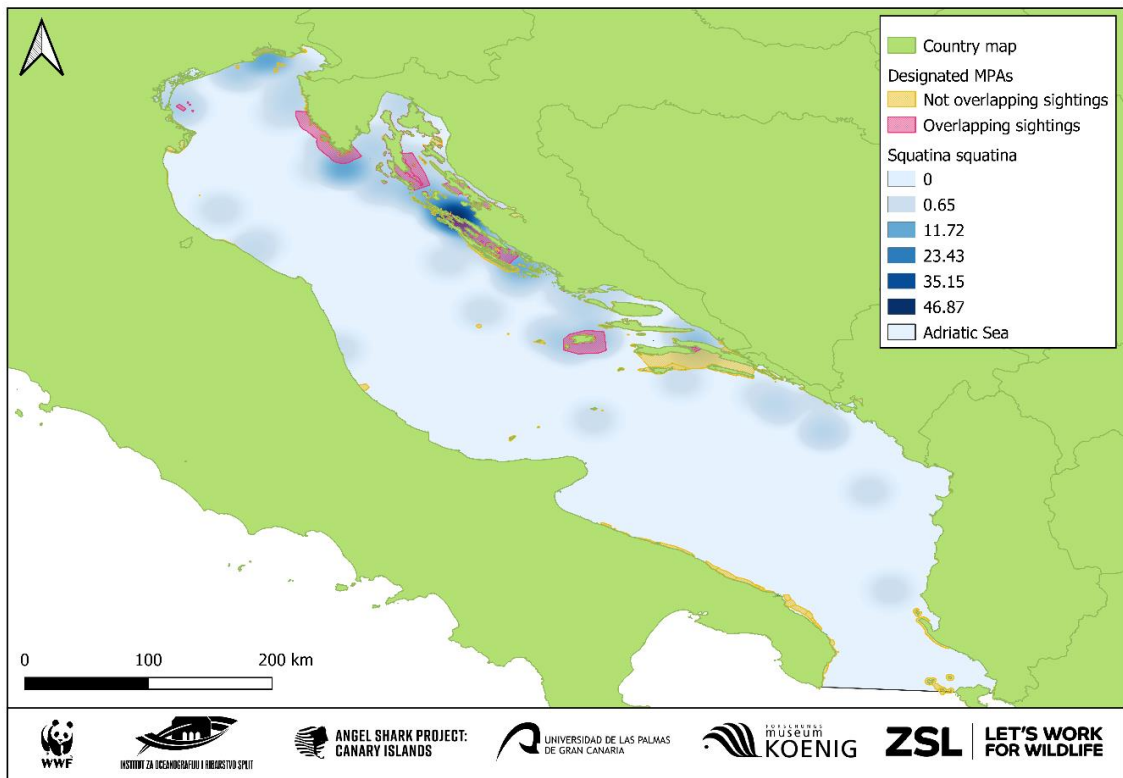
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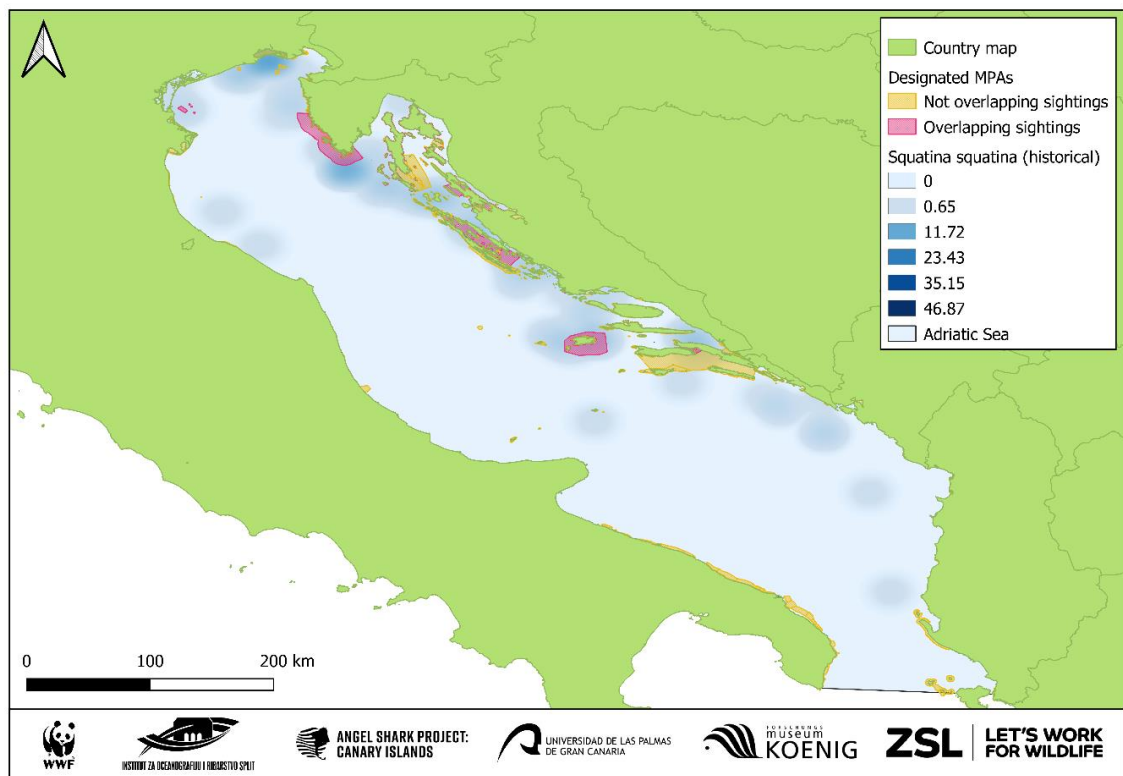
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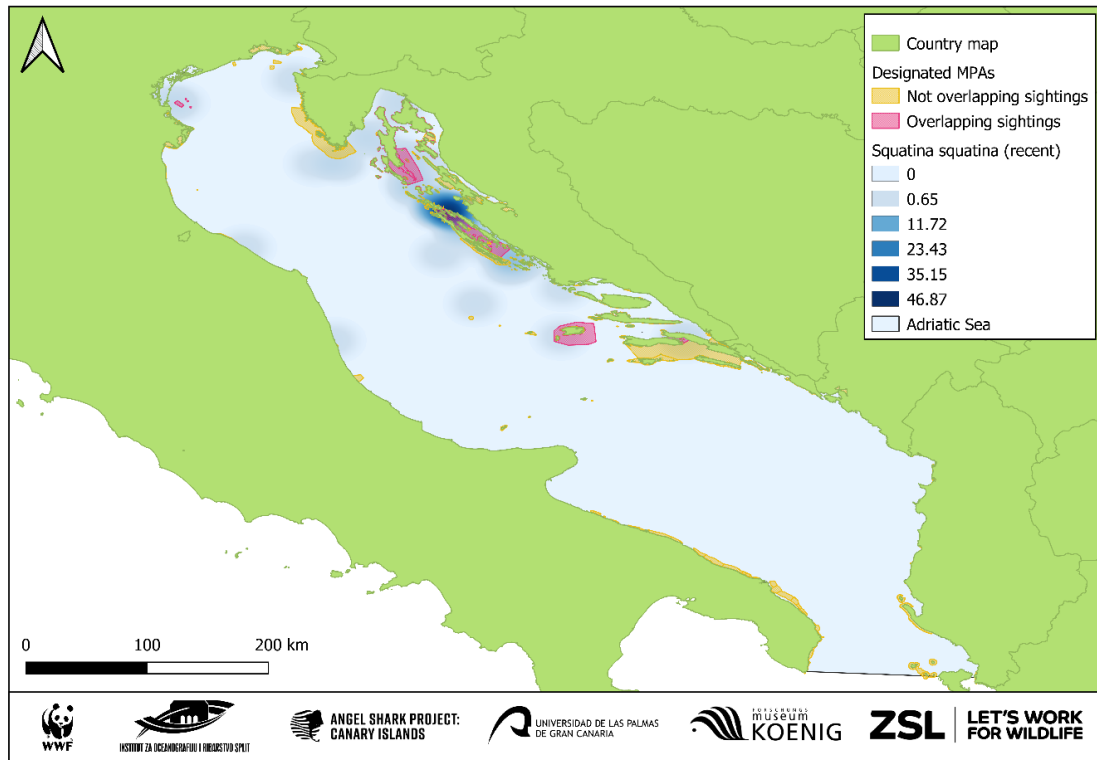
Annex 1 – not for publication



A - Heatmap (25000m radius) of *S. squatina* records, in relation to **designated MPAs** in the Adriatic Sea – not for public use.



B - Heatmap (25000m radius) of historical (1947–2009 inclusive) *S. squatina* records, in relation to **designated MPAs** in the Adriatic Sea – not for public use.



C - Heatmap (25000m radius) of recent (2010–2020) *S. squatina* records, in relation to **designated MPAs** in the Adriatic Sea – not for public use.