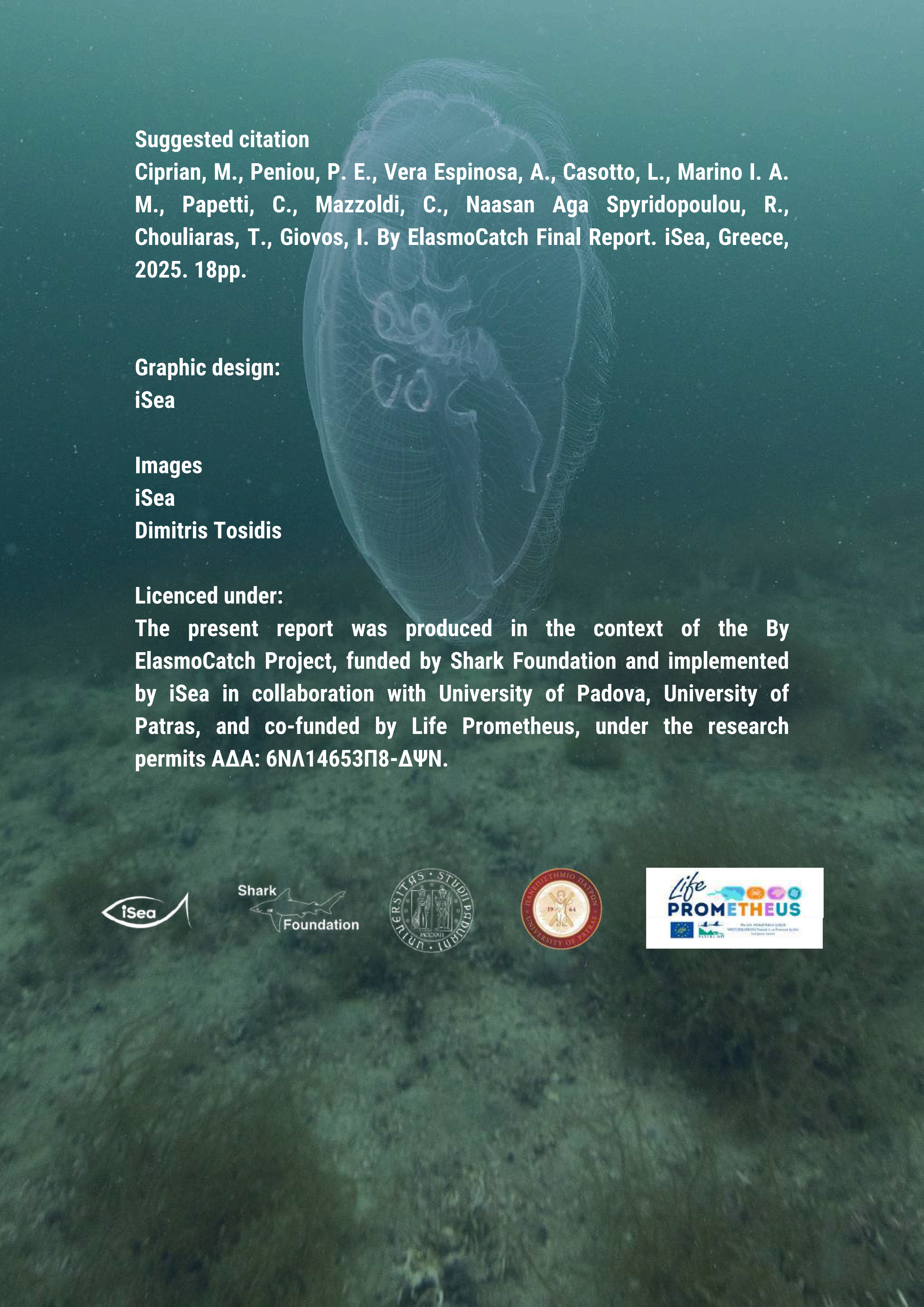
BY ELASMOCATCH

FINAL REPORT

iSea

THESSALONIKI 2025

iSea, 2025



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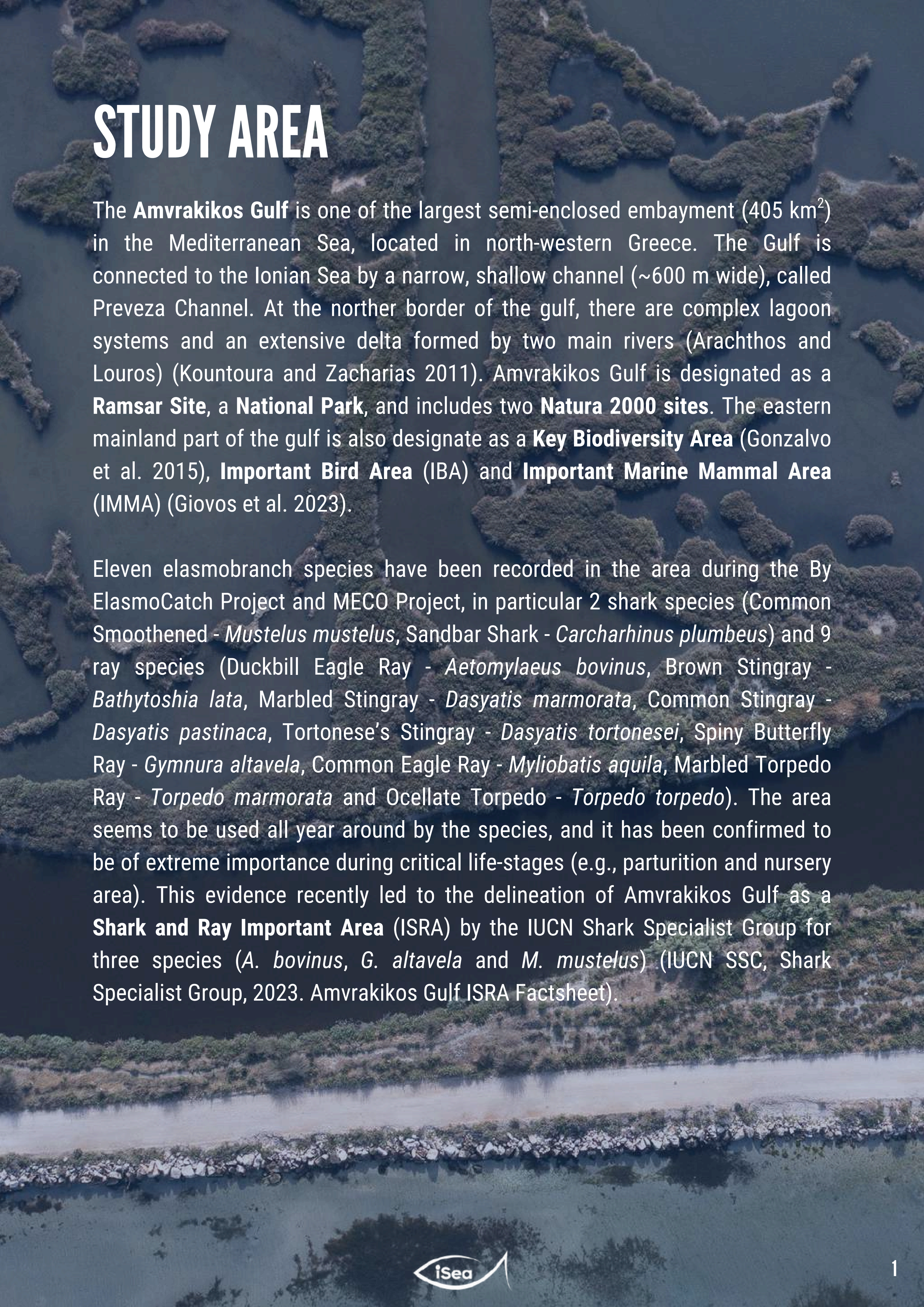
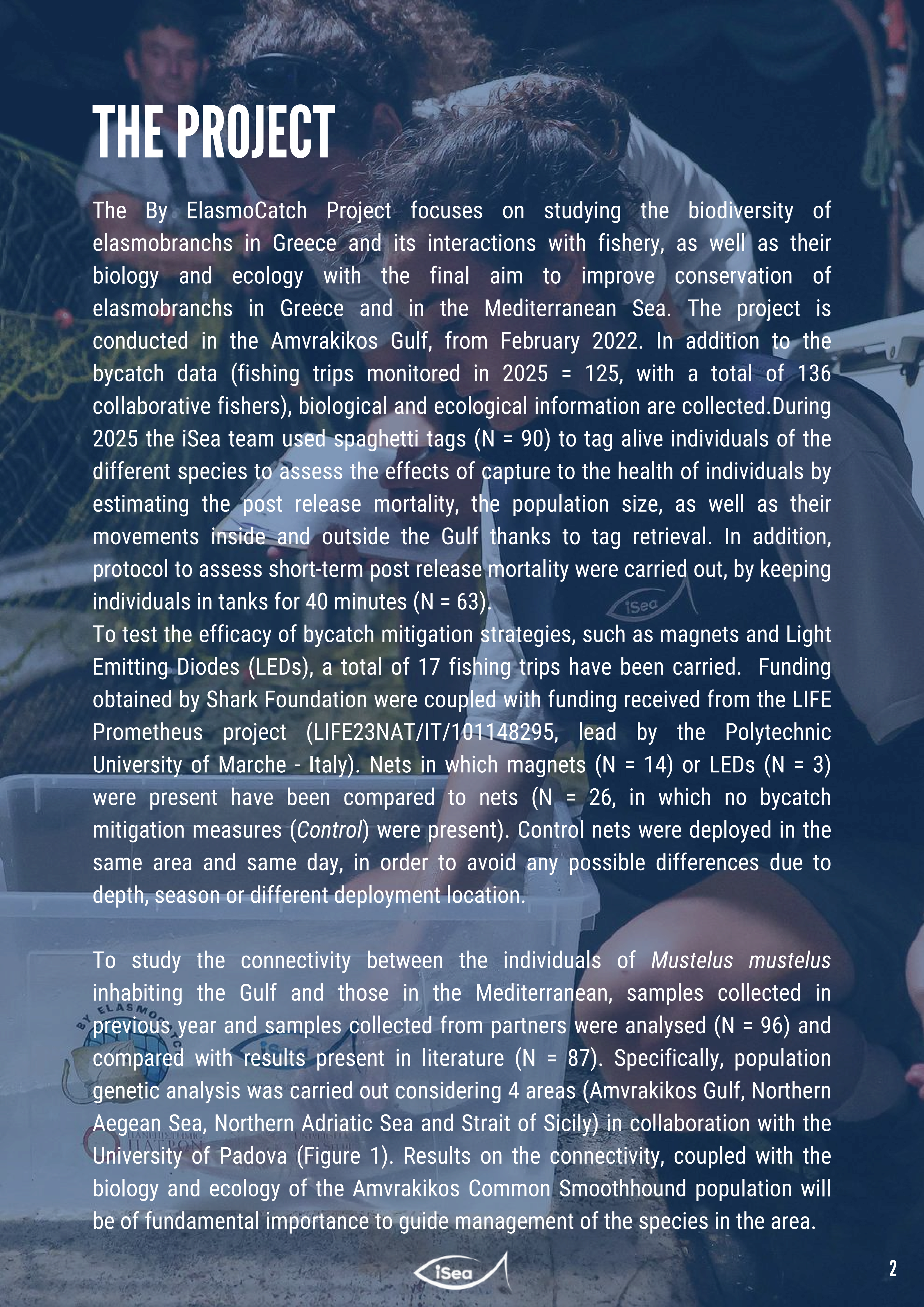


STUDY AREA

The **Amvrakikos Gulf** is one of the largest semi-enclosed embayment (405 km^2) in the Mediterranean Sea, located in north-western Greece. The Gulf is connected to the Ionian Sea by a narrow, shallow channel (~600 m wide), called Preveza Channel. At the norther border of the gulf, there are complex lagoon systems and an extensive delta formed by two main rivers (Arachthos and Louros) (Kountoura and Zacharias 2011). Amvrakikos Gulf is designated as a **Ramsar Site**, a **National Park**, and includes two **Natura 2000 sites**. The eastern mainland part of the gulf is also designate as a **Key Biodiversity Area** (Gonzalvo et al. 2015), **Important Bird Area** (IBA) and **Important Marine Mammal Area** (IMMA) (Giovos et al. 2023).

Eleven elasmobranch species have been recorded in the area during the By ElasmoCatch Project and MECO Project, in particular 2 shark species (Common Smoothened - *Mustelus mustelus*, Sandbar Shark - *Carcharhinus plumbeus*) and 9 ray species (Duckbill Eagle Ray - *Aetomylaeus bovinus*, Brown Stingray - *Bathytoshia lata*, Marbled Stingray - *Dasyatis marmorata*, Common Stingray - *Dasyatis pastinaca*, Tortonese's Stingray - *Dasyatis tortonesei*, Spiny Butterfly Ray - *Gymnura altavela*, Common Eagle Ray - *Myliobatis aquila*, Marbled Torpedo Ray - *Torpedo marmorata* and Ocellate Torpedo - *Torpedo torpedo*). The area seems to be used all year around by the species, and it has been confirmed to be of extreme importance during critical life-stages (e.g., parturition and nursery area). This evidence recently led to the delineation of Amvrakikos Gulf as a **Shark and Ray Important Area** (ISRA) by the IUCN Shark Specialist Group for three species (*A. bovinus*, *G. altavela* and *M. mustelus*) (IUCN SSC, Shark Specialist Group, 2023. Amvrakikos Gulf ISRA Factsheet).



THE PROJECT

The By ElasmoCatch Project focuses on studying the biodiversity of elasmobranchs in Greece and its interactions with fishery, as well as their biology and ecology with the final aim to improve conservation of elasmobranchs in Greece and in the Mediterranean Sea. The project is conducted in the Amvrakikos Gulf, from February 2022. In addition to the bycatch data (fishing trips monitored in 2025 = 125, with a total of 136 collaborative fishers), biological and ecological information are collected.During 2025 the iSea team used spaghetti tags (N = 90) to tag alive individuals of the different species to assess the effects of capture to the health of individuals by estimating the post release mortality, the population size, as well as their movements inside and outside the Gulf thanks to tag retrieval. In addition, protocol to assess short-term post release mortality were carried out, by keeping individuals in tanks for 40 minutes (N = 63).

To test the efficacy of bycatch mitigation strategies, such as magnets and Light Emitting Diodes (LEDs), a total of 17 fishing trips have been carried. Funding obtained by Shark Foundation were coupled with funding received from the LIFE Prometheus project (LIFE23NAT/IT/101148295, lead by the Polytechnic University of Marche - Italy). Nets in which magnets (N = 14) or LEDs (N = 3) were present have been compared to nets (N = 26, in which no bycatch mitigation measures (*Control*) were present). Control nets were deployed in the same area and same day, in order to avoid any possible differences due to depth, season or different deployment location.

To study the connectivity between the individuals of *Mustelus mustelus* inhabiting the Gulf and those in the Mediterranean, samples collected in previous year and samples collected from partners were analysed (N = 96) and compared with results present in literature (N = 87). Specifically, population genetic analysis was carried out considering 4 areas (Amvrakikos Gulf, Northern Aegean Sea, Northern Adriatic Sea and Strait of Sicily) in collaboration with the University of Padova (Figure 1). Results on the connectivity, coupled with the biology and ecology of the Amvrakikos Common Smoothhound population will be of fundamental importance to guide management of the species in the area.

Figure 1: *A) Map of Europe and Mediterranean Sea, showing the areas included in the genetic study. B) Map of Amvrakikos with the location of the main ports.*

BYCATCH MONITORING

RESULTS & DISCUSSION

From January to October a total of 142 fishing trips were monitored, with 96.5% of them using trammel nets (GTR) targeting *Sepia officialis*, fishes or *Penaeus kerathurus* and 3.5% using gill nets (GNS) targeting small-pelagic fishes or *Umbrina cirrosa*. A total of 17 fishing trips using magnets and LEDs (N = 14, N = 3, respectively) were monitored through onboard surveys, and compared to control nets (N = 20, N = 6, respectively) (Figure 2, Table 1). The species recorded in 2025 include 1 shark species (*M. mustelus*) and 8 ray species (*A. bovinus*, *D. marmorata*, *D. pastinaca*, *D. tortonesei*, *M. aquila*, *T. marmorata* and *T. torpedo*).

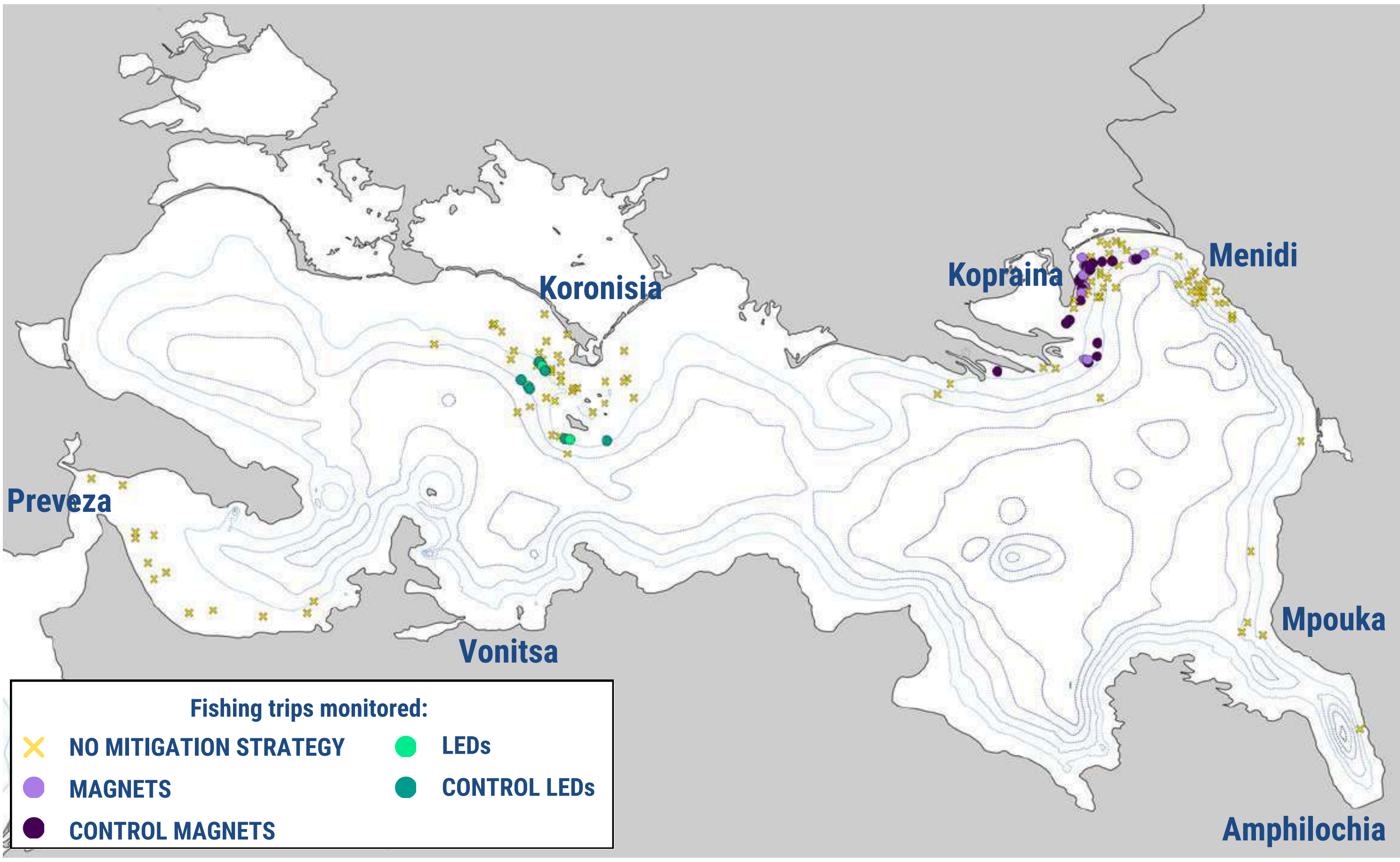


Figure 2: *Map showing the coordinates of the fishing trips monitored (N = 142) from January to October 2025. Different colours of the dots indicate the different bycatch mitigation measures tested on the net and the correspondent controls (purple for magnets, green for LEDs) In yellow X are indicated fishing trips where no bycatch mitigation measures was tested.*

BYCATCH MONITORING

RESULTS & DISCUSSION

Number of collaborative vessels	136
Total number of fishing trips monitored	142
Fishing trips monitored (landing sites)	89
Fishing trips monitored (onboard)	53
Fishing trips using trammel nets (GTR) monitored	137
Fishing trips using gill nets (GNS) targeting small fishes monitored	3
Fishing trips using gill nets (GNS) targeting *Umbrina cirrus*	2
Fishing trips with magnets monitored	14
Fishing trips with LEDs monitored	3
Fishing effort monitored in hours	1106:19
Fishing effort monitored in km of nets	123.841
Species of elasmobranch recorded	8
Specimens of elasmobranchs recorded	487
Specimens of elasmobranchs sampled	449

Table 1: *Summary table of data collected during surveys in landing sites and onboard during 2025.*

BYCATCH MONITORING

RESULTS & DISCUSSION

In order to test the efficacy of magnets and LEDs to reduce bycatch of sharks and rays in Amvrakikos Gulf, bycatch mitigation devices were applied to a portion of the net, and the number of elasmobranchs caught where then compared to the portion of the net with no devices (named '*control*'). Sampling design depending on the different métier (identified by Tzanatos et al. (2006) as a group of fishing operations defined by the combination of fishing gear, target species, area and season) have been delineated depending also on fishers' availability (Figure 3). In particular, in nets targeting Caramote Prawn (*P. kerathurus*) magnets were placed for 100 meters on the lead line, with 1 meter of distance between them, and the control nets were 1100 m long (Figure 3A). In nets targeting cuttlefish (*S. officinalis*) and flatfish (*S. solea*) the magnets were placed for 300 meters on the lead line, with 1 meter of distance between them, and control netsw were 900 m long (Figure 3B). LEDs were placed only in nets targeting *P. kerathurus*, for a total of 100 meters, with 10 meters of distance between them, and the control nets 1000 m long (Figure 3C).

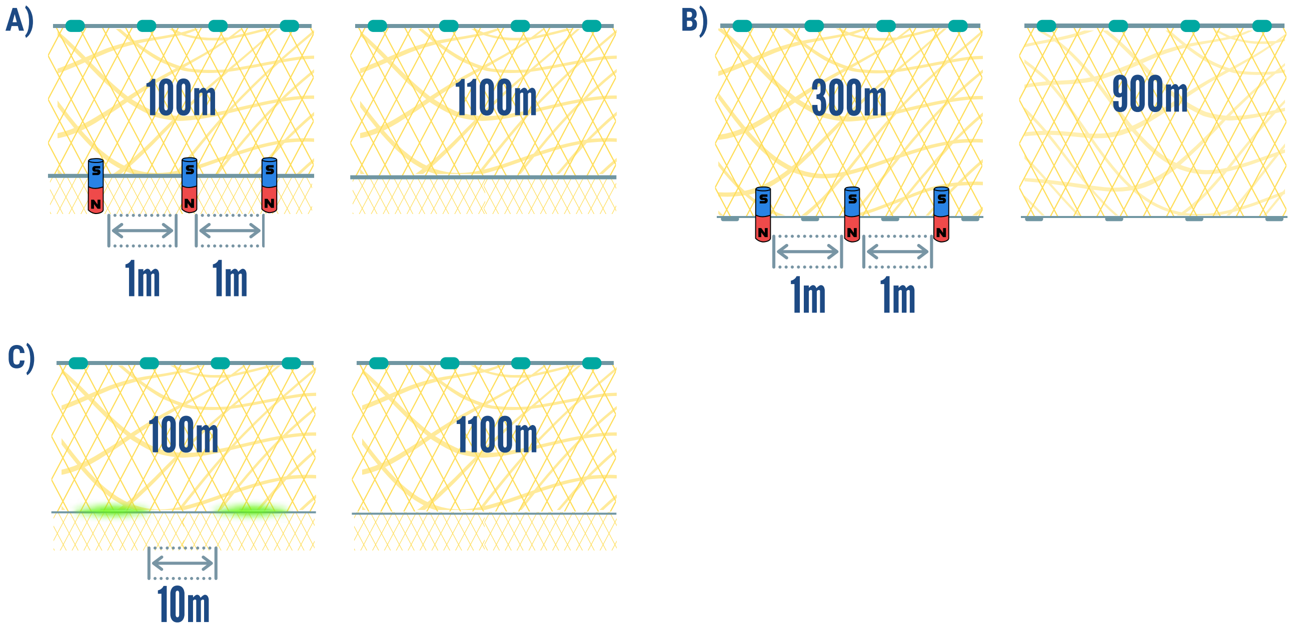


Figure 3: *Net configuration to test: A) magnets in fishing trips targeting P. kerathurus; B) magnets in fishing trips targeting S. officinalis and S. solea; C) LEDs in fishing trips targeting P. kerathurus.*

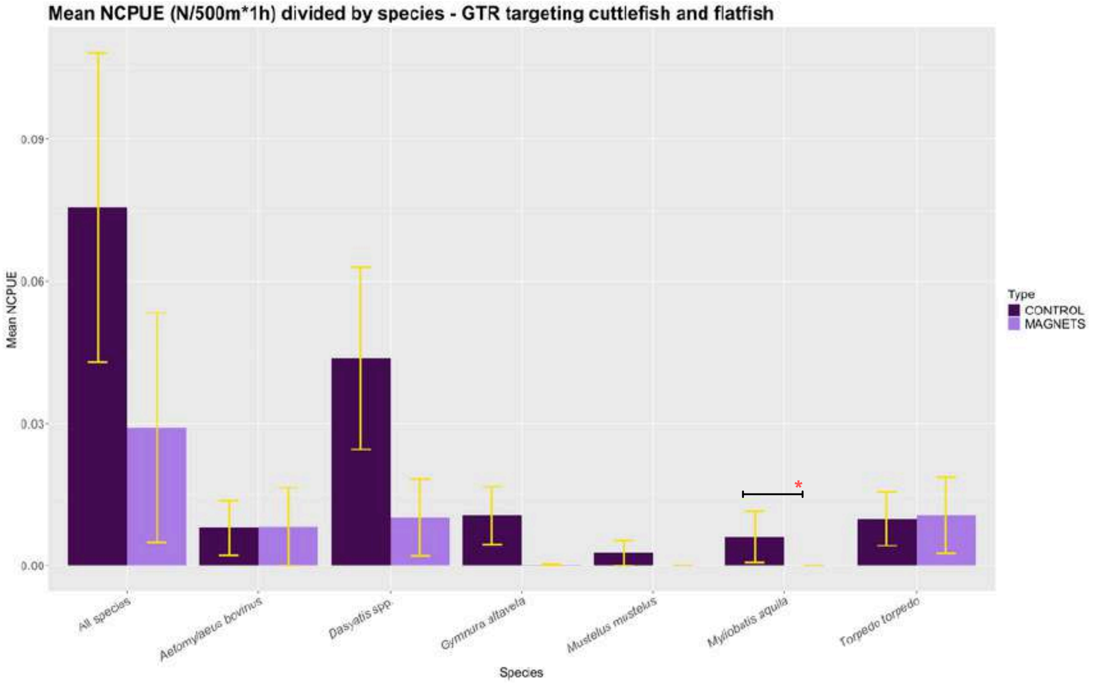
BYCATCH MONITORING

RESULTS & DISCUSSION

Preliminary results obtained did not evidence significant differences in bycatch reduction nets in which magnets or LEDs. In order to compare the different types of métiers, specifically varying in soaking time and net length standardised NCPUE (number of elasmobranchs divided by 500 m of net and 1 hour) was used (Figure 4). The only significant difference found was for the Common Eagle Ray (*M. aquila*) caught in GTR tartgeting cuttlefish and flatfish. The species was caught significantly more in control nets in respect to nets in which magnets were present (Figure 4A). The limited number of trials and the fact that bycatch was not reduced for the other species, must be taken into consideration in the interpretation of the results obtained. Moreover, if considering the Common Eagle Ray, which belongs to the same family of *M. aquila*, bycatch rates seemed to be equal for trials conducted with and without magnets in this métier. For what concern the species *T. torpedo* instead, bycatch rates in nets with magnets were slightly higher than in nets with magnets (Figure 4A, B).

Nets equipped with LEDs had no bycatch of elasmobranchs, but no significant difference was found due to the low number of trials (Figure 4C).

A)



BYCATCH MONITORING

RESULTS & DISCUSSION

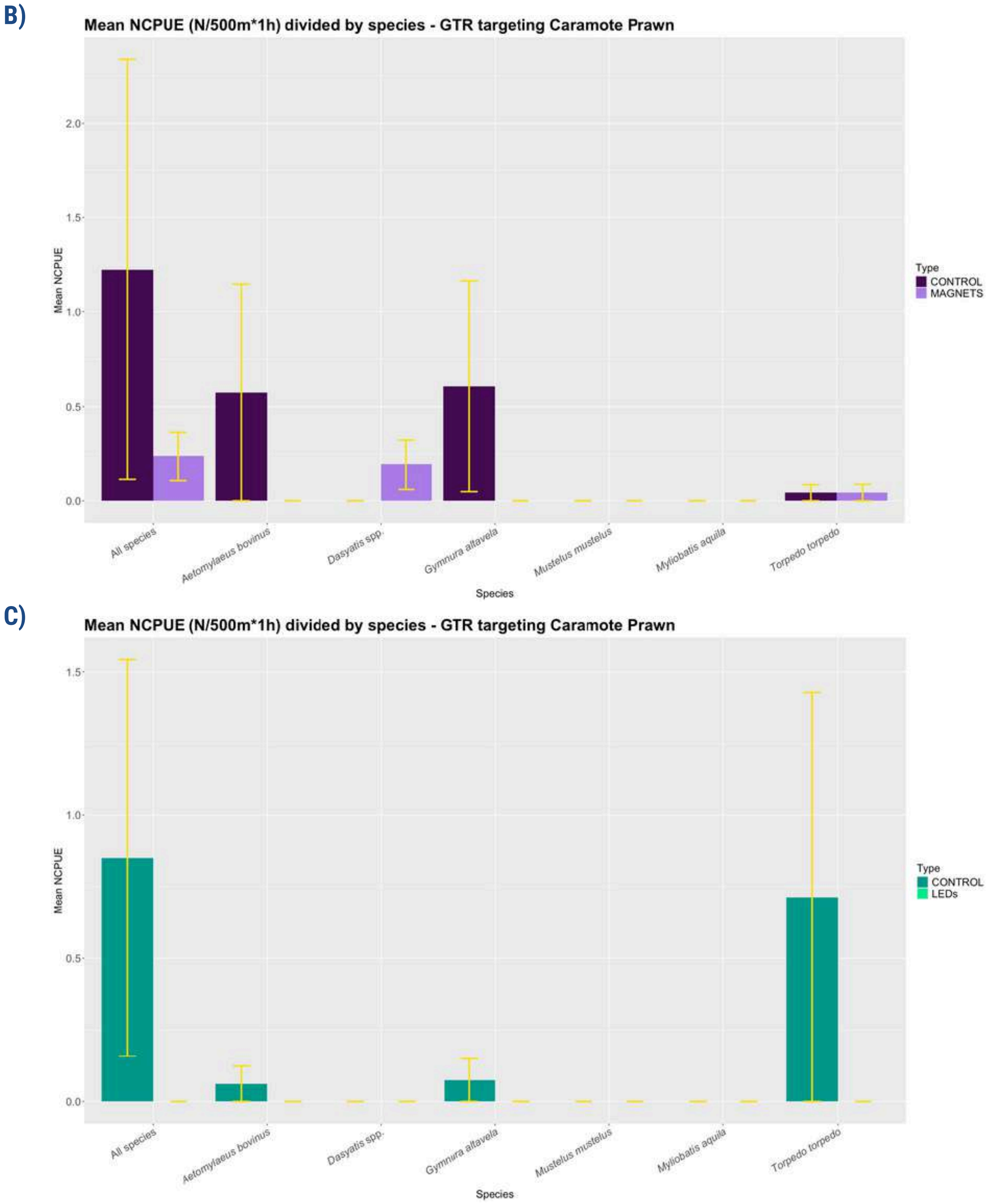


Figure 4: *Mean NCPUE and standard error (yellow bars) for the different métiers: A) NCPUE for magnets (light purple) and control (purple), in fishing trips targeting S. officinalis and S. solea. * indicates p-value = 0.017; B) NCPUE for magnets (light purple) and control (purple), in fishing trips targeting P. kerathurus; C) NCPUE for LEDs (light green) and control (green), in fishing trips targeting P. kerathurus.*

BYCATCH MONITORING

RESULTS & DISCUSSION

A total of 38 *M. mustelus* have been recorded during landing sites and onboard surveys, of which 31 were sampled. All the individuals recorded were smaller than size at maturity, with the 2 biggest individuals caught with trammel nets targeting *S. officinalis* and *S. solea* (Figure 5).

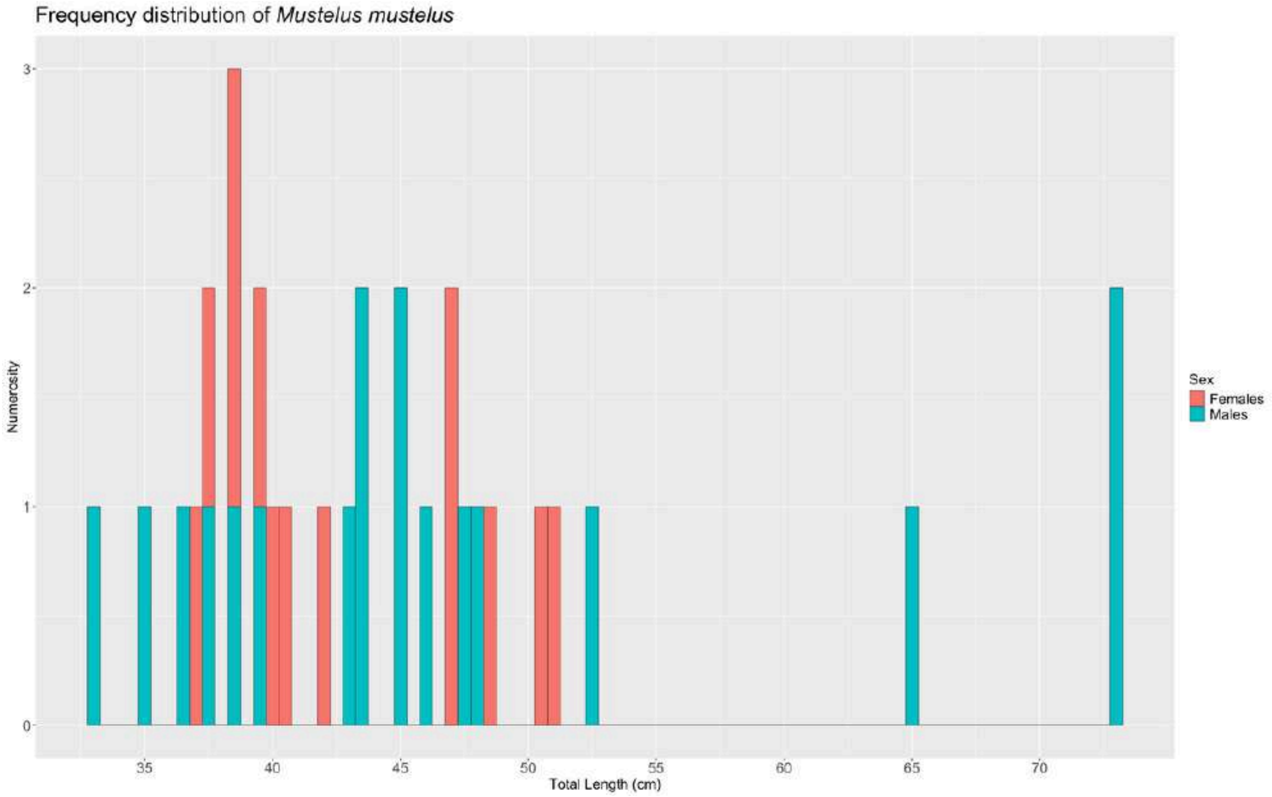


Figure 5: *Frequency distribution plot for the individuals belonging to M. mustelus sampled in 2025.*

For 9 individuals the ***short-term Post Release Mortality*** assessment was carried out by keeping individuals in tanks for 40 minutes, summing up to 36 individuals since 2022. During the stPRM assessment, the conditions of the different individuals seemed to remain stable over time (Figure 6). The 2 individuals that died during the assessment were placed in the tank in a bad condition (*Moribund*), for this reason the ***long-term Post Release Mortality*** of individuals that were classified as “moribund” was probably higher than the one accounted during the 40 minutes (Ellis et al. 2017).

BYCATCH MONITORING

RESULTS & DISCUSSION

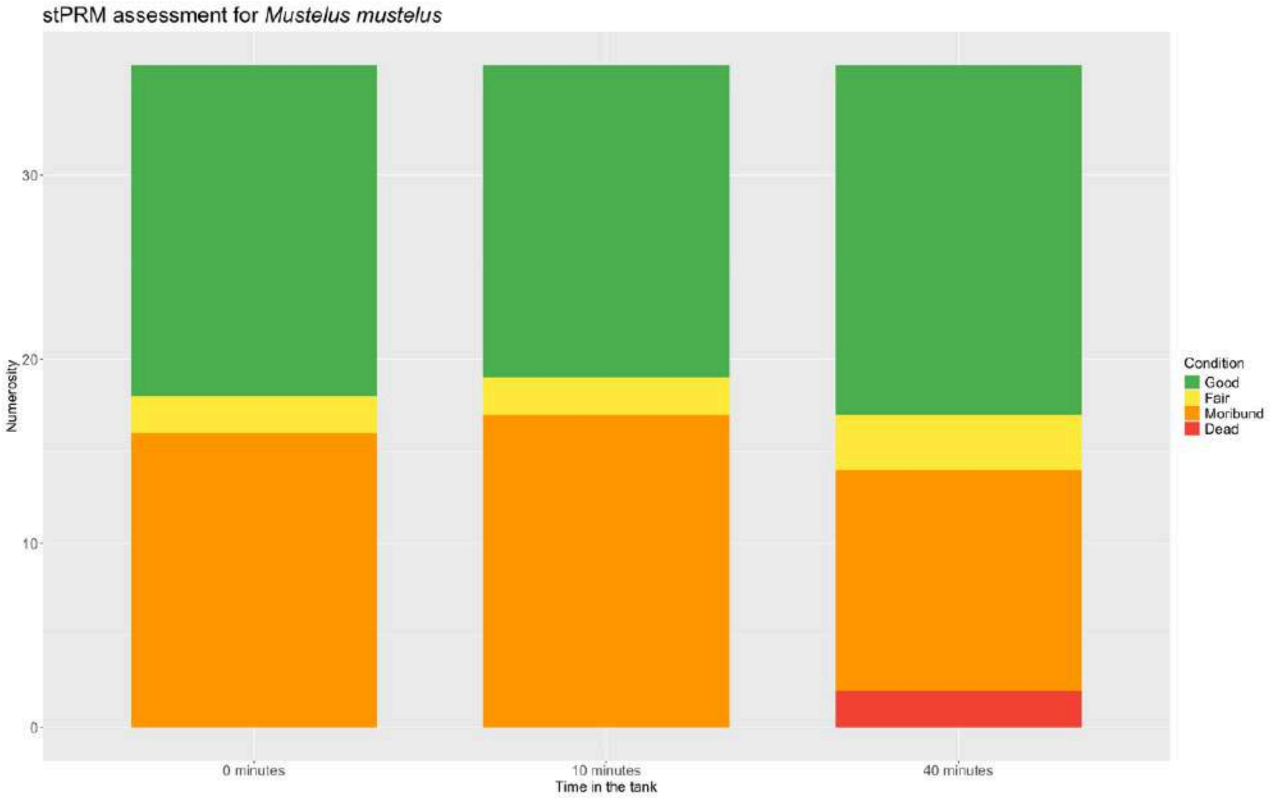


Figure 6: *Short-term Post Release Mortality assessment for M. mustelus (N = 36). Bars indicate the status at 0-minutes, 10-minutes and 40-minutes. Different colours indicate the conditions of the animals: green for Good, yellow for Fair; orange for Moribund and red for Dead.*

Regarding the tagging campaign, a total of 9 individuals were tagged with **conventional tags**, summing up to 65 since 2022. Among these individuals, 9 have been captured again (**13.8 % recaptured rate**), giving us further insights on their habitat use and long-term post release survival. The recapture rate is higher than the recapture rate found in literature for conventional tags, possibly explained by the enclosed nature of the gulf and the strong collaboration built with fishers over the years.

POPULATION GENETIC STRUCTURE

RESULTS & DISCUSSION

A total of 197 samples were obtained by different institutions working with *Mustelus mustelus*. In particular from iSea, a total of 75 samples were collected in Amvrakikos Gulf (from 2022 to 2024) thanks to the By ElasmoCatch Project. In addition to these, other 7 samples were collected in other regions of Greece (Northern Aegean Sea). Thanks to the collaboration with the University of Padova, 70 samples were collected for Common Smoothhound caught in the Northern Adriatic Sea. Samples were also obtained from partners working in the Canary Islands (Atlantic Ocean), for a total of 45 individuals. The samples were sent to the University of Padova in September 2024, when the genetic analysis also started, in collaboration with the laboratory of Professor Chiara Papetti.

DNA extraction was performed for all the samples of Amvrakikos and Aegean Sea, 30 samples of the Canary Islands, and 14 samples from the Northern Adriatic Sea. After DNA extraction, the genetic identification was carried out through mitochondrial markers. However, during this procedure, markers that were previously used to differentiate *M. mustelus* from *M. punctulatus* did not work for samples collected in Greece and in the Canary Islands due to a modification in the site where the marker should have bound. For this reason, delays in the analysis occurred. For the aforementioned reason, in this study, the genetic identification of the species did not took place. This did not create any bias in the analysis because individuals were identified at the species level thanks to morphological features.

All the samples were diluted at a concentration of 20 ng/µl and a polymerase chain reaction (PCR), a fast technique used to "amplify" small segments of DNA was performed, using microsatellite markers. A total of 17 microsatellite-loci were amplified for the analysis.

The products of the PCR were then sent to an external entity (BMR Genomics) for their genotyping.

POPULATION GENETIC STRUCTURE

RESULTS & DISCUSSION

Due to time and availability of the iSea member and laboratory technician, only 1 plate (containing 75 samples from Amvrakikos, 7 samples from the Aegean Sea and 14 samples from the Northern Adriatic Sea, total N = 96) was sent to genotyping. To increase the number of samples for the Northern Adriatic Sea and compare the results with another area of the Mediterranean Sea (Strait of Sicily), the results were compared to those obtained by Barbato et al. (2025). In order to allow comparisons between results a normalisation of the samples had to be carry out. For this reason two sample used by Barbato et al. had to be analysed with the same technique used during our study, and the two datasets had to be subsequently normalised through the program ALLELOGRAM. The total number of samples used was 75 for Amvrakikos Gulf; 7 for Northern Aegean Sea; 62 for Northern Adriatic Sea and 38 for the Strait of Sicily.

Analysis of data obtained started in September. Among the 17 loci used for the analysis, 2 had to be excluded from the analysis because 1 locus (**Mmu9**) was used to confirm the species as *M. mustelus* (and not *M. punctulatus*), and 1 locus (**Mmu5**) was in linkage with another locus. Considering locus that are in linkage would cause a bias in the analysis, as they are inherited together, creating a non-random association. The software Genepop was used to identify linkage between two or more loci.

For each of the 4 populations considered ***Oberserved (Ho)*** and ***Expected Heterozigosity (He)*** as well as ***probability of Hardy-Weinber Equilibrium (HWE)*** were calculated for each locus. The Ho is the proportion of individuals in a population that are heterozygous at a given genetic locus, and it is a direct measure of genetic variation, useful for identifying inbreeding, population bottlenecks or substract, so low Ho can indicate inbreeding, small population size or selective sweeps. The He is the probability that two alleles chosen randomly from the population are different, assuming the HWE (Weir, 1984; Hardy 1908).

POPULATION GENETIC STRUCTURE

RESULTS & DISCUSSION

The HWE is a model stating that allele and genotype frequencies will remain constant across generations with the following assumptions for the population: *i)* must have random mating; *ii)* no mutation; *iii)* no natural selection; *iv)* no gene flow (migration); *v)* large population size. Deviation from HWE can indicate inbreeding (with a lower heterozygosity); population structure or substructure; selection; genotyping errors or recent admixture (so mixture of two or more genetically distinct populations in the last few generations). Ho < He can indicate possible inbreeding or population subdivision; Ho > He can indicate possible heterozygote advantages, negative assortative mating with individuals that prefer mating with more dissimilar individuals; Ho ~ He indicates population close to Hardy Weinberg expectations and a locus with no signs of selection and consistent across populations (Weir, 1984; Hardy 1908). The software Arlequin was used to calculate Ho and He; while Genepop was used to calculate HWE.
Mean Ho and He was calculated for each population, resulting in samples from the **Amvrakikos Gulf** having the a **Ho < He**, and the **lowest Ho and He in respect to the other populations**, supporting the hypothesis of the **population** to be **isolated in respect to the other populations** found **in the Mediterranean Sea**.

Pairwise F_{ST} and correspondent p-values were then calculated for each population to estimate genetic differentiation between them, using Arlequin. The higher the F_{ST}, the more the populations are differentiated. In particular F_{ST} values between 0.00 and 0.05 indicates little differentiation, values between 0.05 and 0.15 indicates moderate differentiation, between 0.15 and 0.25 strong differentiation and > 0.25 very strong differentiation or 2 different species (Wright 1978). Individuals of Amvrakikos Gulf had the highest F_{ST} (Table 2), in particular the highest value was found with individuals from the Strait of Sicily. All p-values were lower 0.001 proving significance of the results obtained.

POPULATION GENETIC STRUCTURE

RESULTS & DISCUSSION

F_{ST} values	Northern Adriatic Sea	Strait of Sicily	Northern Aegean Sea	Amvrakikos Gulf
Norther Adriatic Sea				
Strait of Sicily	0,04486			
Northern Agean Sea	0,05725	0,07971		
Amvrakikos Gulf	0,12869	0,15031	0,10877	

Table 2: *F_{ST} values found for the individuals of the 4 populations investigated.*

F_{ST} values are further confirmed by the AMOVA (Arlequin software) in which the F_{ST} was equal 0.11696, with a p-value equal to 0.

STRUCTURE software was also used in order to cluster the groups and get information on populations structure and how many populations exit in the sample. The software groups individuals into an assigned number of genetic clusters (K) based on their DNA. STRUCTURE was run with K ranging from 1 to 10 (Figure 7) (Evanno et al. 2005). With $K = 2$, it was possible to identify two clearly differentiated groups, with individuals from the Northern Adriatic Sea, the Strait of Sicily and the Northern Aegean Sea clustering together, while individuals of Amvrakikos Gulf grouped separately. When considering higher some substructure emerged in the Strait of Sicily and some background noise in the different groups. The $K = 2$ was supported also by the mean similarity score and the likelihood which values decreased moving on higher K.

The different analysis carried out strongly support the hypothesis that Amvrakikos individuals are isolated in respect to other individuals found in the Mediterranean Sea. This study supports the presence of a genetic structure between these four areas of the Mediterranean. Results of genetic differentiation must be taken into consideration in the fishery management of these stocks, tailoring specific strategies that consider also possible differences in growth rates, reproduction and nursery areas as well as fishing pressures.

POPULATION GENETIC STRUCTURE

RESULTS & DISCUSSION

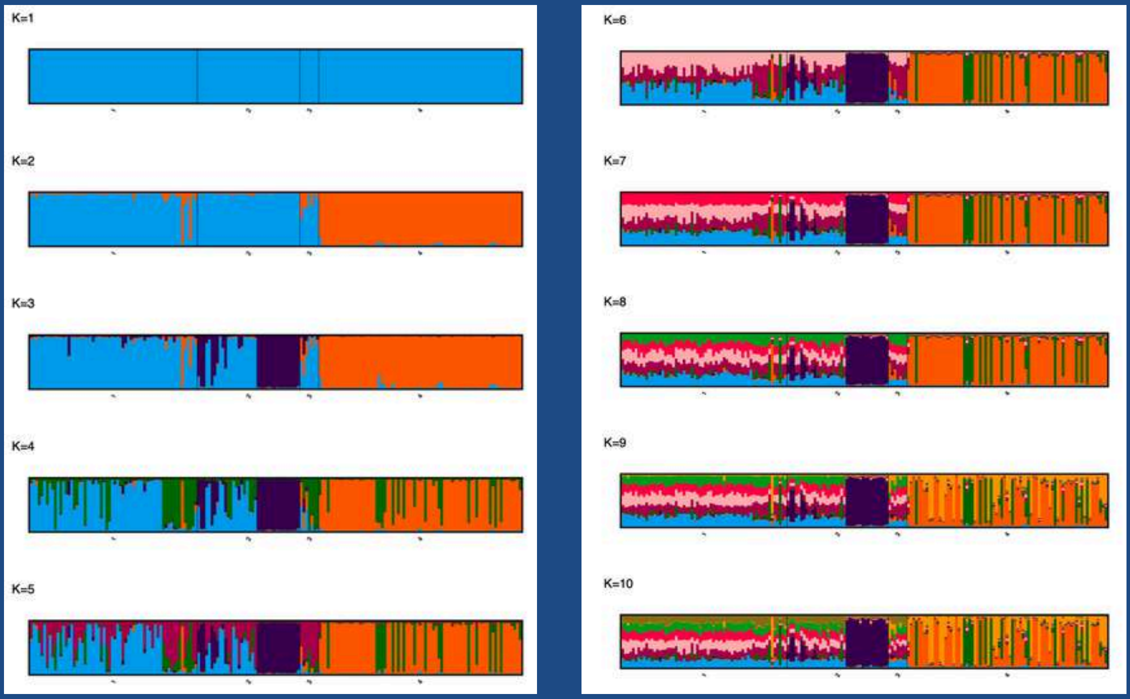
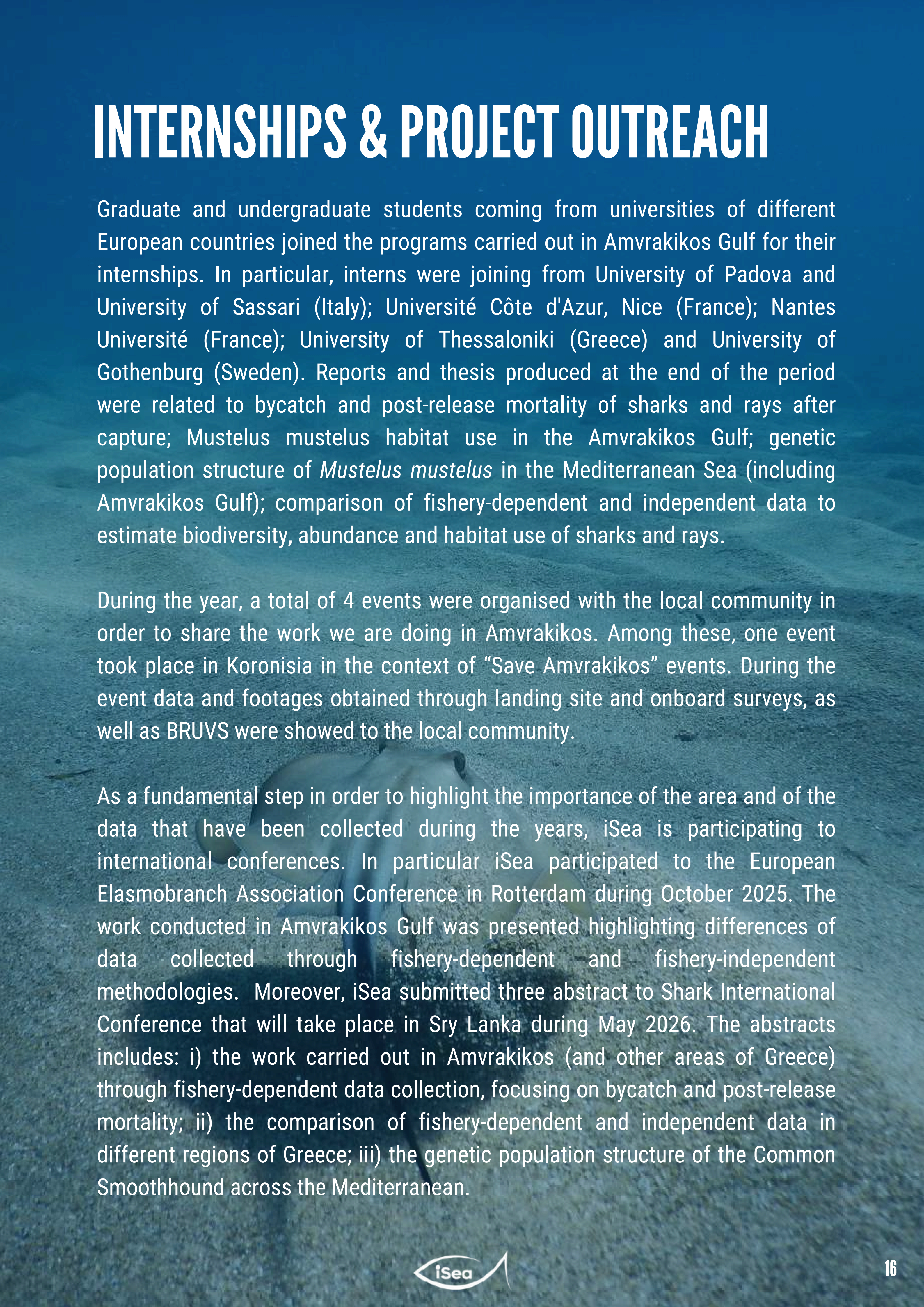


Figure 7: *Structure outputs depending on the different K analysed. Numbers on the bottom of each plot indicate the different populations (1 for Adriatic Sea; 2 for Strait of Sicily; 3 for Aegean Sea; 4 for Amvrakikos Gulf). K = 2 highlight the population of Amvrakikos (orange) as separated in respect to the others (light blue).*

Focusing on our study area, Amvrakikos Gulf, small-scale fishing activities are seasonally targeting *M. mustelus*, and captures of pregnant females have been recorded. In addition to targeted fishery, newborns and young-of-the-year are captured as bycatch during late spring and summer months. As sharks are K-selected species, fishery-associated mortality can lead to a sharp decline of the population. Specifically, in the case of nursery areas, as Amvrakikos Gulf (ISRA factsheet, 2023), this leads to a loss of fertile individuals that support the growth of the population (Conrath & Musick, 2012) and to *recruitment overfishing*, meaning that new generations are caught before reaching the adult population and being able to reproduce (Heupel et al. 2007).

In conclusion, management strategies such as banning of targeted fishery of Common Smoothhound and bycatch mitigation strategies (such as release and bycatch reducing devices such as magnets and LEDs) should be prioritised in the area to prevent local extinction of the species.

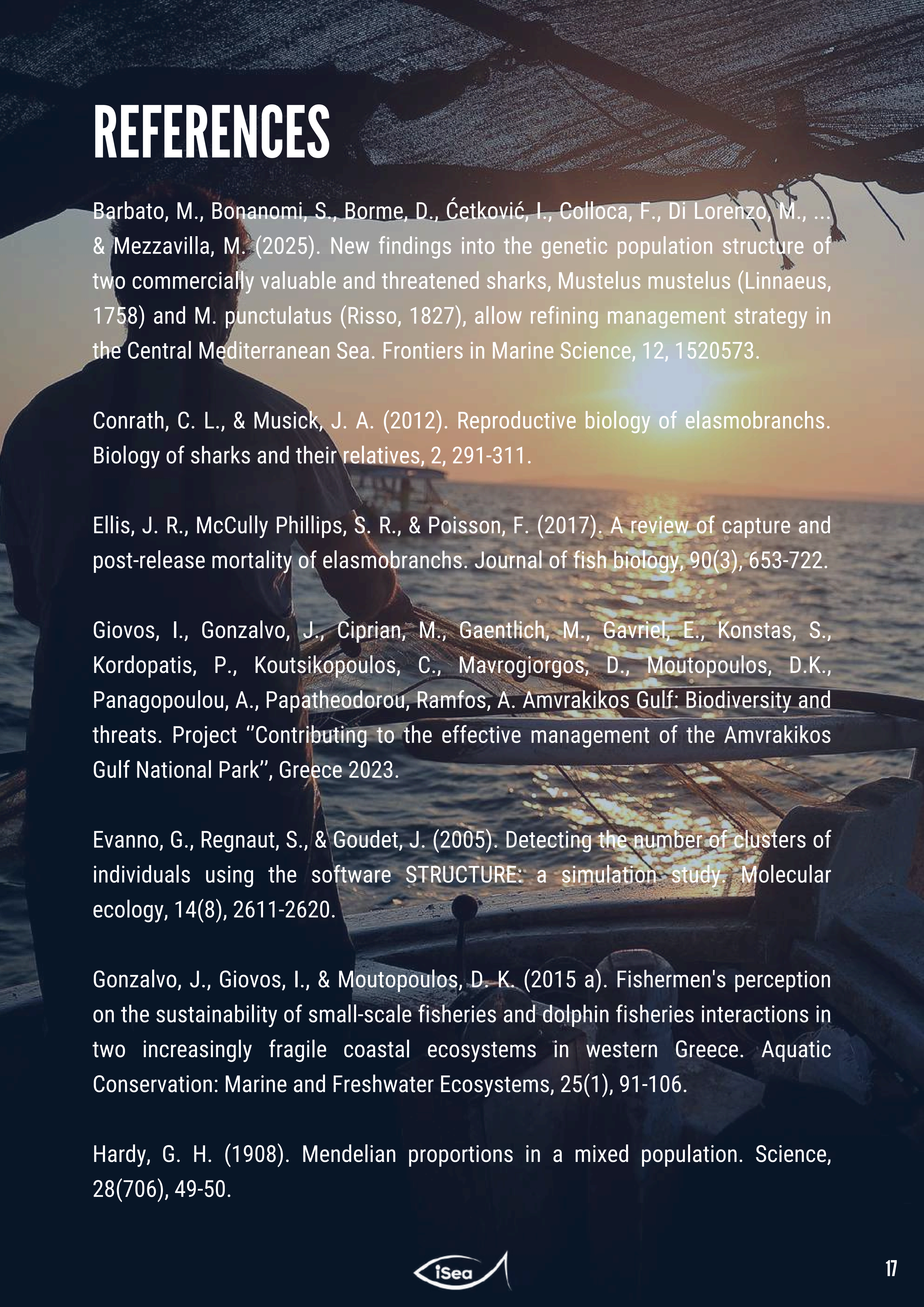


INTERNSHIPS & PROJECT OUTREACH

Graduate and undergraduate students coming from universities of different European countries joined the programs carried out in Amvrakikos Gulf for their internships. In particular, interns were joining from University of Padova and University of Sassari (Italy); Université Côte d'Azur, Nice (France); Nantes Université (France); University of Thessaloniki (Greece) and University of Gothenburg (Sweden). Reports and thesis produced at the end of the period were related to bycatch and post-release mortality of sharks and rays after capture; Mustelus mustelus habitat use in the Amvrakikos Gulf; genetic population structure of *Mustelus mustelus* in the Mediterranean Sea (including Amvrakikos Gulf); comparison of fishery-dependent and independent data to estimate biodiversity, abundance and habitat use of sharks and rays.

During the year, a total of 4 events were organised with the local community in order to share the work we are doing in Amvrakikos. Among these, one event took place in Koronisia in the context of “Save Amvrakikos” events. During the event data and footages obtained through landing site and onboard surveys, as well as BRUVS were showed to the local community.

As a fundamental step in order to highlight the importance of the area and of the data that have been collected during the years, iSea is participating to international conferences. In particular iSea participated to the European Elasmobranch Association Conference in Rotterdam during October 2025. The work conducted in Amvrakikos Gulf was presented highlighting differences of data collected through fishery-dependent and fishery-independent methodologies. Moreover, iSea submitted three abstract to Shark International Conference that will take place in Sry Lanka during May 2026. The abstracts includes: i) the work carried out in Amvrakikos (and other areas of Greece) through fishery-dependent data collection, focusing on bycatch and post-release mortality; ii) the comparison of fishery-dependent and independent data in different regions of Greece; iii) the genetic population structure of the Common Smoothhound across the Mediterranean.



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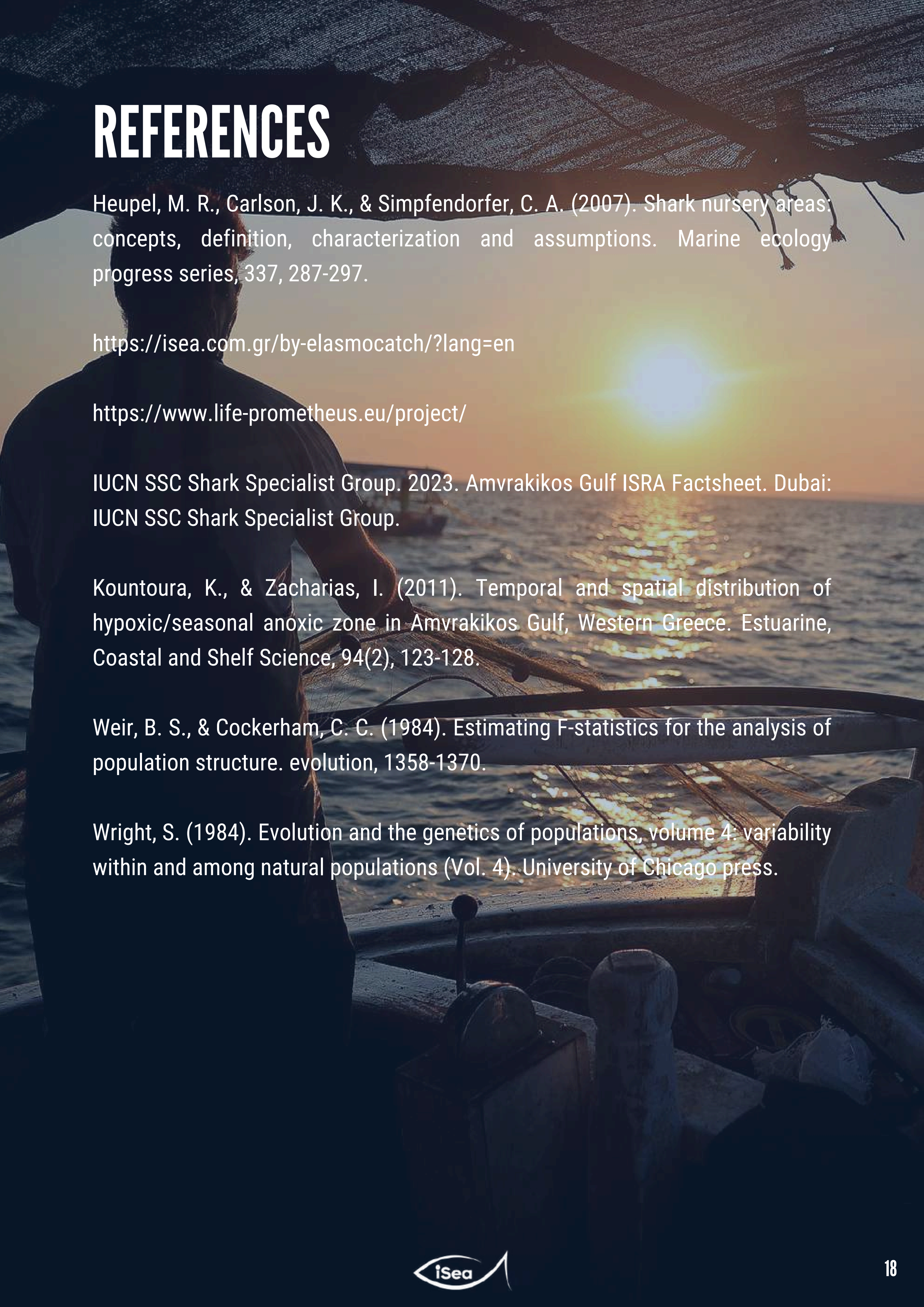

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