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Unveiling Elasmobranch aggregations in the Mediterranean Sea: behavioral, ecological and spatial association insights

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General Overview of the Thesis

Sharks, rays and skates belong to the sub-class of the elasmobranchs, a taxonomic group among the most threatened marine vertebrates worldwide (Dulvy et al., 2014). Like in other marine regions, elasmobranchs are experiencing a significant decline in population in the Mediterranean Sea due to intense anthropogenic pressures, especially unmanaged fisheries which can act both directly with target fishing and indirectly via accidental catches (Walls & Dulvy, 2021) and the depletion of their prey resources. In addition to this, the survival of shark and ray species can be threatened by other drivers, including habitat degradation, pollution, climate change and the loss of critical areas such as breeding and nursery grounds (Dulvy et al., 2024). As all these pressures often act simultaneously and on different spatial scales, identifying where and when shark and ray individuals aggregate can reveal important patterns of vulnerability and resilience to human threats, thus providing a fundamental basis for conservation and management actions. Shark and ray aggregations are temporary gatherings of multiple individuals in specific areas and times, often associated with key biological functions such as reproduction, feeding, parturition, or social interactions (McInturf et al., 2023). These gregarious behaviors may expose grouped individuals to increased vulnerability when overlapping with human activities, even inside marine protected areas (Di Lorenzo et al., 2022; Palacios et al., 2023). Despite their biological importance, Mediterranean elasmobranch aggregations are poorly reported, particularly in terms of structure, drivers, ecological meanings and vulnerability to anthropogenic stresses. This thesis aims to contribute to filling this gap of knowledge by exploring the spatial and temporal dynamics of shark and ray aggregations in the Mediterranean Sea through a multi-scale and multi-source approach. A combination of systematic literature review, citizen science data, field observations, and long-term fishery-independent datasets was employed to investigate different aspects of elasmobranch aggregations, ranging from broad-scale patterns to species-specific case studies.

Accordingly, this thesis is structured into five chapters, each addressing a specific aspect of elasmobranch aggregations in the Mediterranean Sea.

Chapter 1 provides a comprehensive synthesis and classification of the available information on elasmobranch aggregations across the entire basin. By identifying spatial, seasonal, and

ecological patterns, this chapter outlines the current state of knowledge on these phenomena and highlights existing research gaps.

Chapter 2 focuses on the critically endangered common eagle ray (*Myliobatis aquila*), characterizing its aggregation behavior through the integration of published data, social media records, and field observations conducted within a Marine Protected Area in the southern Mediterranean.

Chapter 3 reports new evidence of a reproductive aggregation site for the common stingray (*Dasyatis pastinaca*), underlining the ecological and conservation importance of coastal habitats that serve as critical areas for reproduction and parturition.

Chapter 4 explores ecological aspects at a well-known sandbar shark aggregation site around Lampione Island (Southern Sicily, Italy), contributing to a broader understanding of how shark aggregations may facilitate interspecific interactions (i.e. fish predation), thus potentially affecting ecological dynamics of communities at local scale.

Finally, Chapter 5 investigates the spatial structure of aggregations across demersal elasmobranch species, sexes, and life stages, by analysing thirty years of standardized trawl survey data and applying spatial co-occurrence metrics to reveal long-term and multi-species patterns.

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Chapter 1: Dynamics and Patterns of Mediterranean Elasmobranch Aggregations: A Systematic Review

Abstract

Spatio-temporal patterns of elasmobranchs aggregations in the Mediterranean Sea are poorly documented, with implications for designing effective conservation and management actions aimed at reducing their population decline. This study systematically reviews 225 scientific papers, identifying 28 aggregations and 116 potential aggregations of sharks and rays across the region. Although the biological drivers behind most of these events are of unknown nature, reproduction and feeding are the main purposes occurring in the understood events. Aggregations are most frequently reported in spring and summer, suggesting a higher risk of overlapping with human activity. Preferred shallow habitats are soft-bottoms, rocky areas, seagrass meadows and brackish environments, while seamounts and deep-water (i.e. cold-water) coral reefs are underreported. Most events occur in epipelagic waters (0–200 m), which may reflect both biological preferences and methodological constraints in sampling deeper habitats. Current knowledge on Mediterranean elasmobranch aggregations derives from fishery data, addressing fishery-related aspects, with limited attention to species' ecology, genetics and movement patterns. Research efforts vary across Mediterranean Geographical Sub-Areas as established by the General Fishery Commission of the Mediterranean and Black Seas, with some regions hosting multiple aggregations but lacking continuous scientific monitoring. Given the increasing anthropogenic pressures on marine ecosystems and the ongoing decline of Mediterranean elasmobranch populations, understanding aggregation dynamics is essential for the identification and protection of critical habitats. This review emphasizes the need for enhanced research methodologies, extended spatial monitoring and the development of adaptive, site-specific management strategies to mitigate human-wildlife conflicts, particularly in Mediterranean elasmobranchs' aggregations.

1. Introduction

Elasmobranch aggregations are defined as the co-occurrence of two or more individuals of the same species sharing resources due to a common driver (McInturf et al., 2023). These events are mostly guided by feeding or reproductive cues and supported by a favorable mix of environmental conditions (Crowe et al., 2018; Francis et al., 2015; García et al., 2008). Aggregations may occur in shallow coastal waters, which offer suitable habitats for early-life stages of sharks and rays (Barbato et al., 2023; Grancagnolo et al., 2023) or close to seamounts in the open ocean, where large pelagic species might use this habitat for foraging or stopover during long-distance migrations (Morato et al., 2016; Morato et al., 2008; Weber et al., 2025). Human activities, such as habitat degradation and fishery exploitation, impact both coastal zones and offshore seamounts, impairing the occurrence and the temporal stability of aggregations performed by threatened species (Couturier et al., 2013; Croll et al., 2016; Hall and Roman, 2013). In the Mediterranean Sea, many shark and ray species have experienced a significant population decline and risk of extinction over the past decade (Dulvy et al., 2021; Walls & Dulvy, 2021). This vulnerability is exacerbated by a combination of unsustainable targeted fishing and bycatch, along with gregarious behaviors and K-selected life history traits (late sexual maturity, low fecundity, slow growth rate) (Cattano et al., 2021; Croll et al., 2016; Ferretti et al., 2008). Identifying environmental factors driving aggregations may enhance the ability to locate and predict critical habitats, reducing the risk of mass capture while supporting the design of effective conservation strategies (Jacoby et al., 2012). The socio-cultural heterogeneity and the long history of exploitation of the Mediterranean Sea have been proposed as a limit to the implementation of unified conservation regulations (Badalamenti et al., 2000; Gómez & Maynou, 2020; Pipitone et al., 2014). Despite the presence of Marine Protected Areas (MPAs), which are designed to regulate human activities and protect habitats and species of ecological importance, Mediterranean Partially Protected Areas, that are zones within MPAs where some forms of fishing are permitted, record higher captures of endangered and immature elasmobranchs than unprotected areas (Morey et al., 2006; Di Lorenzo et al., 2022). This conservation paradox highlights the shortcomings of static spatial management tools in addressing the needs of highly mobile species like many elasmobranchs (Grantham et al., 2011; Hyrenbach et al., 2000). Moreover, aggregation dynamics often require species-specific context-dependent management approaches that may diverge from

traditional regulatory frameworks (Chin et al., 2023). Effective management of aggregating species must account for the temporal predictability and site fidelity of aggregations (Speed et al., 2010). For instance, reproductive or nursery aggregations may demand highly restrictive closures (Heupel et al., 2007), while feeding aggregations could be managed through seasonal or gear-specific regulations (Casselberry et al., 2024). Therefore, adaptive, spatially explicit, and species-tailored strategies are essential to ensure the protection of critical aggregation sites (Lea, 2017). In that context, this study aimed to develop and apply a standardized framework for assessing and categorizing literature-based records of elasmobranch occurrences in the Mediterranean Sea. Using available scientific publications, we provided a method to distinguish between aggregations, potential aggregations, and single occurrences based on ecological, spatial, and temporal criteria. When information was available, we classified the biological drivers behind each aggregation event (e.g., reproduction, feeding, migration) and examined seasonality, habitat preferences and bathymetric distributions to describe key factors influencing aggregation formations. Finally, we focused on research topics, methodologies and data sources, to highlight thematic and knowledge gaps across Geographical Sub-Areas (GSA) as established by the General Fishery Commission of the Mediterranean and Black Seas (GFCM), that may hinder effective conservation and management of elasmobranchs aggregations in the Mediterranean Sea.

2. Materials and Methods

2.1 Study Area

The Mediterranean Sea is a semi-enclosed basin comprising multiple marine sub-regions, each characterized by distinct oceanographic dynamics and thermal regimes that contribute to shaping its complexity of species and habitats (Coll et al., 2010) (Fig. 1). The Alboran Sea, in the westernmost end of the basin, has strong biological and physical connectivity with the Atlantic Ocean due to the continuous inflow of waters through the Strait of Gibraltar (Harmelin & d'Hondt, 1993). The Gulf of Lions and Ligurian Sea are among the coldest areas of the western Mediterranean with strong upwelling induced by northern winds that contribute to seasonal productivity pulses (Millot, 1979). The Tyrrhenian Sea has a high mean surface temperature all year round due to its sheltered position, which limits atmospheric mixing and isolates the basin from extreme meteorological conditions (Astraldi et al., 1995). Between the

Balearic Islands and Sardinia, waters are oligotrophic with the lowest productivity levels within the Western Mediterranean Basin (Bosc et al. 2004; Basterretxea et al. 2018). The Strait of Sicily acts as a transitional zone where eastward Atlantic currents interact with westward-flowing Levantine Intermediate Water, generating complex hydrographic dynamics (Schroeder et al., 2017; Sorgente et al., 2011). The area is considered a hotspot of turbulent mixing due to the interactions of the complex bottom topography and water mass circulation, creating local upwellings around banks and seamounts that support high levels of biodiversity (Ferron et al., 2017; Vladoiu et al., 2018). The Northern Adriatic Sea is characterized by seasonal variability, especially during winter a strong winter northeast wind (locally known as "Bora") promotes deep-water formation and vertical mixing (Jeffries & Lee; 2007; Raicich et al., 2013). The influence of the freshwater inputs from the Po River results in low salinity and high productivity conditions that sustain structured trophic webs (Beg Paklar et al., 2005). This area is among the most intensively exploited fishing grounds in the Mediterranean (Barausse et al., 2009; Colloca et al., 2017; Fortibuoni et al., 2017). In contrast, the central and southern Adriatic are deeper and act as transitional zones connecting the Adriatic and Ionian Seas (Manca et al., 2002). The Levantine Sea, at the easternmost area of the basin, is characterized by high evaporation rates that exceed freshwater inputs, resulting in the warmest, most saline and oligotrophic sub-region in the Mediterranean (Krom et al. 1991; Tanaka et al. 2007). The downwelling of highly saline surface waters contributes to the formation of the Levantine Intermediate Water, which spreads westward across the entire basin and exits through the Strait of Gibraltar (Bigg et al., 2003; Lascaratos et al., 1999; Ozsoy et al., 1989). The connection with the Red Sea through the Suez Channel has facilitated the introduction of tropical non-native species, altering biodiversity patterns (Edelist et al., 2013). Along the coasts of Algeria and northern Tunisia, Atlantic-origin species are progressively reduced in richness and abundance from west to east (Bianchi et al., 2012). In the southern and northern Aegean Sea, the overall biodiversity levels are lower compared to the western basin, and the influence of Atlantic-Mediterranean biota is minimal (Bianchi et al., 2012).

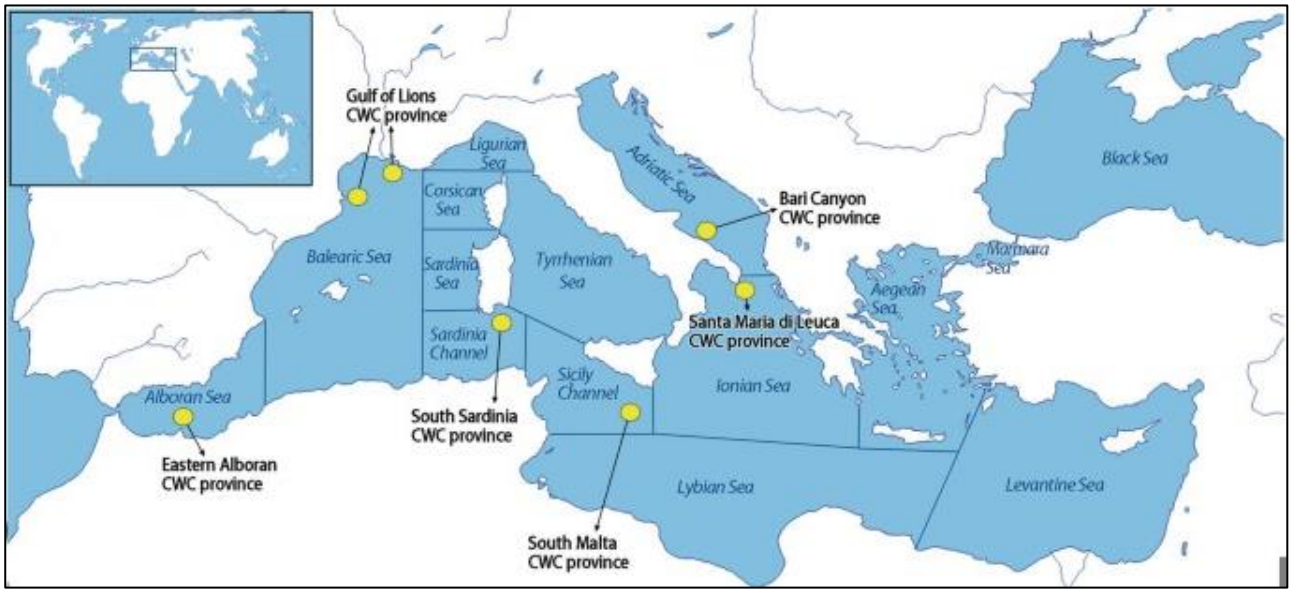


Figure 1. Main sub-basins of the Mediterranean Sea (Orejas & Jiménez, 2019).

2.2 Data collection

The literature search and screening process followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; www.prisma-statement.org), adopting criteria for inclusion and exclusion to guarantee transparency, reproducibility and generalizability in study selection. Bibliographic research was conducted using Google Scholar, Scopus and ResearchGate, constraining the queries to be matched in "Title, Abstract, author keywords" and using as search strings the following keywords: "Mediterranean" and "Elasmobranchs" or "Chondrichthyes" or "Sharks" or "Rays" and "Aggregation". When applicable, the subject area selection was limited to "Environmental Science", "Agricultural and Biological Sciences" and "Earth and Planetary Sciences". Grey literature and materials provided for the identification of Important Shark and Ray Areas (ISRAs) (Kyne et al., 2023; https://sharkrayareas.org/), in the Mediterranean Sea were also included in the analyses since the establishment of ISRAs was reviewed by a panel of scientists and experts. Research articles were used to classify the reported events as aggregations, potential aggregations or single occurrences. Although some macro-areas (i.e., Gulf of Gabes) are known to host multiple aggregation sites for both sharks and rays, no studies have reported mixed aggregations (i.e., simultaneous occurrences of both groups at the same time and location). In such cases, events were treated and classified separately for each species or species occurring within the same broader area. For the purpose of this study, an aggregation was defined by

the matching of four criteria: (1) high abundance, when more than two individuals were observed or captured at the same time, as indicated by McInturf et al., 2024; (2) high occurrence, when abundance data were unavailable, but the species was repeatedly caught or sighted; (3) site fidelity, high occurrence or high abundance recorded only in a specific area; (4) seasonality, for a seasonal pattern in captures or observations. The term observation refers to any empirical evidence obtained through methodologies providing verified detections of species presence and abundance over space and time, including visual records (e.g., underwater, aerial, or video-based observations), tagging data and capture or survey records. Model-based predictions were excluded because they do not represent direct observations of individuals, while environmental DNA (eDNA) records were not considered suitable to detect confirmed aggregations, as they provide information on species presence but not on abundance or the number of individuals. Potential aggregations were defined when at least two criteria were satisfied and no aggregations for events that met only one criterion (Fig. 2). Following McInturf et al. (2024), an aggregation was defined as the grouping of two or more conspecific individuals for a specific purpose within a bounded space and time, since co-occurrence of different species may reflect distinct ecological or behavioral drivers. Information from single occurrences was excluded and was not considered for further analysis.

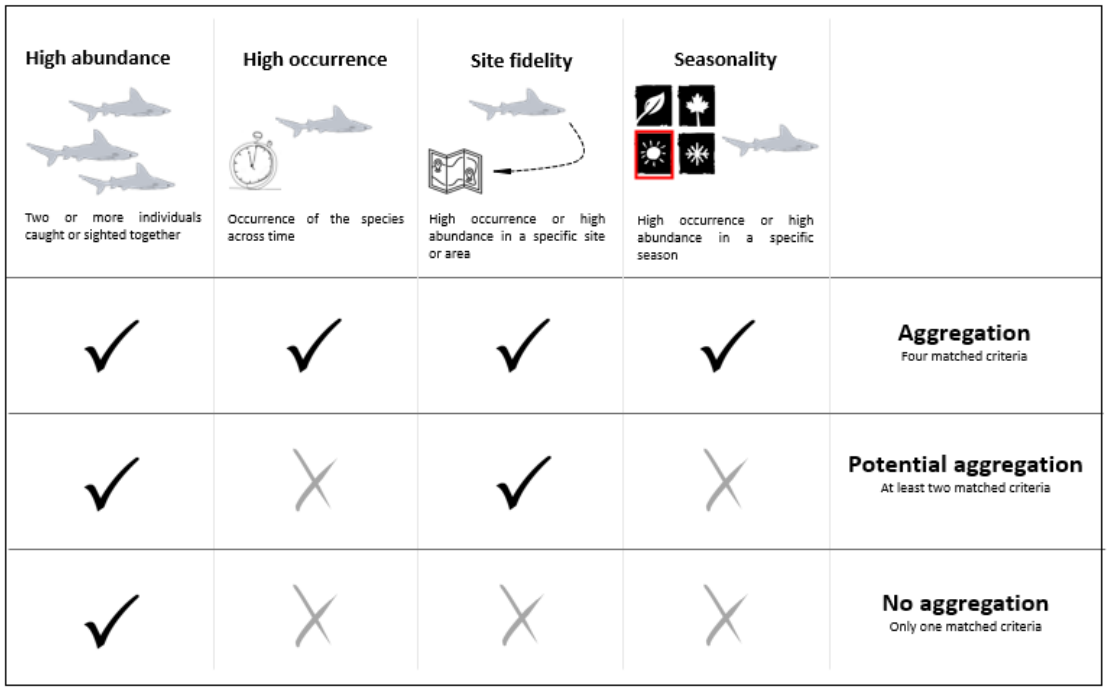


Figure 2. Criteria used to classify papers and assess aggregations, potential aggregations and single occurrences.

For each research article, information was noted about the species, study area (then grouped according to their corresponding General Fisheries Commission for the Mediterranean - GFCM - Geographical Sub-Areas - GSAs), habitat type, depth, research topic, and data collection methodologies. Within the ISRA framework, "C Criterion" refers to "Aggregation Areas", defined as sites where sharks or rays gather for key biological purposes such as mating, parturition, feeding, resting, or seasonal movements (Kyne et al., 2023). The "C Criterion" provided by the ISRA framework was used both for aggregations and potential aggregations to categorize reproduction, resting, movement, feeding, or unknown events. According to available depth information, aggregations and potential aggregations were assigned to the epipelagic zone (from 0 to 200 m), the mesopelagic zone (from 200 to 1000 m) and to the bathypelagic zone (more than 1000 m). Habitats were classified in sand/mud, cold coral reefs, seagrass meadows, brackish waters, artificial habitats (mussel farms, power plants, tuna farms), seamounts, channels (including canyons and ridges) and rocky outcrops. Research topic categories were classified as: (i) "Biology": growth, development, reproduction and physiology; (ii) "Conservation and Management": policies, management measures, Marine Protected Areas (MPAs), and mitigation strategies; (iii) "Ecology": interspecific interactions, diet, and behavior; (iv) "Habitat Use" spatial distribution and environmental drivers; (v)"Record": checklists and single observations; (vi) "Fishery": impacts of fishing, stock assessment, population trends, bycatch, and alternative gears; (vii) "Genetics": phylogeny, molecular taxonomy. Methodologies were categorized into: (i) "Fishery-dependent data": data derived from commercial or experimental fisheries; (ii) "Stomach content and isotopes": techniques used for trophic ecology studies; (iii) "Aerial, BRUVs, and UVC": grouped as non-invasive direct observation techniques for abundance estimation; (iv) "LEK and Citizen Science": community-based approaches for monitoring spatial and temporal occurrence; (v) "Models": statistical and computational approaches for species distribution predictions and environmental parameter assessments; (vi) "Microbiome": microbial community analysis in sharks and rays for ecological investigations; (v) "Tags": conventional, acoustic, data loggers, and satellite tags for movement and habitat use studies.

2.3 Data analysis

Statistical analyses were grouped according to two main objectives:
(1) to assess differences in the occurrence and characteristics of aggregation events across taxa, habitats, and environmental factors; and
(2) to evaluate study characteristics and research efforts across the Mediterranean Sea. In all ecological analyses (aggregation purpose, seasonality, habitat and depth), the response variable was the number of aggregation events, each representing an independent occurrence as reported in the reviewed literature. When a single publication described multiple events, each was treated as a separate record. Analysis related to research topics, methods and effort were based on the number of published papers.

2.3.1 Aggregation occurrence and ecological patterns

Differences in the number of events reporting aggregations, potential aggregations and single occurrences between sharks and rays were tested using Chi-squared tests (χ^2 $p<0.05$), with post-hoc pairwise comparison adjusted using Bonferroni correction. A Separate Chi-squared test was performed within sharks and rays to evaluate intra-group differences. After establishing that no significant differences existed between sharks and rays in the overall number of aggregation events, the dataset was analyzed jointly for all elasmobranchs. This approach prevented data over-fragmentation, ensuring sufficient sample sizes and statistical robustness across analyses. Aggregation purposes, categorized according to the ISRA criterion C (feeding, movement, reproduction, resting and unknown), were analyzed separately for aggregations and potential aggregations. The response variable considered was the number of aggregation events assigned to each category. Proportions were expressed as percentages and tested using a Likelihood Ratio G-test, dealing with expected values lower than five. Fisher's Exact test with Bonferroni correction was applied post-hoc to account for low-frequency categories and control Type I errors. Seasonal variation in aggregations and potential aggregations was tested through Chi-squared tests with Bonferroni adjusted post-hoc correction for multiple comparisons, based on the number of recorded events per season. Differences among habitats were based on the number of events for each habitat and were assessed using exact Chi-square test (Monte Carlo simulation, $p<0.05$) and pairwise Fisher's

Exact test with Bonferroni corrections to account for data with small sample sizes and to control the risk of Type I errors. Bathymetric preferences (epipelagic 0- 200 m; mesopelagic 200 – 1000; bathypelagic >1000) were based on the number of events in each depth range and were evaluated separately with Exact Binomial test verifying the null hypothesis of uniform distribution across depths strata (probability of aggregations or potential aggregations in each zone of 1/3). A 95% confidence interval was estimated to describe the range of presence probabilities.

2.3.2 Research effort and study characteristics

Differences among research topics and methodological approaches were evaluated using Chi-squared tests and Bonferroni corrected post-hoc Pairwise test, using the number of published papers as the response variable. To explore spatial patterns of research effort and aggregation occurrence, a Principal Component Analysis (PCA) was performed on z-score transformed data (number of aggregations, aggregated species and published papers per GSA). Only principal components with eigenvalues > 1 were retained for interpretation. A Hierarchical Clustering analysis (Ward's method, Euclidean distance) identified GSA groups with similar patterns and the optimal number of clusters was determined using the Silhouette method. GSAs clusters were visualized in a dendrogram. The same analysis was repeated for potential aggregations. Research effort was quantified as the number of publications reporting confirmed or potential aggregation records within each GSA (n = 131 studies), representing the spatial distribution of aggregation-focused research across the Mediterranean. The variability across GSAs in the number of events, publications and species performing gregarious behaviors was separately illustrated in three separate maps for aggregations and potential aggregations. All statistical analyses were performed in R (R Core Team, https://cran.r-project.org) and the spatial mapping and visualization of aggregation and potential aggregation events were conducted in QGIS 3.28.15 (QGIS Development Team, 2024. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org").

3. Results

3.1. General overview

A total of 246 research papers were retrieved from the reference list, complemented by 27 additional sources identified through bibliographic searches on Scopus, ResearchGate and Google Scholar. 19 documents were excluded due to irrelevance (based on title and abstract content) or because they were not available online (e.g., unavailable technical reports). The remaining 254 articles were reviewed to confirm aggregations and collect data for event characterization. Of these, 142 papers were excluded from further analysis as they described only single occurrences (271 total events: 130 involving sharks and 141 rays). The remaining 131 studies reported 28 confirmed aggregations (13 involving sharks and 15 involving rays) and 116 potential aggregations (70 for sharks and 46 for rays). The overall screening and selection process followed the PRISMA framework and is summarized in Fig. 3.

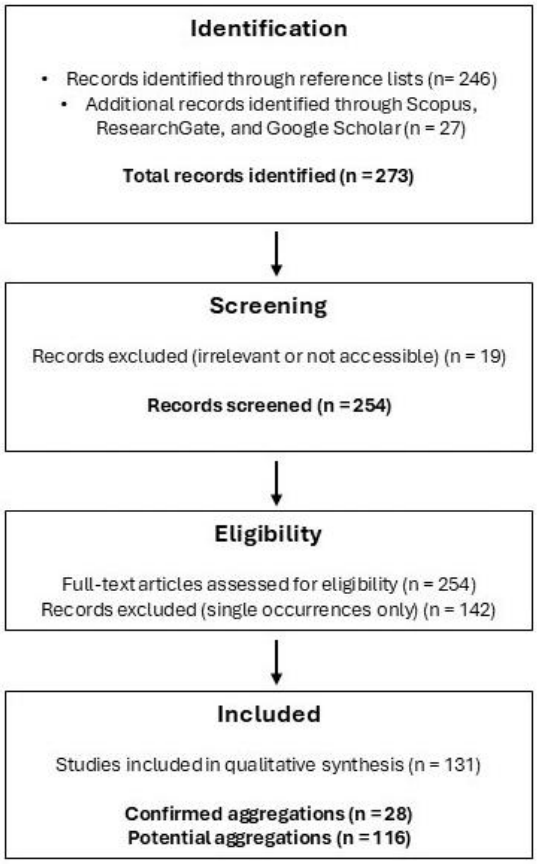


Figure 3. PRISMA flow diagram summarizing the identification, screening and inclusion of studies in the systematic review on shark and ray aggregations in the Mediterranean Sea.

Significant differences were found among the number of aggregations, potential aggregations, and simple occurrences (χ^2, $p < 0.05$). Post-hoc pairwise comparisons with Bonferroni correction confirmed significant differences between each category, with single occurrences

being the most frequently reported ($p < 0.05$). No significant differences were observed between sharks and rays within any of the three categories ($p > 0.05$) (Fig. 4).

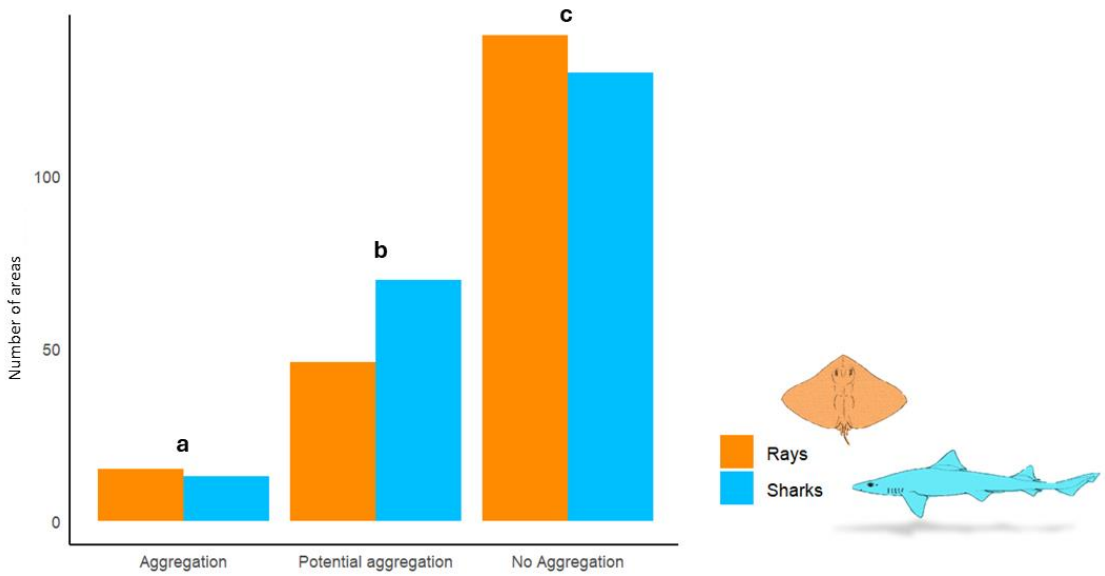


Figure 4. Comparison between the number of areas with aggregations, potential and occurrence of sharks and rays in the Mediterranean Sea. The histogram shows the number of confirmed aggregations, potential aggregations, and non-aggregated occurrences of sharks (in blue) and rays (in orange) in the Mediterranean. Letters indicate the results of the post-hoc pairwise comparison test.

3.2 Aggregation purposes

Among aggregation events, 43% were of unknown nature, 40% were for reproductive purposes and 14% for feeding (Fig. 5A). For 55% of potential aggregations, purposes were not understood, 30% were for reproduction and 13% for feeding (Fig. 5B). Resting aggregations were not detected in aggregations nor in potential aggregations.

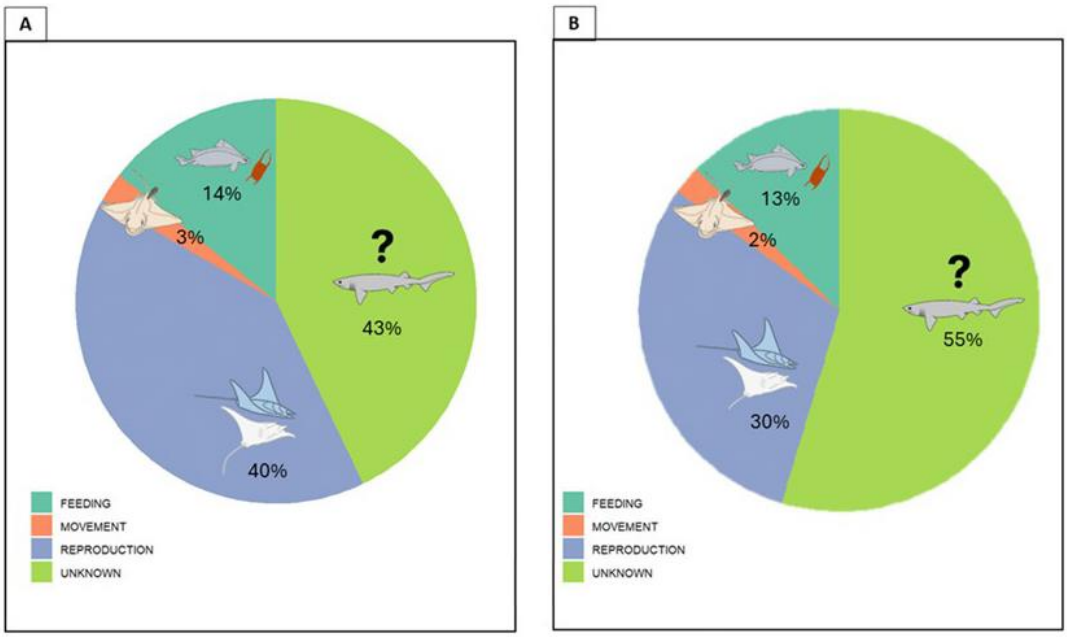


Figure 5. Proportion of aggregation types in the Mediterranean Sea: proportion of aggregation types in aggregations (A) and potential aggregations (B)

Aggregation purposes significantly differed among categories (G-test, $p < 0.05$), with the largest contributions from aggregations of unknown nature, reproductive and feeding events (Fisher's Exact test with Bonferroni correction, $p < 0.05$) (Fig. 6A). Potential aggregation types differed significantly (G-test, $p < 0,05$), confirming a non-uniform distribution across categories. Post-hoc Fisher's Exact Test with Bonferroni correction indicates that all categories are statistically different except for resting and movement, with the predominance of unknown events ($p > 0.05$) (Fig. 6B). The "resting" category was excluded from the analysis, as no events of this type were recorded.

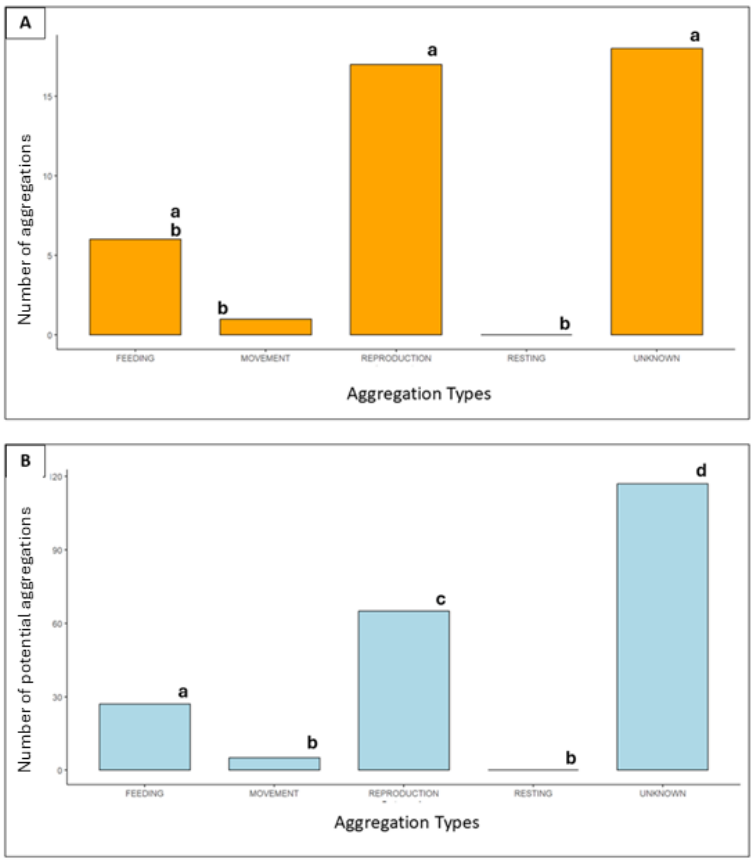


Figure 6. Number of aggregation types and statistical differences: (A) histogram shows the number of papers reporting each aggregation type in aggregations, (B) shows the distribution for potential aggregations. Letters indicate statistical differences of the post-hoc Fisher's Exact test with Bonferroni correction.

3.3 Seasonal trends

The Chi-squared test revealed significant differences in Seasonal trends of occurrences significantly differed (χ^2, $p < 0.05$), with spring and summer being the most frequently reported seasons for both aggregations and potential aggregations (Fig. 7A; 7B).

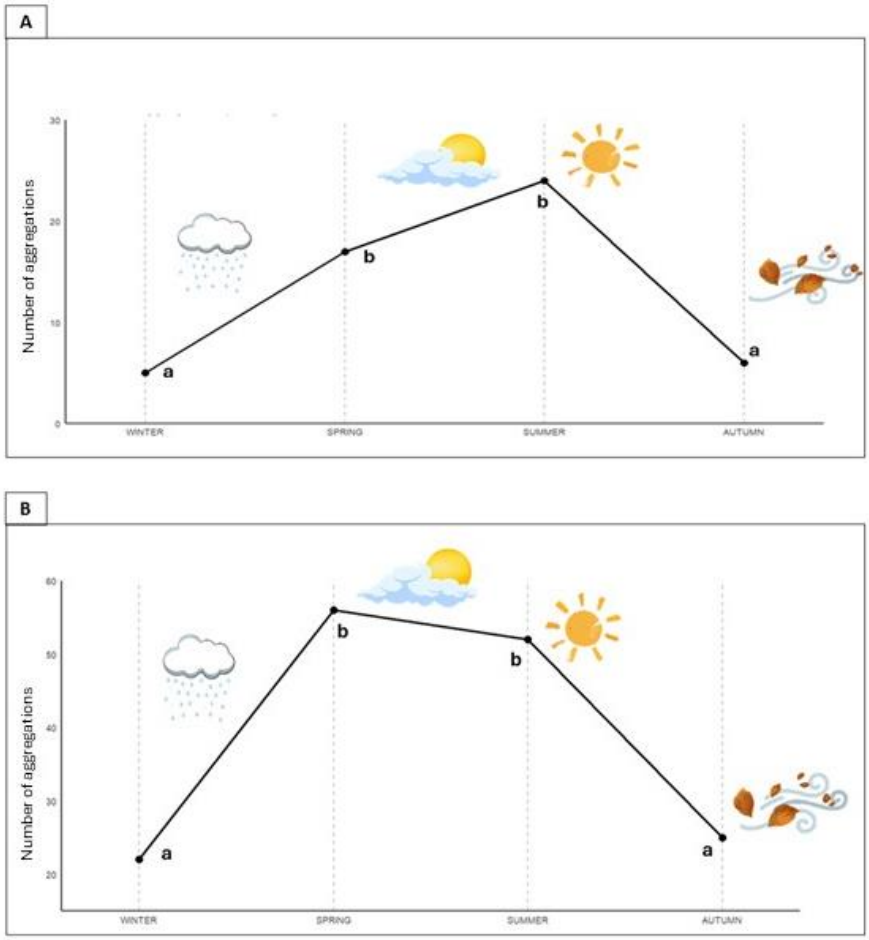


Figure 7. Seasonal trends of aggregations and potential aggregations: (A) Number of aggregations across the different seasons (Winter, Spring, Summer, Autumn).
(B) Number of potential aggregations across the different seasons. Letters above the data points indicate statistically significant differences according to post-hoc analysis.

3.4 Depth ranges

The epipelagic zone (0 – 200 m) showed a higher-than-expected proportion of aggregations compared to the expected distribution (Binomial test, $p < 0.05$), with 93% estimated presence probability and a 95% confidence interval of 0.66 – 0.99 (Fig. 8A). In the mesopelagic zone (201- 1000 m), the proportion of aggregations significantly differs from the expected distribution (Binomial test, $p < 0.05$) with an occurrence probability of 0.07 and a confidence interval of 0.0018 – 0.338 (Fig. 8A). No aggregations were recorded in the bathypelagic zone (> 1000 m), and the Binomial test confirmed a deviation from the expected uniform distribution ($p < 0.05$), with a probability of 0.0 occurrence and a confidence interval of 0.00 – 0.23 (Fig. 8A). For potential aggregations, the epipelagic zone also showed higher-than-

expected proportion of events (Binomial test, $p < 0.05$), with an estimated presence probability of 72% (95% confidence interval of 0.56-0.84) (Fig. 8B). The mesopelagic range did not show a deviation from an expected uniform distribution ($p > 0.05$). No potential aggregations were found in the bathypelagic zone, which distribution differed significantly from uniform (Binomial test, $p < 0.05$; estimated presence probability of 0% in a confidence interval of 0.0 – 0.08) (Fig. 8B).

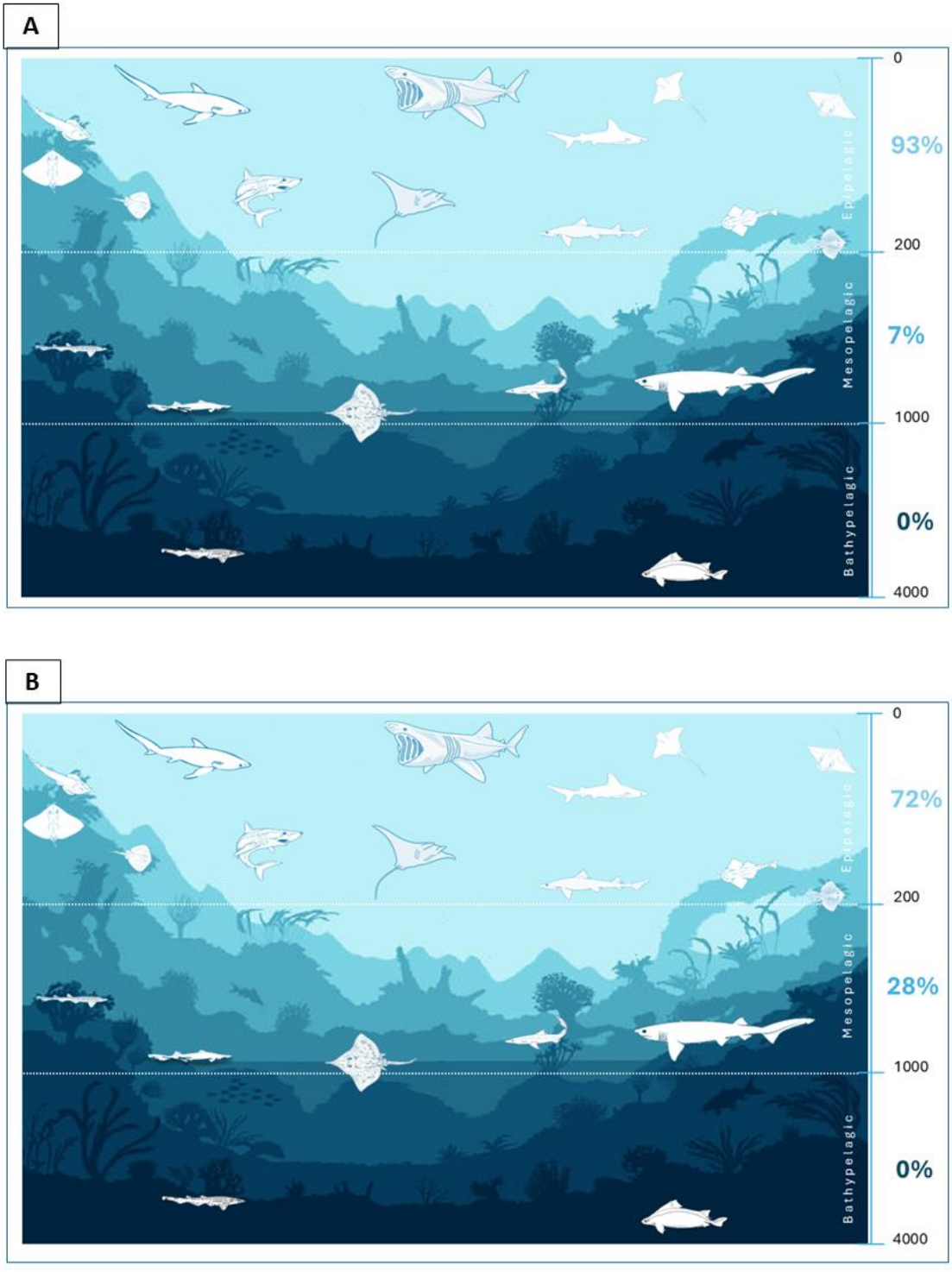


Figure 8. Depth preferences in aggregations and potential aggregations: (A) Aggregations across three bathymetric zones (epipelagic: 0–200 m, mesopelagic: 201–1000 m, bathypelagic: >1000 m). (B) Potential aggregations across three bathymetric zones (epipelagic: 0–200 m, mesopelagic: 201–1000 m, bathypelagic: >1000 m).

3.5 Habitat preferences

Rocky substrates (23%), artificial habitats (23%) and sand/mud (21%) were the most frequent habitats in aggregation sites (Fig. 9.1). For potential aggregations, sand/mud was the most frequently reported (32%), followed by brackish waters (24%) (Fig. 9.2). Differences across habitats were significant both for aggregation and potential aggregation (Exact χ^2 test, $p < 0.05$). Post-hoc Fisher's exact test with Bonferroni correction indicated that cold water corals and seamounts were the least reported habitats hosting aggregations (Fig. 9.1). For potential aggregations, rock, sand/mud, seagrass and brackish waters were the most frequently reported habitats (Fisher's Exact with Bonferroni correction, $p< 0.05$) (Fig. 9.2).

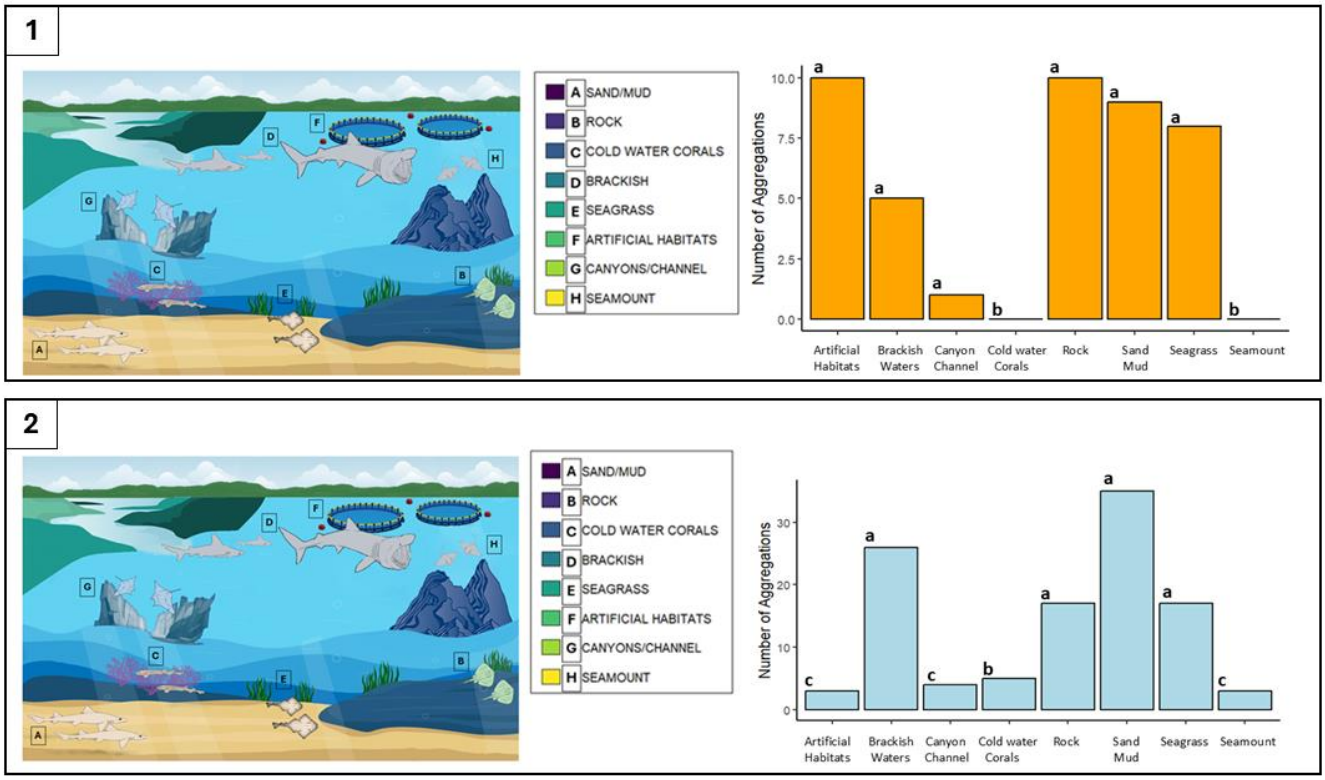


Figure 9. Aggregations and potential aggregations across different Mediterranean habitats: (1) Number of aggregations across habitats. (1) Number of potential aggregations across habitats. Illustrations on the left depict the main habitat categories identified: (A) sand/mud, (B) artificial habitats, (C) cold-water corals, (D) rocky reefs, (E) seagrass meadows, (F)

brackish waters, (G) canyons/channels, and (H) seamounts. Letters above bars are the results of the pairwise Fisher's exact test with Bonferroni correction**.**

3.6 Research topics and methodologies

The number of publications statistically differs across research topics (χ^2, $p < 0.05$). Post-hoc pairwise comparison with Bonferroni correction indicated that biology, ecology, conservation and management and habitat use share the same number of references, forming a single cluster. Fishery-related topics were the most frequent, while genetics was the least represented (Fig. 10).

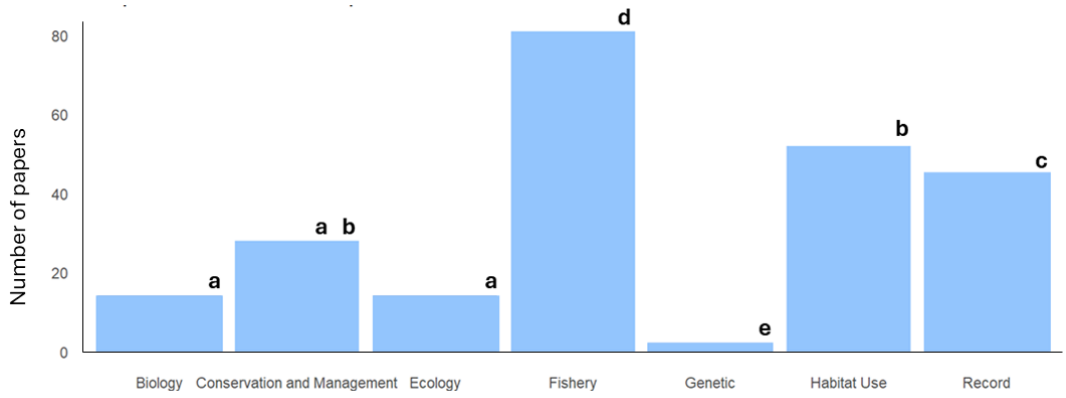


Figure 10. Comparison of research topics based on the number of published papers for aggregations and potential aggregations. Letters represent the results of the post-hoc test, significant differences are represented by different letters.

The number of studies significantly differed among methodologies (χ^2, $p < 0.05$). Post-hoc pairwise comparisons (Bonferroni correction) revealed that some fishery-related methods were the most applied, followed by CS and LEK, both in aggregations and potential aggregations studies (Fig. 11).

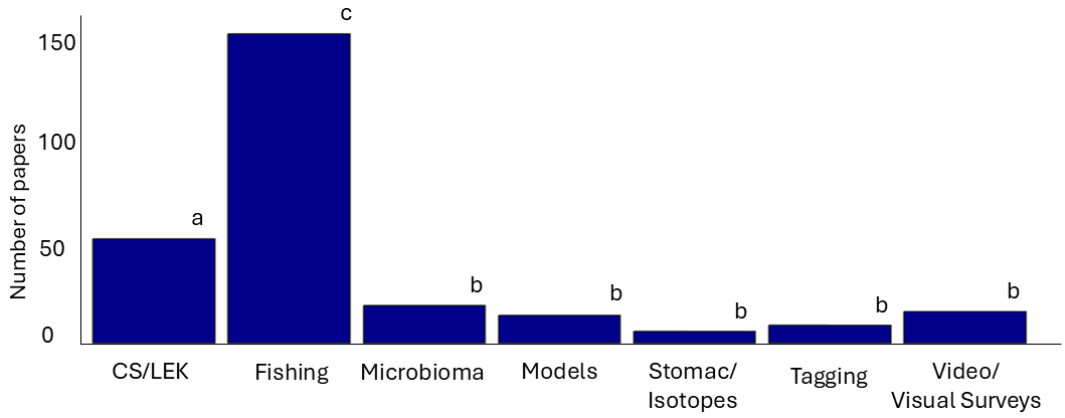
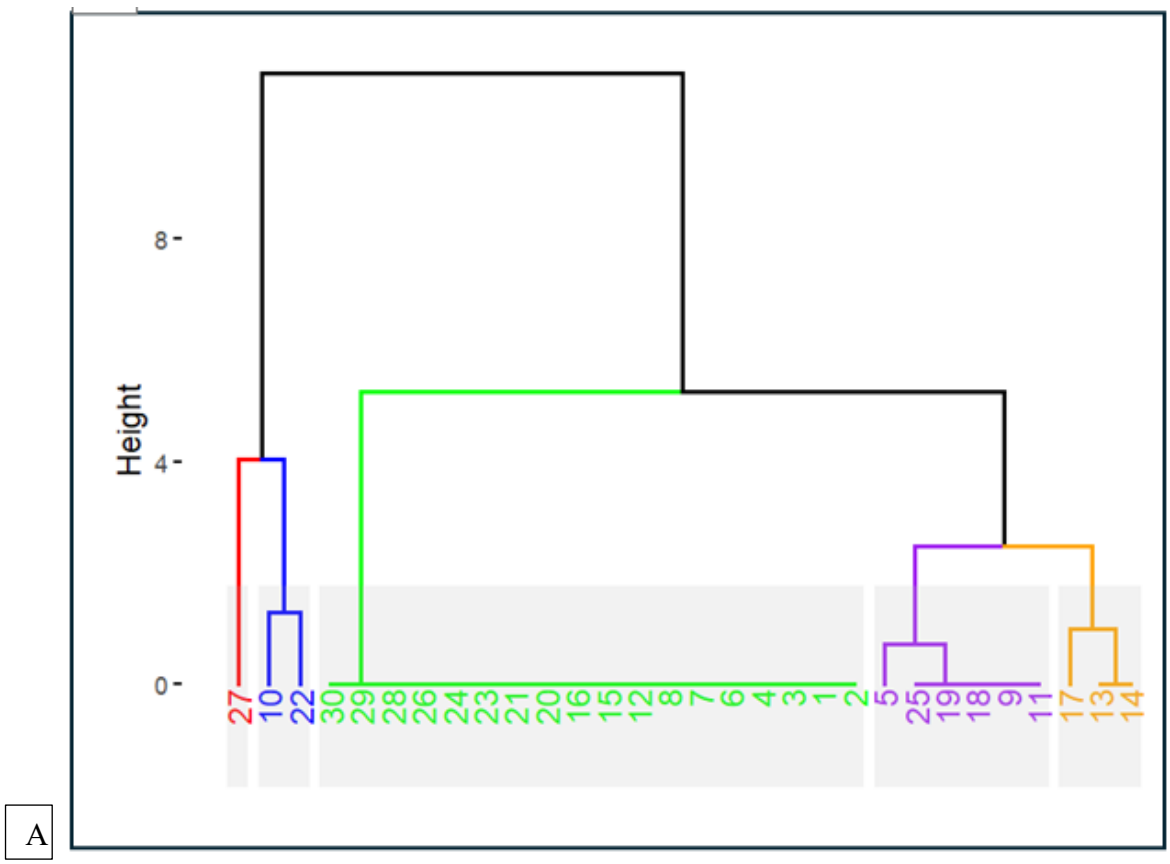


Figure 11. Comparison of research methods based on the number of published papers for aggregations and potential aggregations. Letters represent the results of the post-hoc test, significant differences are represented by different letters.

3.7 Spatial occurrences and research efforts across GSAs

Hierarchical clustering analysis identified five clusters based on the number of aggregations, aggregated species and related publications (Fig. 12A). Cluster 1 included 18 GSAs (1, 2, 3, 4, 6, 7, 8, 12, 15,16, 20, 21, 23, 24, 26, 28, 29, 30) with no aggregations occurrence nor related studies. Cluster 2 included six GSAs (5, 9, 11, 18, 19, 25) with low aggregation presence (mean 1,17) and low publication counts (mean 1); Cluster 3 comprised GSAs 10 and 22, showing intermediate aggregations (mean 5) and published papers (mean 3); Cluster 4 included GSAs 13, 14 and 17, with higher aggregation occurrences (2.3) and medium research effort (mean 2); GSA 27 was isolated as fifth cluster, showing the highest aggregation presences (mean 7) and research effort (mean 8). The PCA performed on aggregations across GSAs showed that the first two principal components (PC1 and PC2) explained 99.2% of the total variance (PC1: 96.9%, PC2: 2.3%). The PCA biplot indicated that the number of species, aggregations and papers were positively correlated along PC1. For potential aggregations four clusters were identified by the silhouette method (Fig. 12B). Cluster 1 (16 GSAs: 1,2,3,4,6,8,12,13,19,20,21,23,25,26,29,30) showed low number of potential aggregations (mean 1.31), species richness (mean 1.56) and research effort (mean 1.25). Cluster 2 (11 GSAs: 5, 7, 9, 10, 11, 14, 15, 17, 18, 27, 28) had intermediate values (mean potential aggregations: 7.27, species: mean 6.45, papers: 4.73). Cluster 3 (three GSAs: 16,24) showed high number of potential aggregations (mean 18.5) and number of species (mean 17.5) and an intermediate number of published papers (mean 9.5); Cluster 4 (GSA 22) characterized by

an intermediate number of potential aggregations and species richness (mean 7 and 6 respectively) and by a high research effort (mean 14). The PCA on the potential aggregations showed that the first two PCs accounted for 98.7% of the total variance (PC1: 85.4%, PC2:13.3). PC1 was driven by the number of aggregations and species, while PC2 was more influenced by research effort. The PCA biplot indicated that GSAs with high potential aggregations and species richness clustered separately from those with few publications. Fig. 13 illustrates the differences across the three analyzed variables (number of aggregations, number of species, number of papers) for both aggregations (panels A, C, E) and potential (panels B, D, F).



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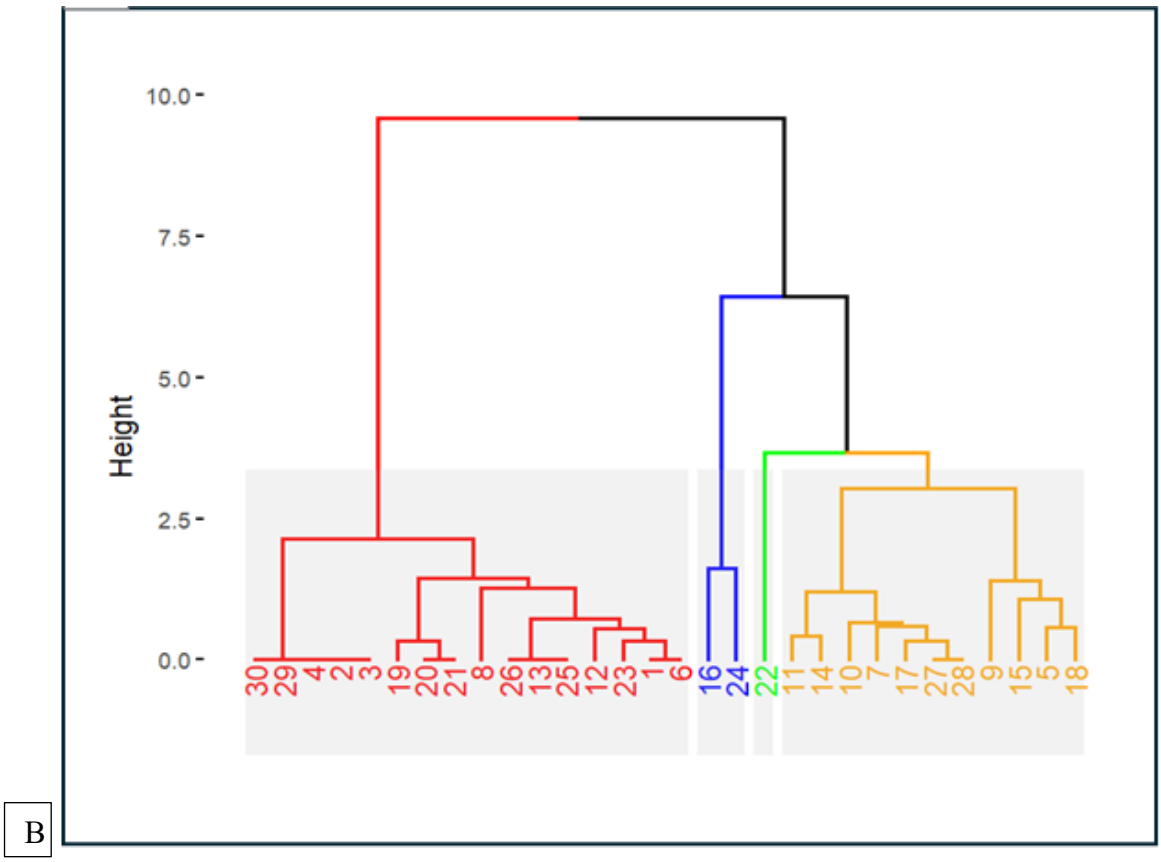

Figure 12. Hierarchical clustering and Principal Component Analysis of aggregations and potential aggregations across GSAs. (A) Dendrogram representing the hierarchical clustering of GSAs based on the number of aggregations, papers and species; (B) Dendrogram representing the hierarchical clustering of GSAs based on the number of potential aggregations, papers and species. The colors indicate the different clusters identified through the silhouette method.

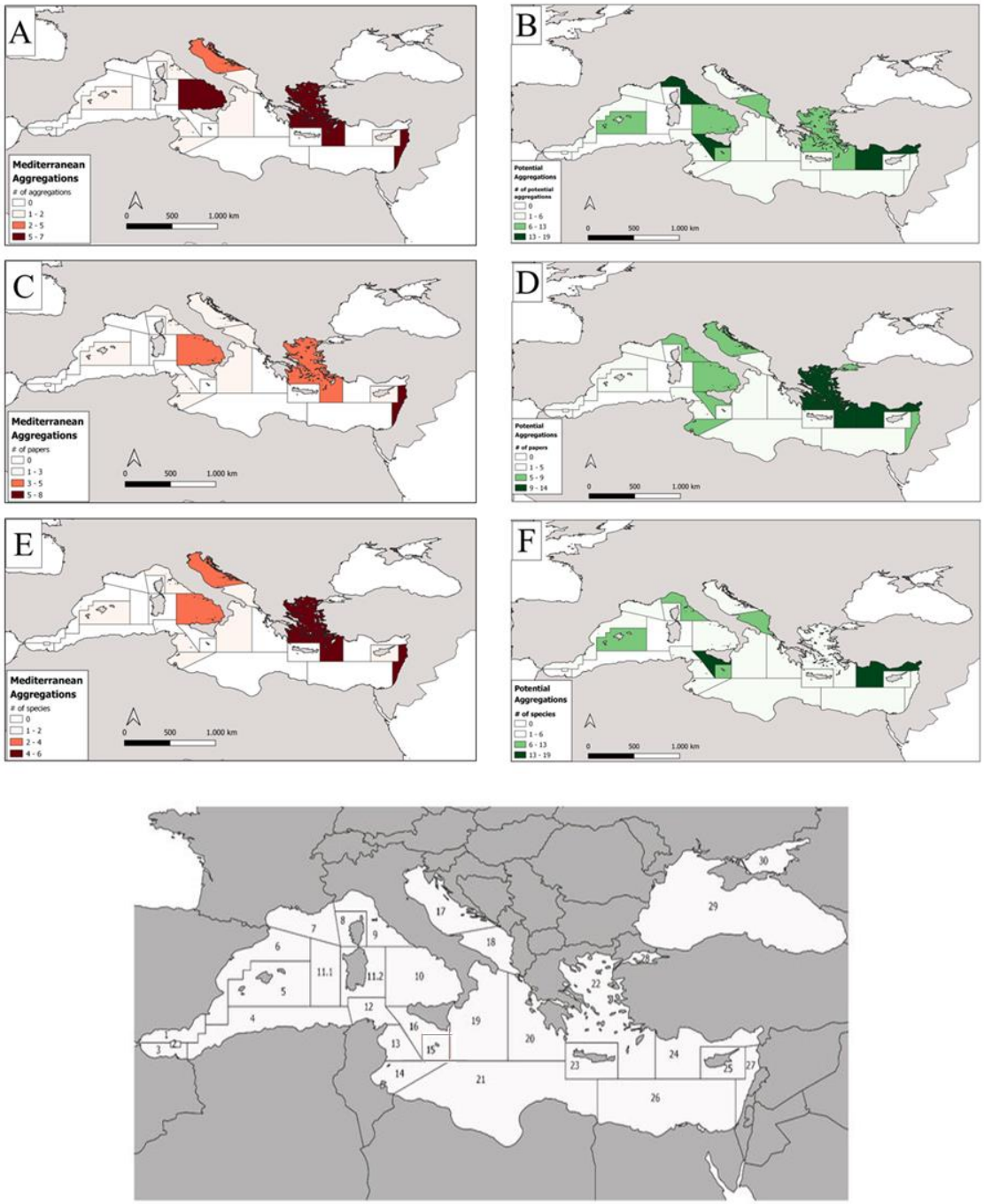

Figure 13. Spatial Distribution of three variables across Mediterranean GSAs**.** Spatial distribution of aggregations (left panels: A, C, E) and potential aggregations (right panels: B, D, F). The maps provide a visual representation of the aggregation patterns concerning: (A) and (B) number of events per GSA; (C) and (D) number of published papers related to aggregations or potential aggregations; (E) and (F) number of species performing aggregations or potential aggregations. The reference map on the bottom illustrates the spatial

extent and numbering of the GFCM Geographical Sub-Areas (GSAs) used in this study (adapted from Borg et al., 2023).

4. Discussion

This study synthesized the available literature on elasmobranch aggregations in the Mediterranean Sea, providing an overview of their occurrence, typology, and the diversity of research sources documenting them. By systematically reviewing published records, our results revealed that most potential aggregation events were reported only once in the literature, rather than being confirmed as recurrent aggregations. This pattern could indicate that aggregative behaviors can be relatively uncommon (Crowe et al., 2018) but they might also reflect biases in research design and reporting (McInturf et al., 2023). The lack of significant differences in the recorded events between sharks and rays suggests that, at least in terms of frequency of reports, both groups may display similar aggregation patterns. However, these findings must be interpreted considering that the predominance of single occurrences could be due to opportunistic sampling, where isolated events are more likely to be reported than systematic observations of gregarious behaviors (Barone & Friedman, 2021). The absence of standardized criteria has contributed to challenges in identifying aggregations across regions and species. To address these difficulties, this study proposes a practical and replicable framework for classifying aggregation events into confirmed, potential and single occurrences. Such an approach provides a basis for the use of different data sources and can serve as a reference for the identification of critical habitats, especially in data-poor regions where monitoring is limited (Cattano et al., 2023).

4.1 Aggregation purposes

A challenge that emerged from this review was the assessment of the biological purposes of elasmobranch aggregations. Indeed, understanding the biological purposes of aggregations is important, as different purposes are associated with distinct conservation and management implications (Baum et al., 2003; de Mitcheson & Colin, 2011; Jacoby et al., 2012). A large proportion of aggregation and potential aggregation events documented in the literature are reported without clear information on their function, likely reflecting the difficulties of studying elasmobranch behavior through direct observation (Gruber & Myrberg, 1977;

McInturf et al., 2023). Feeding aggregations often overlap with fishery grounds, such as areas exploited by pelagic longlines, making sharks and rays vulnerable to bycatch and generating human-wildlife conflicts (Carbonara et al., 2023; Garibaldi, 2015; Papageorgiou et al., 2022). Reproductive aggregations are often philopatric, meaning that individuals repeatedly return to mate or give birth to the same area (Flowers et al., 2016). This type of knowledge is key either to inform management actions or to feed conservation planning models, as effective management measures for feeding aggregations such as modifications to fishing gear and deployment aimed at by-catch reduction or increased post-catch survival (Carruthers et al., 2009; Coelho et al., 2012, 2013; Poisson et al., 2016; Ward & Myers, 2007) may offer a flexible alternative to permanent spatial closures (Poisson et al., 2010; Santos et al., 2009; Watson & Bigelow, 2014), but in turn may be inadequate for reproductive and nursery areas that require precautionary approaches (Leathwick et al., 2008; Senko et al., 2014) and longer term protection (Alverson et al., 1994; Dunn et al., 2011). Clarifying the purposes of elasmobranch aggregations is therefore essential for tailoring management and conservation actions to species' ecological needs. Identifying aggregation habitats is pivotal as they be integrated into spatial management plans to improve protection effectiveness (Rohner et al., 2025). Our synthesis highlights that addressing this gap should be a priority for future research.

4.2 Seasonality, Depth and Habitat preference

Seasonality, depth preferences, and habitat selection influence the occurrence of elasmobranch aggregations. Our synthesis revealed that most reported events are concentrated in spring and summer and documented in shallow, epipelagic zones. The seasonality observed aligns with observed patterns of distribution and collective behaviors adjusted in response to abiotic factors (Jorgensen et al., 2012; Schlaff et al., 2014). The coincidence of peak aggregation periods with heightened human activities, such as fisheries and tourism, further increases the vulnerability of these events (Cattano et al., 2021; Pichot et al., 2025), as concentrated groups of individuals in shallow coastal areas become more exposed to targeted or incidental fishing pressure (Saidi et al., 2023). Moreover, the predominance of shallow-water records is consistent with reproductive behaviors of some species, even large pelagic species often approach coastal habitats to give birth, exposing

juveniles to intense fishing pressure (Lloret et al., 2020; Maioli et al., 2024; Mancusi et al., 2023). Despite these insights, some caveats of the present study need to be discussed. The scarcity of records from mesopelagic and demersal environments potentially reflects methodological limitations, such as the difficulties of monitoring deep-sea habitats, rather than a true absence of aggregations (Daley et al., 2015; Finucci et al., 2018; Rodríguez-Cabello et al., 2016). Similarly, the ecological importance of seamounts and cold-water coral reefs in the Mediterranean is still poorly understood (Bo et al., 2020; Bo et al., 2021), despite evidence mostly from other marine regions suggests that such habitats sustain feeding aggregations or act as migratory stopovers (Morato et al., 2010; Weber et al., 2025). Ongoing coral mass mortality events further underscore the urgency of investigating these potential associations before they disappear (Cerrano et al., 2020; Chimienti et al., 2020; Ponti et al., 2018). Finally, soft-bottom habitats, often overlooked in conservation planning, are underrepresented in aggregation studies despite supporting feeding and reproductive aggregations of endemic and threatened batoid species (Bilgili et al., 2023; Chaikin et al., 2020; Kadri et al., 2013; Navarro et al., 2013). Understanding the interplay between seasonality, depth distribution, and habitat preferences is essential for designing adaptive management strategies that account for both natural drivers and human impacts (Di Sciara et al., 2025; López et al., 2025). Conservation actions should expand beyond traditionally protected environments, incorporating understudied habitats such as deep-sea, soft bottom and seamounts.

4.3 Research topics and methodologies

The study of elasmobranch aggregations in the Mediterranean Sea has relied mostly on fishery surveys, which are the main source of long-term standardized data. However, alternative low-impact methods emerged as accessible and cost-effective tools for detecting aggregations, particularly in data-poor regions where resources for systematic monitoring are limited by logistical or financial limitations (Barbato et al., 2021; Bargnesi et al., 2020; Giovos et al., 2019; Giovos et al., 2021). When carefully managed, these approaches can extend monitoring capacity and foster community involvement in conservation (Cattano et al., 2023). Similarly, tagging techniques may offer valuable insights into the study of elasmobranch aggregations structures and patterns (Zemah-Shamir et al., 2022). A multi-approach can enhance ecological

knowledge, guide adaptive management, and contribute to the design of effective conservation strategies (Goodman et al., 2024; Marshall et al., 2023). Despite their potential, Citizen science and LEK data can be prone to biases and without careful oversight, there is a risk that human presence at aggregation sites may promote site exploitation rather than conservation (Colloca et al., 2024; Zemah-Shamir et al., 2019; Zemah-Shamir et al., 2025). Tagging studies, though powerful, are underutilized in the Mediterranean and often limited in scale due to financial and logistical constraints (Welch et al., 2025). Future research should aim to combine different survey methods to overcome current limitations. An integrated multi-method framework would help in enhancing conservation strategies by linking aggregation patterns to population dynamics and management needs.

4.4 Aggregations and research efforts across GSAs

Our analysis of spatial clustering and PCA highlights geographic imbalances in research coverage across Mediterranean GSAs. While some areas have been well studied, many GSAs remain poorly represented, which prevents the development of evidence-based conservation strategies at regional level. GSA 27 emerged as a hotspot, with numerous monitored aggregations and a high number of published studies. Other areas, GSAs such as 16 and 24, show potential aggregation records but lack systematic investigations, suggesting that gaps in knowledge may reflect research bias rather than a true absence of aggregations. The correlation between research effort, species richness, and aggregation reports indicates that where more scientific work is carried out, aggregations are more often detected. Therefore, scientific effort plays a central role in shaping our understanding of elasmobranch gregarious behaviors. This uneven research effort has direct management consequences. Areas with confirmed aggregations are likely to overlap with regions already subject to some management measures, while GSAs with only potential aggregations may remain largely unmanaged, exposing species to unregulated exploitation pressures (Cashion et al., 2019; Shakman et al., 2023). Further assessment of the spatial correspondence between aggregation sites and existing management zones is needed to verify this pattern. Moreover, existing regulations, such as the 3-mile fishing restriction, have proven insufficient to ensure sustainability (Colloca et al., 2017.b; Riginella et al., 2022). The lack of systematic monitoring in several GSAs creates blind spots where threatened species continue to be

targeted during aggregations, worsening population declines (Echwikhi et al., 2014; Louhichi et al., 2004). Proposed alternatives, such as shark tourism, may offer opportunities for non-extractive economic use (Vianna et al. 2012), but they also risk introducing new pressures if not carefully designed and regulated (Healy et al., 2020). Addressing these imbalances requires a precautionary and adaptive approach. Regional authorities should prioritize poorly studied GSAs where potential aggregations are reported, implementing management measures even in the absence of complete data (Koehler et al., 2022; Mejía-Falla et al., 2019). Best-practice guidelines and collaborative frameworks with local stakeholders are essential to ensure that initiatives, including ecotourism, contribute to management rather than harm (Gallagher & Huveneers, 2018; Montero-Quintana et al., 2020). For example, the Strait of Sicily, already recognized as a hotspot of shark and ray diversity (Di Lorenzo et al., 2018; Geraci et al., 2021), highlights the need for area-specific strategies, since distinct life stages may use different subzones within the same region (Enajjar et al., 2015; Cattano et al., 2021). Overall, reducing geographic biases in research and adaptive, area-specific management is an essential step toward protecting aggregation sites across the Mediterranean.

5. Conclusions

This review provides the first synthesis of elasmobranch aggregation records in the Mediterranean Sea, offering both a methodological framework for classification and a regional overview of patterns and knowledge gaps. Aggregations are a vulnerable phase of some elasmobranchs' life cycles and understanding their spatio-temporal dynamics is important for ensuring long-term population resilience. Our findings highlight the predominance of single records over confirmed aggregations, which may reflect both the rarity of such behaviors and biases in reporting and study design. The high proportion of events with unknown function underscores the need for targeted ecological and behavioral research to identify feeding and reproductive areas. Seasonal and habitat-related trends, together with the uneven distribution of research across GSAs, stress that conservation strategies must integrate species-specific biology, regional context, and emerging environmental pressures.

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Chapter 2: Distribution and characterization of threatened common eagle ray aggregations in the Mediterranean Sea: opportunities for regional conservation actions

This chapter is at the second round of review:

Grancagnolo, D., Cattano, C., Turco, G., Aglieri, G., Milazzo, M. (under review). *Myliobatis aquila* (Linnaeus, 1758) aggregations in the Mediterranean Sea: distribution, characterization and opportunity for conservation. *Aquatic Conservation Marine Freshwater Ecosystems*

Abstract

Many sharks and rays aggregate in critical habitats to feed, mate, or give birth. These predictable events, often involving dozens to thousands of individuals, represent important and vulnerable phases in their life cycles. However, fishing activities and unregulated tourism can threaten these aggregations, disrupting natural behaviors. Identifying when and where these events occur is essential to inform conservation strategies and spatial management. In this study, we combined systematic literature review and social media data mining to investigate spatial and temporal aggregation patterns of the critically endangered common eagle ray (*Myliobatis aquila*) across the Mediterranean Sea. We identified seven aggregation sites and extracted biological indicators useful for their characterization. We conducted a four-year underwater video survey at Marettimo Island (Italy), within the Egadi Islands Marine Protected Area, to describe a previously poor documented aggregation and assess potential impacts from underwater tourism. Up to 100 individuals were recorded in the summer months, with interannual site fidelity. Aggregations were female-dominated, with 4% of females observed pregnant. Courtship and mating behaviors were documented for the first time in this species. Our observations revealed a significant decline in eagle-ray abundance in the presence of scuba divers, indicating potential disturbance from human activity. This study provides critical insights into the aggregation pattern of a threatened species and highlights the need for management in high-use coastal areas. We propose a set of biological indicators to support the identification and monitoring of aggregation events, especially in data-poor regions, offering a valuable tool for marine conservation planning.

1. Introduction

Sharks, rays, and chimaeras (class Chondrichthyes) are adapted to a wide range of environments and play multifunctional roles in marine ecosystems (Dedman et al., 2024; Stein et al., 2018). Targeted and unintentional fishing is the main driver of Chondrichthyes depletion, with exploitation rates exceeding their slow life-history (Dulvy et al., 2021; Pacoureau et al., 2021). The Mediterranean Sea, with its long history of exploitation, is a hotspot for elasmobranchs extinction, experiencing a faster decline than other marine regions (Dulvy et al., 2014). Over half of the species are assessed as threatened with extinction by the International Union for Conservation of Nature (IUCN) (Dulvy et al., 2016; Walls & Dulvy, 2021). Although less studied than sharks, Batoids face a similar decline and a lack of historical data prevents an appropriate assessment of their status (Geraci et al., 2021; Milazzo et al., 2021). Many elasmobranchs form transient aggregations, defined as the co-occurrence of two or more individuals in space and time due to the use of a common driver, such as breeding and foraging (McInturf et al., 2023). These predictable gatherings can increase the exposure to anthropogenic threats, such as targeted fishing and by-catch (Croll et al., 2016; Litvinov, 2006), as well as tourism in the form of shark- and ray-watching (Cattano et al., 2021; Shamir et al., 2019). Identifying spatio-temporal dimensions of elasmobranch aggregations and assessing anthropogenic impacts is central to informing conservation strategies targeting these threatened species. Marettimo Island (southern Italy), within the Egadi Islands Marine Protected Area (MPA), hosts an aggregation of the common eagle ray (hereafter referred to as eagle-ray) *Myliobatis aquila* (Linnaeus, 1758) (Grancagnolo & Arculeo, 2021). The species is viviparous, with a breeding period between September and February, a gestation of 6-8 months, and forms groups in summer (Manfredi et al., 2010; Serena, 2005). Like other K-selected elasmobranchs, unmanaged fishing activities led eagle-rays to a steep decline in abundance and a reduction in their distributional range (Ferretti et al., 2005; La Mesa et al., 2016). Signs of recovery were observed in the Northern Adriatic Sea, with an 80% increase in the frequency of occurrence in the Mediterranean International Trawl Surveys (MEDITS) from 1996 to 2019 (Maioli et al., 2023). However, the paucity of data from other Mediterranean areas suggests adopting a precautionary approach following the Mediterranean or the more recent global IUCN assessments, which classified the species as Vulnerable

(Serena et al., 2016) and Critically Endangered (Jabado et al., 2021), respectively. In 2023, the third Important Shark and Ray Areas (ISRA) regional expert workshop classified the Egadi Archipelago as a "reproductive aggregation site" for the eagle-ray (Kyne et al., 2023; https://sharkrayareas.org/), based on both social media and field-based data. Although the area is within an MPA, the presence of a reproductive aggregation highlights the need for site-specific management measures. Building on studies supporting conservation through diverse data sources (Batista et al., 2024; Cattano et al., 2023), we combined social media data mining, literature review and on-site underwater surveys to (1) identify potential aggregations spots of eagle-ray; 2) collect data on a set of biological (abundance, size, and sex ratio) and behavioral variables of congregating individuals; 3) assess impacts of scuba diving; 4) proposed a dashboard of biological indicators supporting the early identification of other aggregations where conservation actions could be implemented.

2. Materials and methods

2.1 Literature search and social media analysis

Scientific papers were searched on Scopus, Web of Science and Google Scholar, constraining the queries to be matched in "Title, Abstract, author keywords" and using as search strings the following keywords: "*Myliobatis aquila"* or *"Common-eagle-ray"* and "aggregation" and "Mediterranean". When applicable, the subject area selection was limited to "Environmental Science", "Agricultural and Biological Sciences" and "Earth and Planetary Sciences". We compiled a database containing information on location, seasonality and survey methods. Only data belonging to peer-reviewed journal articles and reviews were considered. Records from scientific literature were combined with social media sightings to provide an overview of potential eagle-ray aggregations across the Mediterranean region. Mediterranean records of eagle-rays were examined across social media platforms (Instagram, Facebook and Youtube) to identify areas with occurrences of individuals over multiple years. Aggregation was considered as the co-occurrence of two or more individuals within a specific spatio-temporal scale triggered by environmental drivers (McInturf et al., 2023). Visual contents (videos/photos) were searched in English, Spanish, Italian and French to gather information on location, year, maximum number of individuals recorded together in the same frame,

number of males (identified by claspers and supra-orbitary tubercles according to Capapè et al., 2007; Capapè & Quignard, 1974), number of females (when ventral view allowed sex determination) and evidences of pregnant females (assessed by the symmetrical swellings on the back and on the belly of each individual following Chaikin et al., 2020). Inter-annual sightings were mapped to visualize the spatial distribution of potential aggregations in the Mediterranean Sea.

2.2 On-site surveys in the eagle-ray aggregation of Marettimo Island

2.2.1 Description of the study area

The Egadi Archipelago is in the north-western part of the Strait of Sicily and encompasses the islands of Favignana, Levanzo, and Marettimo (Fig. 1). The “Egadi Island” MPA was established in 1991 and comprises four distinct protection levels. Zone A is a "fully protected area", where only diving and swimming are permitted. Zones B and C correspond to" general" and "partial" protection, respectively, where regulated artisanal fishing and recreational activities (such as diving and boating) are allowed. D zone, where trawling and purse seine fisheries are permitted with prior authorization. The aggregation of eagle-rays occurs within the B zone, in "Cala Martina" off the southernmost sector of Marettimo Island (Fig. 1). This site features a shallow rocky substrate at 15-20 m depth, characterized by sedimentary structures among *Posidonia oceanica* ((Linnaeus) Delile 1813) patches and vertical paleo-cliff structures emerging from a sandy bottom at 35 m depth (Lo Iacono, 2003).

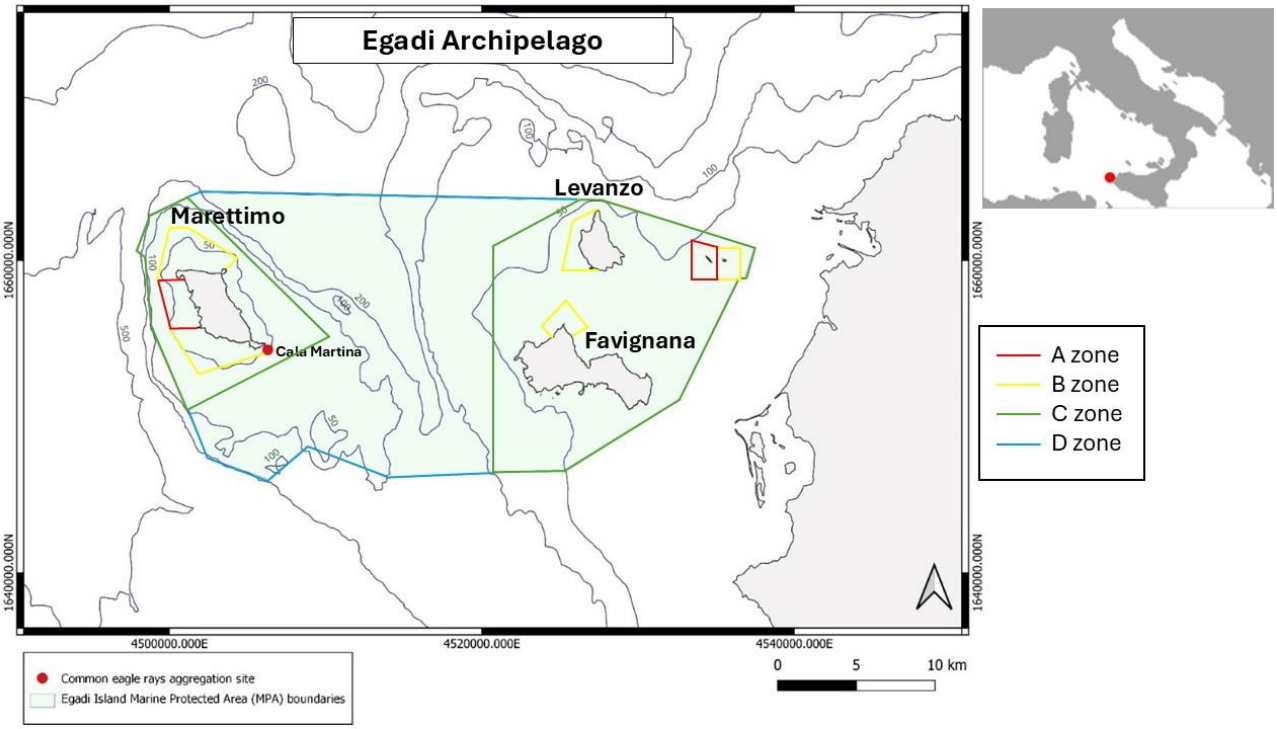


Figure 1. Map of the Egadi Archipelago (north-western Sicily, Italy) showing the boundaries and zonation (A–D) of the Egadi Islands MPA. The red dot indicates the location of the common eagle ray aggregation site at Cala Martina, off the southeastern coast of Marettimo Island. The inset on the top-right map shows the position of the study area within the Mediterranean Sea.

2.2.2 Video surveys

Surveys were conducted over four consecutive summers from 2020 to confirm the seasonal recurrence in the area and to characterize eagle-rays' demographic structure and behavior**.** Preliminary visual surveys in late spring and early summer (May–July) 2020/2021 did not report eagle-rays in the area. As a result, all subsequent surveys in 2022/2023 were scheduled between August and October, when aggregations were confirmed to occur. Two video sampling techniques were employed: Unbaited Remote Underwater Video systems (URUVs) and Diver Operated Video systems (DOVs). URUVs consisted of a single GoPro Hero 8 camera housed in a horizontally oriented waterproof case fixed to the bottom using a weighted tripod. DOVs comprised an aluminum bar holding two GoPro Hero 8 cameras in a single or stereo-view, previously calibrated with CAL software (www.seagis.com.au). We deployed 77 URUVs during daylight hours (08:00 AM/06:00 PM) at depths from 23 to 28 meters (Tab.1). Three URUVs at a time, spaced at least 150 meters apart, were positioned on the

seafloor and recorded continuously for a minimum of 60 minutes each (Osgood et al., 2019). Videos from URUV deployments were used to monitor co-occurrence patterns and to characterize the aggregation of eagle-rays in terms of behavior and demographic structure.

Table 1. 77 URUV deployments by month and year at Marettimo Island. Number of URUVs deployed during daylight hours (08:00–18:00) at depths ranging from 23 to 28 meters, between 2021 and 2023.

Year	August	September	October
2021	2	6	0
2022	63	4	0
2023	0	0	2

Videos from URUVs deployed in 2022 were analyzed to assess the effect of scuba diver presence on eagle-ray aggregation dynamics. Ten URUV deployments were conducted under high-impact conditions (with Open Circuit (OC) divers in the site - 12:57:19 hh:mm: ss) and nine under low-impact conditions (deployed by a single technical-diver using Closed-Circuit-Rebreather (CCR) - 11:43:31 hh:mm: ss). All deployments took place in the morning, between 08:00 and 14:00, to avoid potential behavioral variation linked to environmental or diel cycles. For each video, the maximum number of individuals observed simultaneously (MaxN), the aggregation time (total duration in seconds during which multiple eagle-rays were present in the field of view), and the number of scuba divers were recorded and standardized on 60 minutes of video duration. A total of 142 10-minute DOV (both single and stereo-cameras) transects were conducted in the area where eagle-rays seasonally congregate (Tab.2). These transects were carried out by divers using both Open Circuit (OC) and Closed-Circuit Rebreather (CCR) systems. In total, we analyzed 131:47 hours of video footage, of which 106:43 hours were obtained from URUVs and 25 hours from DOV recordings. Footage collected from 2020 to 2023 was used to characterize the aggregation in terms of group behavior and sex ratio. Size class estimates were performed using calibrated camera data from 2022 and 2023 only.

Table 2. 142 DOV transects by month and year at Marettimo Island.

Year	August	September	October

2020	1	0	0
2021	4	6	0
2022	99	10	3
2023	0	0	19

VLC software (VideoLAN; https://www.videolan.org/vlc/index.it.html) was utilized by a single annotator to count the number of sightings (the number of times eagle-ray individuals were in the field of view) and MaxN (the maximum number of individuals in a single frame; Willis & Babcock, 2000) as a conservative metric to estimate the relative abundance of eagle-rays in the aggregation area (Whitmarsh et al., 2017). Although sightings may involve repeated observations of the same individual and do not provide a reliable estimate of species abundance, this measure was used to explore spatio-temporal trends in species activity. Disk-Width (DW) of individuals detected by DOVs was measured using EventMeasure (www.seagis.com.au) to estimate their body size (Fig. 2A). Only measures with a residual mean square (RMS) error below 20 mm were considered to maximize the ratio of precision of the measurement to the length of a fish at 10% (Goetze et al., 2019). The different specimens were successively categorized for each sex into the three main life stages (Juveniles, Sub-Adults, and Adults) following Capapè et al. (2007). Males were identified by the presence of claspers and supra-orbital tubercles (Capapè et al., 2007; Capapè & Quignard, 1974) and considered sexually mature with elongated, longer than pelvic fin length claspers (Capapè et al., 2007) and a DW length above 32 cm (Fig. 2B), while females had a DW length ≥43 cm (Last et al., 2016). Reproductive behaviors in males were classified as courtship, pre-mating (chasing conspecifics, contact or biting), and mating, following Tricas (1980) (Tab.3). Mating events were identified by the presence of males displaying the dorsal erection of claspers (medially to 90°) during copulation, as previously reported for *Myliobatis californica* (Gill, 1865) and *Rostroraja eglanteria* (Bosc, 1800) (Tricas, 1980; Libby and Gilbert 1960). Reproduction in eagle rays and bat rays occurs in the water column while swimming, as occurs in large sharks, rather than demersal Rajidae and Dasyatidae (Tricas, 1980). Advanced pregnancy was inferred in females with significant posterior-dorsal swelling (Chaikin et al., 2020) (Fig. 2D). Special body marks (scars, tail morphology, humps) were used to assess seasonal site fidelity of the same individuals over years (McCoy et al., 2018; Kohler et al., 2023).

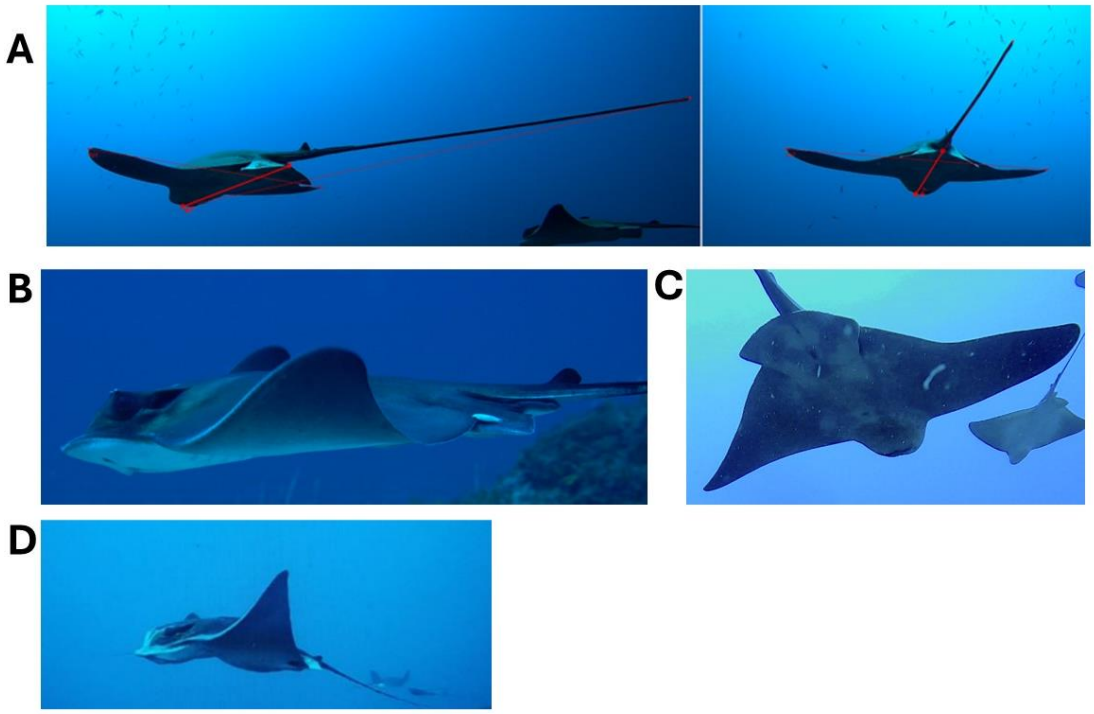


Figure 2. Visual cues used to assess biological indicators of eagle rays. (A) Example of body size measurements obtained with Seagis software (total length, disk length, disk width). (B) Male with elongated claspers and supra-orbital tubercles. (C) Female without claspers. (D) Pregnant female with distended abdomen.

Table 3. Description of courtship and reproductive behaviors observed in male *M. aquila*.

Behavior	**Description**
Courtship	A sexually mature male performs erratic swimming with changes in direction and spinning movements (pirouettes), possibly to attract the attention of a female.
Chasing	The male increases swimming speed and actively pursues a female, trying to maintain proximity.
Contact	The male attempts to establish physical contact by approaching the female from below and gently biting her ventral surface.

Mating	The male positions himself in a dorso-ventral alignment with the female and rotates one clasper approximately 90° to insert it into the female's cloaca for copulation.

2.3. Data analysis

To validate the use of social media for the detection of eagle-ray aggregations and for the collection of demographic information, the frame corresponding to the MaxN was selected from each shared video. Individuals in that frame were sexed, and a female-to-male (F/M) ratio was calculated for each video. To assess deviations from an equal sex ratio, a binomial test ($p=0.5$) was used for each site. For comparisons between field-based datasets (DOV and URUV) and social media data, non-parametric Wilcoxon rank-sum tests ($\alpha=0.05$) were used to test differences in F/M ratios. Each site was tested against both Marettimo DOV and URUV datasets. A χ^2 test was used to test the null hypothesis of no differences among seasons or months in eagle-ray sightings across the Mediterranean basin. The total number of individuals sighted per season and months were used to represent seasonal variations. When the assumptions for the χ^2 test were not met (no expected frequencies <1 and no more than 20% of expected frequencies <5), a Monte Carlo simulation with 10000 permutations was used to estimate the p-value. In cases of a significant p-value in the χ^2 test, post hoc pairwise comparisons between seasons and months were performed using chi-squared tests and Bonferroni correction. The individual contribution of each season and month to the total variance of sightings was assessed using the difference of the residuals. The monthly sighting values were standardized (scaled between 0 and 1) for each site to compare locations with different survey effort or sighting magnitude.

Data collected through direct field observations (both by URUVS and DOVs) in Marettimo were compared to the Mediterranean regional aggregation trends. The percentage of pregnant females was calculated for each site by dividing the number of sighted pregnant females by the total number of female sightings. Differences in sex abundance in Marettimo were tested using the Wilcoxon Mann-Whitney test for non-normally distributed data ($\alpha=0.05$). For each DOV transect, the video frame corresponding to the maximum MaxN value was selected ($n=24$) and the number of males and females was counted within that frame. A $\chi 2$ test was

used to assess whether the sex ratio was different from parity within each life stage. Differences in abundance among adult, sub-adult and juvenile life stages were evaluated for males and females using the Kruskal-Wallis test (α=0.05). Life stage classification was based on 25 and 21 video frames showing the maximum MaxN per transect for females and males (MaxNfem and MaxNmale, respectively). A Dunn's post hoc test with Bonferroni correction was used to identify pairwise differences among life stages. The frequency of reproductive interaction categories was compared using a $\chi 2$ test to evaluate whether some behaviors occurred significantly more than others. The percentage of pregnant females was calculated by dividing the number of sighted pregnant females by the total number of sighted females and compared to the social media dataset. Frequencies of occurrence of eagle-rays identified by natural marks were calculated as the ratio between the number of videos with sightings of the same individual and the total number of video footage. To investigate the effects of scuba divers' presence on the aggregation, MaxN and aggregation time recorded from URUVs deployed under low and high impact conditions were compared using the non-parametric Mann-Whitney U test. Additionally, a linear regression analysis was conducted between the number of divers and the MaxN of eagle-rays observed within the aggregation. A piecewise regression analysis was performed to identify potential thresholds where the rate of decline of the eagle-rays' numbers became more pronounced. The segmented package (Muggeo, 2008) was used to estimate breakpoints in the data. The initial linear regression model was specified as the starting point, and the segmented function was applied to detect significant changes in slope. All statistical analyses were performed in R (R Core Team, https://cran.r-project.org) and the spatial mapping and visualization of aggregation and potential aggregation events were conducted in QGIS 3.28.15 (QGIS Development Team, 2024. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org").

3. Results

3.1 Literature review and social media content

Ten fishery-dependent studies published between 2007 and 2023 reported on high densities of eagle-rays in four areas of the Mediterranean Sea, with six of them using the term "aggregation" to define the events. Nine studies showed a higher abundance of individuals in

the continental shelf (0-50 m) of the Strait of Sicily (n=3), northern Adriatic Sea (n=3) and western Mediterranean (n=3) between late spring and early autumn (Fig. 3). All social media contents (n=194) were uploaded by scuba divers and identified the Santa Croce Bank (n=65; 34% of the observations), the Tor Paterno shoal (n=36; 19%), Cabo de Palos (n=29; 15%), Marettimo island (n=28; 14%), the Medes Islands (n=20; 10%), Pianosa Island (n=13; 7%) and Benidorm (n=3, 2%) as areas with inter-annual evidence of aggregations (Fig. 3). Due to the limited number of observations from Benidorm, this site was excluded from statistical comparisons with other sites. Marettimo showed the highest number of individuals recorded together at the same time in social media footage (up to 36 ind. per footage; MaxN), followed by Santa Croce Bank and Pianosa (MaxN=17) and by Cabo de Palos (MaxN=16) (Fig. 3). The oldest videos were taken in Medes Islands and Pianosa Island in 2010, when the presence of six and two specimens, respectively, was documented. Since 2010, observations of multiple eagle-rays, indicating aggregation events, were recorded annually across the study sites, except for 2011, when only single individuals were reported, each from a single video recorded at Medes Islands and Cabo de Palos. In the other years, aggregations were observed at study sites with different occurrences (from 2012 to 2022 at Tor Paterno; in 2010–2012, 2017, 2019, and 2021–2022 at Medes Islands; from 2013 to 2022 at Santa Croce; in 2010, 2013, 2015, 2019, 2021, and 2022 at Pianosa; from 2008 to 2012 and from 2015 to 2023 at Cabo de Palos; in 2017 at Marettimo; and continuously from 2019 to 2023).

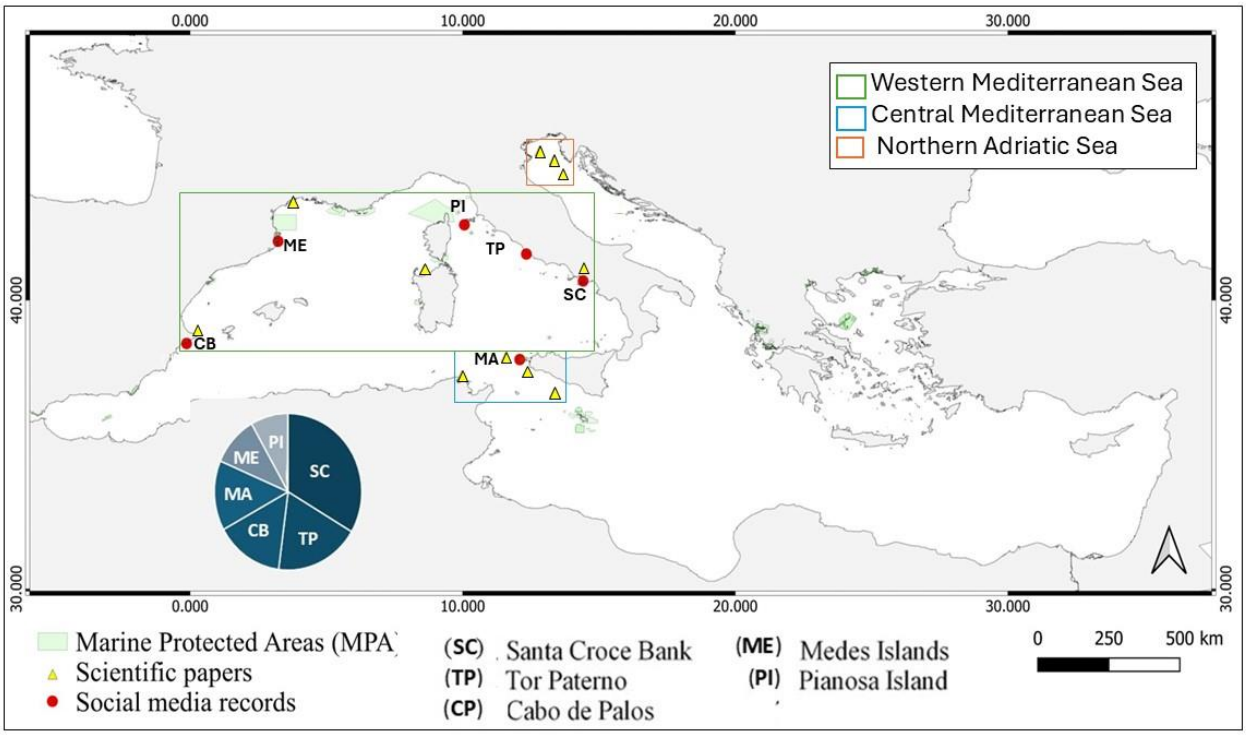


Figure 3. Central – Western Mediterranean Aggregations of eagle rays: Identified aggregations based on scientific literature and social media. The pie chart represents the proportion of videos uploaded for each site.

Visual content uploaded on social media platforms reported 488 aggregated individuals sighted in summer, 76 in autumn, 9 in spring, and 10 in winter. The $\chi 2$ test revealed significant differences between seasons ($\chi 2$ $p<0.05$), with the residuals indicating that summer contributes the most to the total variation (residual=33). The post hoc pairwise with Bonferroni correction showed that all seasonal comparisons were significant (post hoc pairwise $p<0.001$), except for spring and winter (post hoc pairwise $p>0.05$). The $\chi 2$ test performed across months revealed a significant difference in the number of sightings ($\chi 2$ $p<0.05$). September is the major contributor to the total variation (residual=29.74), followed by October (14.64) and August (8.25), while other months have negative residuals, with the lowest values in January, March, and April (7.01). Post hoc pairwise comparisons with Bonferroni correction confirmed that sighting frequencies from July to October are significantly higher than those in January–June and November–December. The strongest differences were observed between September compared to all other months. The binomial tests revealed significant female-biased distributions at most sites (Tab.2).

At five of the six locations, the proportion of females was higher than expected by the null hypothesis of a 1:1 sex ratio. Cabo de Palos (Mean MaxN F=2, Mean MaxN M=0; $p<0.05$), Medas Islands (Mean MaxN F=1, Mean MaxN M=0; $p<0.05$), Marettimo (Mean MaxN F=4, Mean MaxN M=0; $p<0.05$), Pianosa Island (Mean MaxN F=2, Mean MaxN M=0; $p<0.05$), and Santa Croce Bank (Mean MaxN F=1, Mean MaxN M=0; $p<0.05$) have a significantly higher numbers of females recorded.

Only Tor Paterno (Mean MaxN F=1, Mean MaxN M=1; $p>0.05$) did not show a significant deviation, indicating a balanced sex ratio. The total mean female-to-male ratio across all sites was twelve females for each male. A video captured in Santa Croce Bank on August 22nd, 2015, documented two individuals with mature claspers, representing the maximum number of males recorded together in social media content. Females were sighted 224 times across all study sites, with a maximum of seven individuals recorded simultaneously on August 24th, 2013, in Pianosa Island.

3.2 Biological characterization of the eagle-ray aggregation in Marettimo Island

Since 2020, URUVs and DOVs surveys carried out in the aggregation area of Cala Martina recorded a total of 6231 sightings. The largest group of individuals was recorded in October 2023 by URUVs (MaxN=101 ind. h^{-1}), along with the maximum number of males (MaxN=4) and females (MaxN=75). No eagle-rays were observed during spring and earlier summer months, despite regular diving activity by local diving centers operating around the island and annual targeted surveys (First author personal observation). For 589 sightings, it was possible to assign the sex to the recorded individuals (43 sightings of males and 546 of females). Moreover, size estimates were possible for 434 sightings in 2022 (of which 3 males and 17 females) and for 123 sightings in 2023 (of which 4 males and 35 females). The average female-to-male ratio was 14 females for each male in DOV transects and 21 females for each male in URUVs. The Wilcoxon rank sum test confirmed significant differences in the MaxN of males and females recorded during 10 minutes of DOV transects (Wilcoxon rank sum $p<0.05$) (Fig. 4). Differences between sightings of males and females were also confirmed by the χ^2 test within all three life stages (χ^2 $p<0.05$). The sex ratio, calculated on the mean MaxN of the three main life-stages, revealed 10 juvenile females for each male, 15 sub-adult females for each male, and 11 adult females for each male (Fig. 4). The Kruskal-Wallis revealed

significantly different abundances between the three life-stages in males (Kruskal-Wallis $p<0.05$). The posthoc Dunn's test showed that males are mostly adult (530-1330 mm DW) in both 2022 (17 sightings and MaxN=3) and 2023 (37 sightings and MaxN=4) (Fig. 4.1). Sub-adults (490-500 mm DW) were observed nine times in 2022 and 10 times in 2023 (MaxN: 1 and 1 respectively), while juvenile males (340-420 mm DW) were only recorded in 2023, with 6 sightings and MaxN 1. Adults and sub-adults male sighted and sized in 2023 (n=47) and 2022 (n=22 sightings) fell within the size classes reported by Capapé et al., 2007 as corresponding to sexual maturity (DW greater than 500–540 mm) off the coast of Languedoc. Moreover, calcified claspers extending beyond the pelvic fins were observed, even among smaller individuals (490 mm DW), supporting the identification of these specimens as mature males. Immature females (DW below 480-580 mm) were recorded both in 2022 and 2023 (9 sightings in 2022; 11 sightings in 2023). The Kruskal-Wallis test found statistically different abundances between life stages in females (Kruskal-Wallis $p<0.05$), mostly represented by adults (740 – 190 mm DW) as revealed by the Dunn's test (Fig. 4.2). The aggregation site hosted pregnant females, with 97% of sightings occurring in August in 2021, 2022 and 2023. Four pregnant eagle-rays were filmed together in August 2022, which represented the highest number observed in a single frame. Pregnant females were sighted in 4% of total sightings across the on-site dataset (both in URUVs and DOVs). Two mature females were identified by natural marks in 2020 and 2021 and resighted in 2022 and 2023 (Fig. 4).

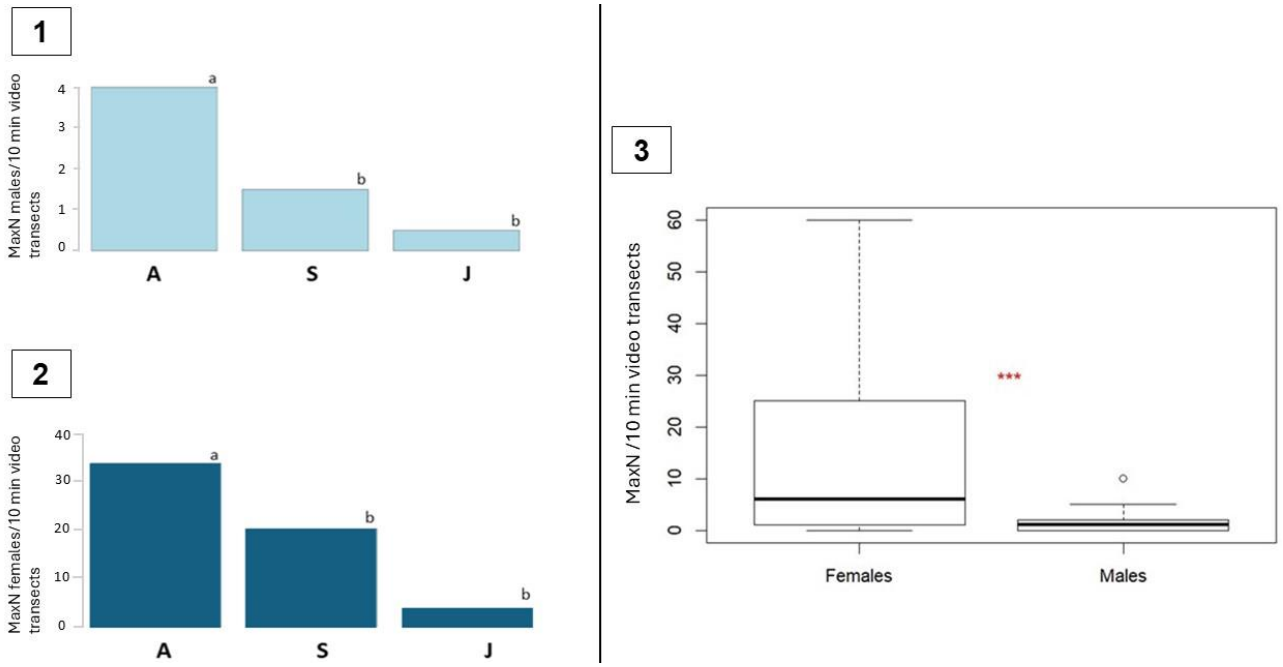


Figure 4. Population structure in Marettimo aggregation site: Left panels: Differences in life stages (A: adult; S: sub-adult; J: juveniles) between males (1) and females (2) sized in 2022 and 2023. Letters indicate the results of Dunn's test. Panel (3): Differences in the maximum number of females and males recorded by DOV transects in 2022 and 2023. Statistical significance was based on the results of the Wilcoxon rank sum test.

A mature female, identified by a dorsal deformation, was recorded in 12 videos between 2021 and 2023, with a frequency of occurrence of 2% (Fig. 5). Another mature female, identified by the absence of a tail and for white stripes over pelvic fins and head, appeared in 35 videos between 2020 and 2023, with a frequency of 6% (Fig. 5).

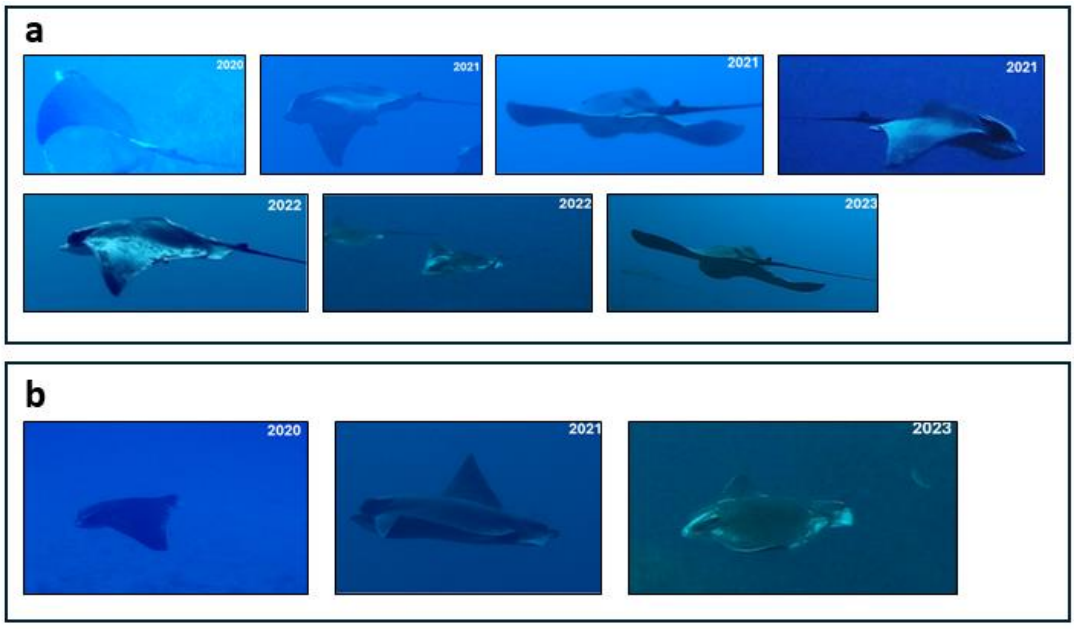
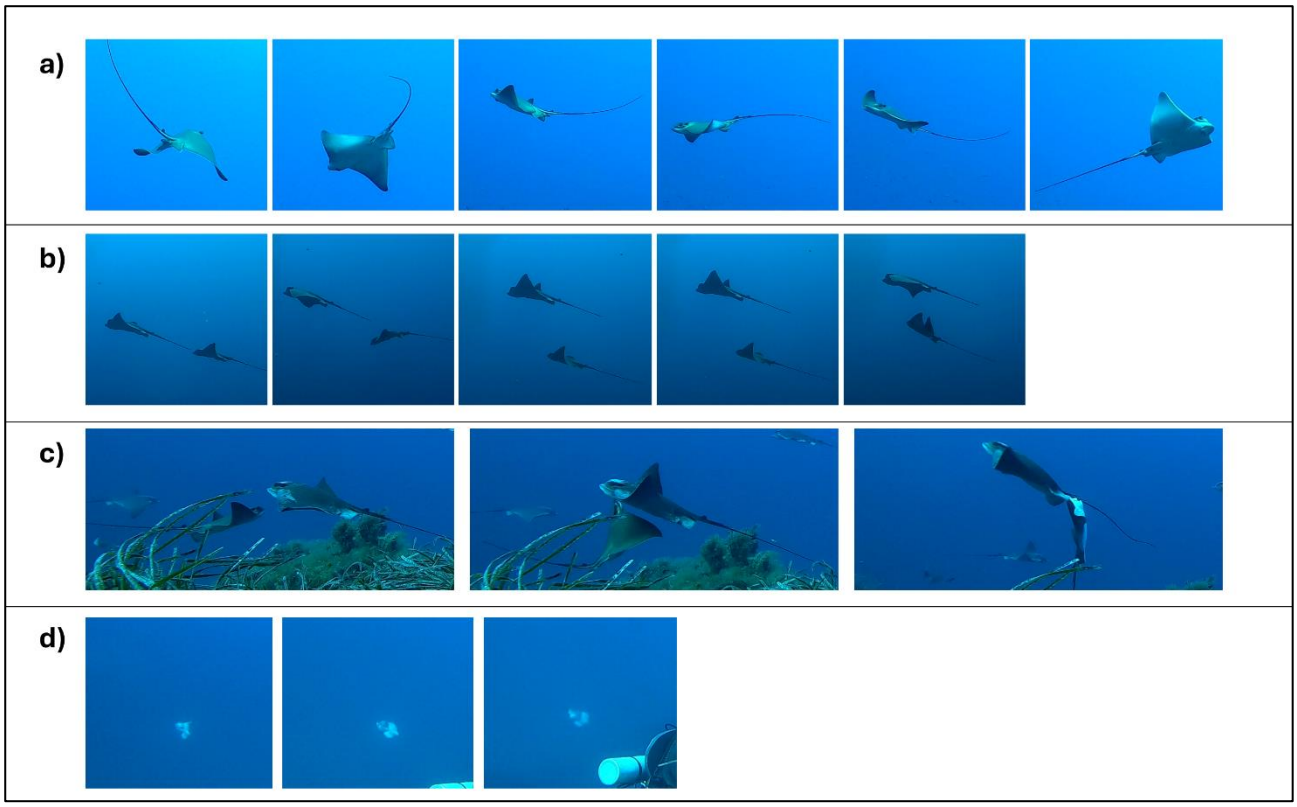


Figure 5. Individuals identified by natural marks at Cala Martina site across years. a) A female *M. aquila* with a dorsal deformation resulting in a hump; b) A *M. aquila* with a cut tail and white stripes over pelvic fins and head.

A total of 134 reproductive interactions were documented in summer 2022 on 78 DOV and 67 URUV surveys (n=104 events on 65 DOV and 63 URUV surveys in August, n=25 events on 10 DOV and 4 URUV surveys in September, n=5 events on 3 surveys in October) and 34 events on 14 DOV and 2 URUV surveys in October 2023. The $\chi 2$ test performed on the number of times each reproductive behavior was observed showed statistical differences (χ^2 $p<0.05$), with chasing of conspecific females being the most common mating phase

documented (n=91 in 2022; n=15 in 2023), followed by courtship displays (n=38 in 2022; n=17 in 2023). We observed males contacting females by biting them in the ventral area (near the pectoral and pelvic fins) in seven events in 2022 and two in 2023, while mating was recorded on three occasions in August 2022 (Fig. 6).



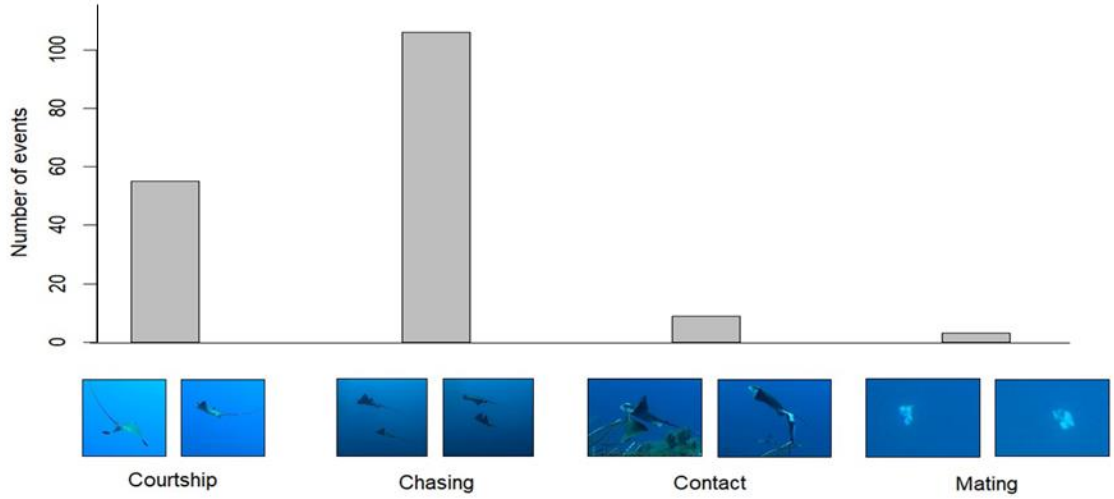


Figure 6. Top panel: Number of courtship/mating interactions recorded by DOVs and URUVs in Marettimo aggregation spot. a) Courthip; b) Chasing; c) Contact; d) Mating. Bottom panel: Number of courtship/mating interactions recorded by DOVs and URUVs in Marettimo aggregation spot.

3.3. Interactions with recreational scuba diving (ray-watching) in Marettimo Island

According to the Egadi Islands MPA visitor register, the number of scuba divers visiting the eagle-ray aggregation spot increased from 266 in summer 2021 to 537 in 2022, likely due to the high probability of encountering eagle-rays in the wild and to the spread of video content on social media. Significant differences were found by the Wilcoxon tests between low- and high-impact diver conditions (Wilcoxon test $p<0.05$) (Fig. 7 a; 7 b). A linear regression between the number of divers at the aggregation site and the average maximum number of eagle-rays showed a significant negative relationship between the two variables. The regression model explained 50% of the variation, indicating a statistically significant inverse correlation ($r^2=0.55$; $p=0.013$) (Fig. 7c). A piecewise regression analysis identified a significant threshold at approximately 5 divers (±1.01 SE). Below this threshold, the decline in the number of eagle-rays is less pronounced (Fig. 7d). When the number of divers exceeds the breakpoint, the number of rays in the aggregation remains stable, suggesting that diver presence beyond this threshold does not further affect aggregation size.

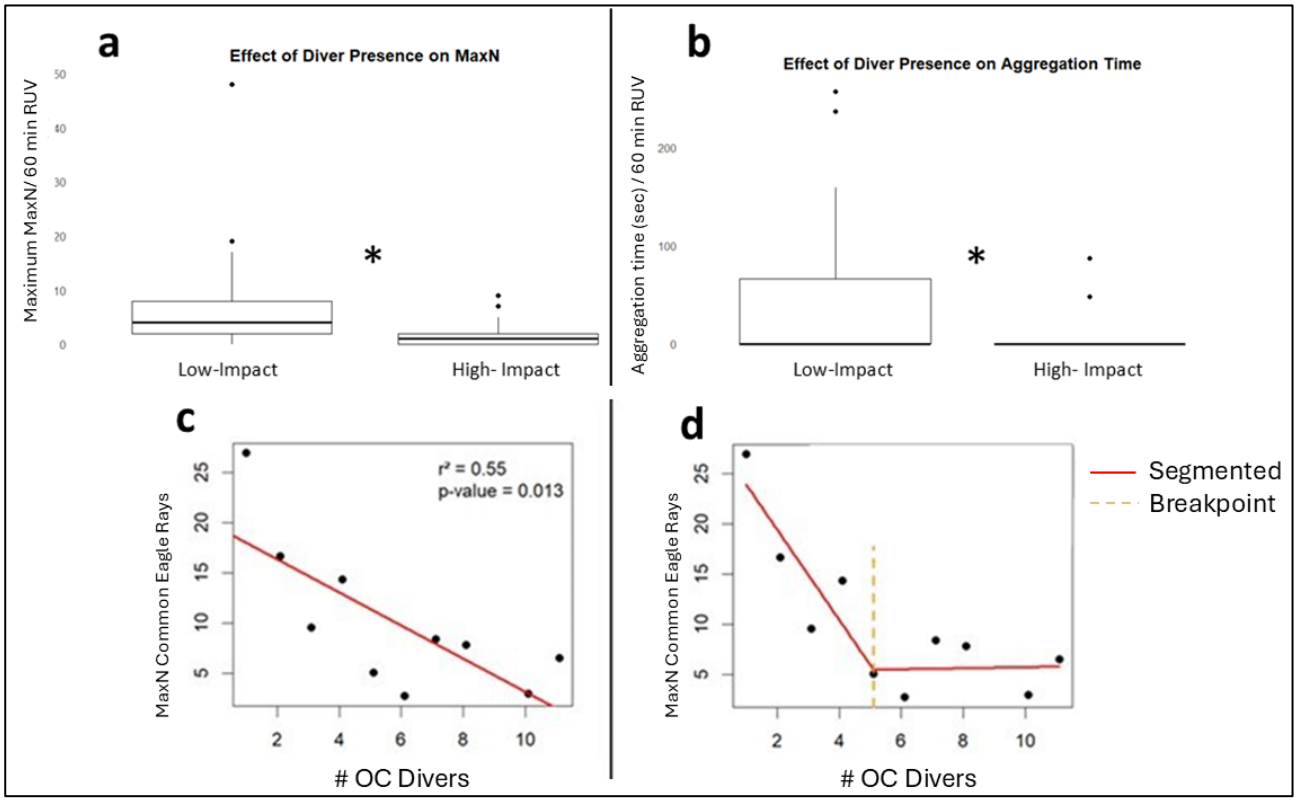


Figure 7. **Impact of diver presence on eagle-rays aggregation:**

(a-b) MaxN and aggregation duration (sec.) recorded during URUV deployments under low- (presence of single CCR technical diver) and high-impact (presence of OC divers) conditions. * Indicate statistically significant differences based on Wilcoxon rank-sum tests ($p<0.05$).

(c) Linear regression showing the relationship between the number of divers and the mean MaxN of eagle-rays ($R^2=0.55$, $p=0.013$).

(d) Segmented regression analysis identifying a breakpoint at 5 divers, beyond which the number of rays significantly drops.

3.4 Biotic indicators from field surveys to track aggregations through social media

Social media contents are aligned with field observations in seasonal patterns and sex ratios. Peaks in sightings of aggregated individuals occurred from summer to early autumn across all monitored sites, in both social media and field-based datasets (Fig. 8).

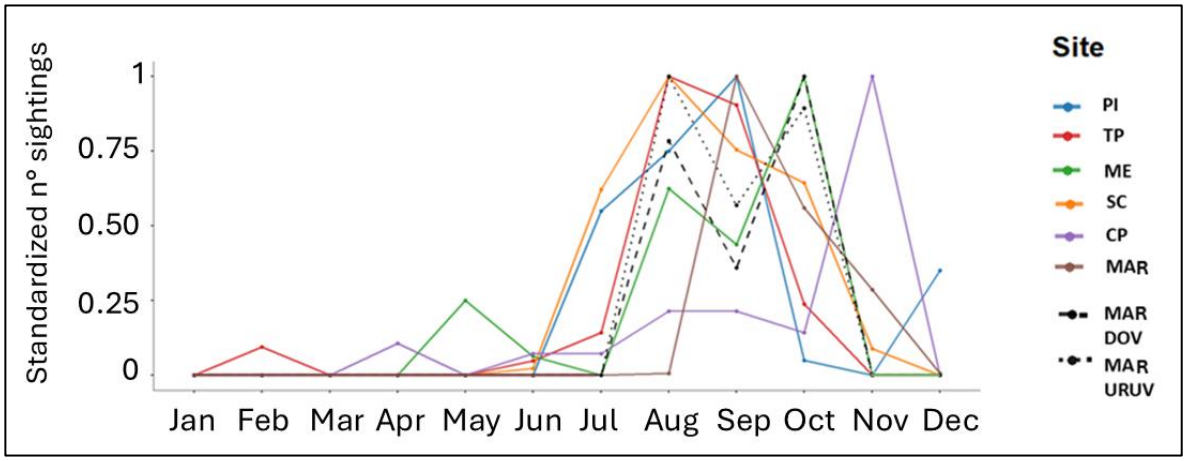


Figure 8. **Scaled monthly sightings of eagle-rays across Mediterranean sites**. Colored lines indicate sites identified through social media data mining: PI (Pianosa Island), TP (Tor Paterno), ME (Islas Medas), SC (Santa Croce Bank), CP (Cabo de Palos), and MA (Marettimo). Dashed lines indicate field-based observations from Marettimo, using DOV (black dashed line) and URUV (black dotted line).

Field surveys using video methods were conducted during the summer and autumn months in 2022 and 2023, as no individuals were reported in the area during visual surveys in the late spring and early summer of 2020 and 2021. The female-to-male (F: M) ratios derived from social media for Pianosa, Medes Islands, Cabo de Palos, and Marettimo were not significantly different from those collected via URUVs at Marettimo (Wilcoxon rank-sum tests, $p>0.05$). For Tor Paterno and Santa Croce sites, the sex ratio was comparable to that recorded in Marettimo by DOV (Wilcoxon rank-sum tests, $p>0.05$). Overall, the sex ratio patterns derived from social media are generally consistent with those obtained through field-based methods, although the degree of alignment varies depending on the observation system used (DOV and URUV) (Tab.2). All the aggregation sites identified by data mining hosted pregnant females. The highest percentage of pregnant females (51%) was observed at Santa Croce Bank, followed by Medas Islands (33%), Pianosa Island (29%), and Tor Paterno (27%). Lower percentages were recorded at Cabo de Palos (10%) and Marettimo (4%) (Fig. 9). The proportion of pregnant females recorded in Marettimo was about 4% across all techniques (10 pregnant sightings on 262 female sightings by URUVs, 11 pregnant sightings on 284 female sightings by DOVs, and 4 pregnant sightings on 106 female sightings on social media), calculated relatively to the total number of female sightings with each technique. This

proportion was higher in other locations, especially in Santa Croce, where over half of the female sightings involved gravid individuals.

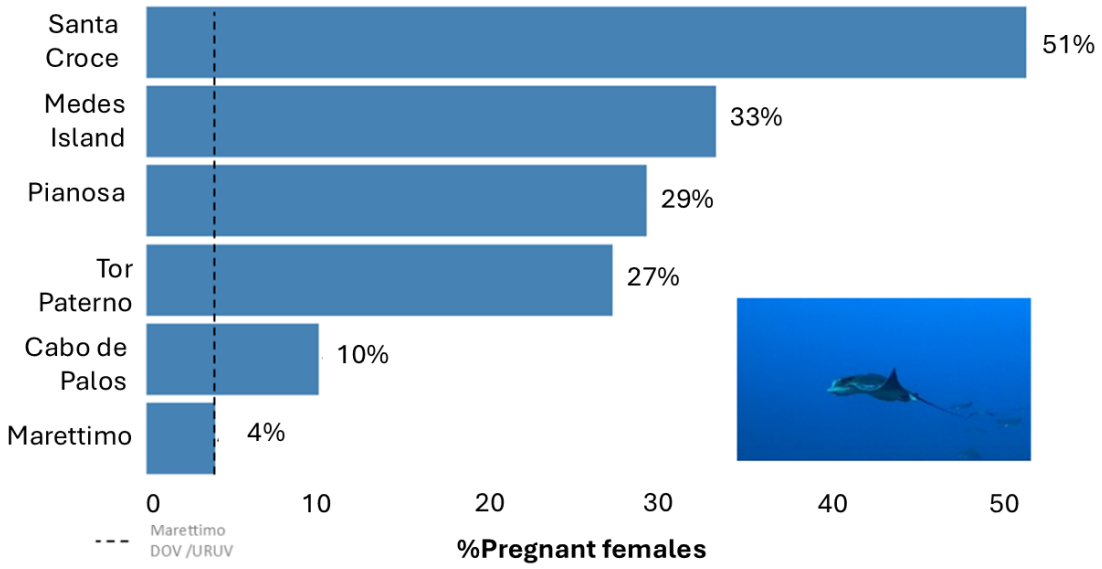


Figure 9. Pregnancy evidence: Percentage of pregnant females of eagle-ray at each area identified by social media data mining. Bars are the proportion of gravid females relative to the total number of sighted females per site. The vertical dashed line represents the percentage recorded in Marettimo through field observations. A pregnant female, with posterior-dorsal swelling, recorded in Marettimo in August 2022, is shown in the photo. Courtship and mating behaviors in social media footage were observed only once at Tor Paterno and Marettimo. These behaviors were more frequent in field observations, with 75 interactions recorded via DOVs and 38 via URUVs in Marettimo (Tab.4).

Table 4. Monthly maximum MaxN ($MaxN_{max}$) of eagle rays recorded at different Central-Western Mediterranean sites based on social media (a) and field observations using DOV and RUV (b) in Marettimo island. For each site, data include monthly $MaxN_{max}$, female-to-male sex ratio (F:M), percentage of pregnant females and number of courtship/mating events. The results of Wilcoxon tests comparing social media and field observations sex ratios with DOV and RUV

a)

MaxN from social media footage																	
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**F:M**	**% of pregnant females**	**Courtship/ Mating Events**	**Wilcoxon test MA DOV**	**Wilcoxon test MA RUV**
MA	-	-	-	-	-	-	1	36	18	22	-	2	08:01	29	0	*	
TP	-	2	-	-	1	2	4	2	4	-	-	-	01:01	27	1		*
ME	-	-	-	3	1	-	2	3	6	-	-	-	03:01	33	0		
SC	-	-	-	-	1	9	10	6	17	3	-	-	02:01	51	0		*
PI	-	-	-	-	-	10	5	17	1	-	-	5	--	10	0	*	
CP	-	-	-	3	1	2	2	6	2	16	-	12	06:01	4	1	*	

b)

MaxN from DOV and RUV in Marettimo island															
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**F:M**	**% of pregnant females**	**Courtship/ Mating Events**
MA DOV	-	-	-	-	-	-	-	55	**92**	80	-	2	04:01	4	75
MA RUV	-	-	-	-	-	-	-	48	39	**101**	-	-	08:01	4	38

4. Discussion

This study integrated scientific literature, social media, and multi-year field data to identify the spatio-temporal distribution of eagle-ray aggregations in the Mediterranean Sea, offering a replicable framework for data-poor regions. Data from scientific literature relied on fisheries-dependent sources gathered through monitoring landings, Local Ecological Knowledge, and scientific fishery surveys, aligning with the results of prior studies (Bousquet et al., 2024; De Santis et al., 2025). Social media analyses revealed aggregations with seasonal recurrences in six locations of the western and central Mediterranean Sea, four of them

already recognized as “potential aggregation sites” or “reproductive aggregations” ISRA (Murcia Eastern Coast, Santa Croce Bank, and Egadi Archipelago), highlighting the efficacy of opportunistic content in supporting spatial conservation for endangered elasmobranchs (Bradai et al**.,** 2012; Ferretti et al., 2008). Scuba divers were the sole video uploaders and, as taking and sharing underwater images is easier and more frequent than conducting scientific surveys, opportunistic footage posted on social platforms is a powerful and low-cost method for monitoring marine ecosystems (Otero et al., 2024; Roberts et al., 2023). Social media content are useful data sources for studying elasmobranchs (Cattano et al., 2023; Giovos et al., 2019) but require validation through standardized on-site surveys (Vianna et al., 2014; Ward-Paige & Lotze, 2011). The Marettimo case study provided a dashboard of biotic indicators (such as relative abundance, life stages and sex ratio) that, when coupled with social media content, can preliminarily identify conservation interest sites, as in other studies targeting elasmobranch distribution (Boldrocchi & Storai, 2021; Kabasakal, 2021). Our results suggest that juvenile stages of both sexes were less abundant than sub-adult and adult stages during Summer, similar to a study conducted in Northern Patagonia (Argentina) that revealed the presence of *Myliobatis goodei* (Garman, 1885) juveniles year-round and adults entering a bay in austral spring and summer, speculating this spot may serve as a mating ground (Molina & Lopez-Cazorla, 2015). The sex ratios estimated from social media and at the Marettimo site were skewed toward females. An unbalanced sex ratio is a diffuse response to different social, thermal or foraging requirements in many elasmobranchs (Magurran & Macias Garcia, 2000; Wearmouth & Sims, 2008). Some sites showed high percentages of gravid individuals, exceeding those observed during surveys in Marettimo. This overrepresentation may be due to behavioral factors, as larger and slower-moving pregnant females are more likely to be approached by divers. However, this also raises concerns about disturbance during vulnerable reproductive phases. While interannual records of more than 100 courtship and mating interactions were recorded in field surveys, only two videos from social media captured reproductive behaviors (courtship or mating), suggesting that divers' presence may inhibit such interactions, which are underrepresented in opportunistic footage. As a result, although social media is useful for identifying aggregation areas and general demographic patterns, it is less reliable for behavioral studies that require controlled, minimally invasive observation protocols. Elasmobranchs' gregarious tendencies support

ecotourism activities worldwide (Vaudo et al., 2017), enforcing conservation through public awareness and engaging stakeholders (Ward-Paige, 2017). However, unmanaged ecotourism can be detrimental to aggregations due to an increase in visits to aggregation sites (Ripple & Beschta, 2006). Since 2019, social media content has spread information on the presence of congregated eagle-rays in Marettimo, resulting in an increased presence of divers. According to our findings, the number of congregated eagle-rays decreases significantly with more than five scuba divers at the site. This suggests that unregulated tourism can interfere with natural behaviors, as observed in other Mediterranean elasmobranchs aggregation spots (Cattano et al., 2021; Shamir et al., 2019). We recognize some limitations of the present study. We caution that our findings did not consider environmental factors, as well as both small-scale and long-range movements. Future research should be focused on collecting crucial information for triggering and predicting the formation of aggregations in additional areas and to understand the overlaps with human activities (Klimley et al., 2022). Moreover, divers' records are biased toward specific depth ranges, popular diving sites and most favorable seasons, even if in our case study, seasonal aggregation patterns are consistent with literature-based data from fisheries and scientific surveys (Afonso & Rodriguez, 2015; Barbato et al., 2021; Bonanomi et al., 2018; Consoli et al., 2016; La Mesa et al., 2016). Finally, our social media-based dataset does not include records from North Africa or the Levantine Basin, reflecting the less developed scuba diving industry in North Africa, resulting in fewer videos shared online from those regions, and the different environmental conditions that may play a role in shaping the species' distribution. According to the *Habitat Suitability Map* available on FishBase (Scarponi et al., 2018), areas of the eastern Mediterranean show lower suitability for eagle-rays. Published literature suggests that the species is generally rare in the eastern Mediterranean, scattered records were reported from the Aegean Sea (Kallianiotis et al., 2020; Roditi & Vafidis, 2022). Despite this, our study demonstrates that footage by recreational divers can provide a set of useful biotic indicators such as the relative abundance of congregating individuals, their sexual maturity and sex dominance in different locations and over time.

4.1. Implications for conservation

This study underscores the importance of identifying and monitoring aggregation sites for threatened elasmobranchs as a conservation priority in the Mediterranean Sea. Seasonal

aggregations occur in predictable coastal areas, making them vulnerable to human disturbances such as unregulated scuba diving. The integration of social media data with scientific monitoring offers a cost-effective approach to detecting these events, especially in data-poor regions. Although our direct scientific surveys are locally confined to the "Egadi Islands" MPA, the identification of other potential Mediterranean aggregations through data mining emphasizes the urgency to enhance efforts in collecting data and to mitigate human impacts with site-based measures. Our results highlight the risk that increased visibility may attract uncontrolled tourism, potentially altering natural behaviors. We recommend site-specific management measures, such as codes of conduct for divers and temporal restrictions during peak aggregation periods. The adoption of good practices to regulate the eagle-ray watching activities could serve as an example for other Mediterranean elasmobranchs aggregation hotspots. Although MPAs can be effective tools to protect endangered species (Di Franco et al. 2018), specific conservation strategies for elasmobranchs must include actions targeting aggregation areas, especially when these sites serve as reproductive grounds. No Mediterranean MPAs were designed to specifically limit human impacts on elasmobranchs aggregations (Cattano et al. 2021; Di Lorenzo et al., 2022). These recommendations gain relevance considering recent policy advances of the 23rd Meeting of the Contracting Parties to the Barcelona Convention, when the species was added to Annex II. Moreover, we proposed biological indicators derived from social media that can support early identification and prioritization of critical habitats for targeted protection. For instance, the 2002 Recovery Plan for the Grey Nurse Shark (*Carcharias taurus* (Rafinesque, 1810)) in Australia, where *critical habitats* were identified, even when initially based on anecdotal or opportunistic observations (Cochran et al., 2019; Lynch et al., 2013).

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Chapter 3: Preliminary evidence of a potential reproductive aggregation area of the common stingray Dasyatis pastinaca (Linnaeus, 1758) (Chondrichthyes – Dasyatidae) in the Central Mediterranean Sea

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Abstract

Shallow and coastal aggregations of batoids are poorly documented in the Mediterranean Sea, despite being likely threatened by multiple anthropogenic pressures. We report the first observations of a potential summer reproductive aggregation of the common stingray (*Dasyatis pastinaca*). The recurrent presence in previous years of several pregnant females, a mature male and a female with fresh bite wounds was recorded in shallow waters by recreational scuba divers and scientists in Scilla Bay (Strait of Messina, Italy), a location with unique oceanographic features in the Central Mediterranean Sea. These observations suggest that the area could be used as a parturition and mating site in early summer, and this is significant for the conservation of this Vulnerable species.

1. Introduction

The identification of spatio-temporal patterns of distributions related to critical life stages of threatened elasmobranchs is key to their effective conservation and potential recovery from direct and indirect human-induced threats (Hyde *et al.,* 2022). This is particularly true within the Mediterranean Sea, a known hotspot of extinction risk for sharks and rays with a concerning decline of their populations and no signs of improvement in their conservation status for many years (Dulvy *et al.,* 2014; Dulvy *et al.,* 2016; Milazzo *et al.,* 2021). Among elasmobranchs, batoids have received less scientific attention compared to other vertebrate groups (Aschliman *et al.,* 2012; Bräutigam *et al.,* 2015) and are often studied via fishery-dependent approaches, which fail to capture their abundance and distribution, especially in shallow waters (Ismen, 2003; Morey *et al.,* 2006; Ismen *et al.,* 2007; Geraci *et al.,* 2021; Ruiz-García *et al.,* 2023). Alternative low-impact field surveys, such as direct underwater observations, led to the description of a coastal seasonal aggregation of the common eagle ray *Myliobatis aquila* (Linnaeus, 1758) in Western Sicily (Grancagnolo & Arculeo, 2021) and citizen science has proven to be a valuable tool in detecting rare or temporal events, like reproductive aggregations that may occur over short temporal windows and in restricted areas (Clapis-Garla et al., 2022). Many elasmobranch species are known to form seasonal aggregations for a variety of biological purposes, including feeding, thermoregulation, migratory stopovers, and reproduction (Papastamatiou et al., 2022). Among these, reproductive aggregations are particularly significant as they reflect critical phases of the species' life cycle that are especially sensitive (Carrier & Pratt, 1998; Osgood & Baum, 2015). Here, we specifically refer to the definition of reproductive areas proposed by Hyde *et al.* (2022) for elasmobranchs: "*…areas where a regular or predictable presence of mature sharks and rays has been recorded for mating, and/or where pregnant females aggregate*".Identifying reproductive areas and the ecological drivers that support them is essential to implementing targeted conservation strategies, particularly in a region as heavily exploited as the Mediterranean (Karampetsis et al., 2022). Reproductive aggregations can represent hotspots of key biological activity and potential conservation bottlenecks, given their vulnerability to spatially and temporally concentrated human impacts such as fishing, coastal development, and tourism (Hueter et al., 2005). High relative abundances of the common stingray *D. pastinaca* have been reported during late spring and early summer off

the Balearic Islands and along the Israeli coast, with a balanced sex ratio of mature individuals, evidence of courtship behaviours and presence of gravid females and juveniles (Morey *et al.,* 2006; Chaikin *et al.,* 2020). In this context, our study reports the first characterization of a potential reproductive aggregation of the common stingray *D. pastinaca* in the Strait of Messina (SoM) – Central Mediterranean Sea -, an important migration route for several cartilaginous fish with a high frequency of records of Critically Endangered species (Canese *et al.,* 2011; Malara *et al.,* 2021; Bargnesi *et al.,* 2022). This study aimed to characterize the recurrent seasonal aggregation of the common stingray within Scilla Bay, using low-impact survey techniques. By combining underwater observations with reports gathered through the involvement of local divers, we documented repeated occurrences of the species across multiple years. Sightings included mature individuals of both sexes, pregnant females, and behavioral cues indicative of reproductive activity. These findings provide the first evidence supporting the identification of this area as a potential reproductive site for *D. pastinaca*, with important implications for species conservation and spatial management in the region.

2. Materials and methods

2.1. Study area

The study was conducted in the shallow waters of Scilla Bay, located on the Northern coast of the SoM (Italy; Fig. 1). The SoM is a narrow channel with an articulated bottom topography and strong tidal currents that separates Sicily Island from the Italian peninsula and connects the Tyrrhenian and the Ionian seas.

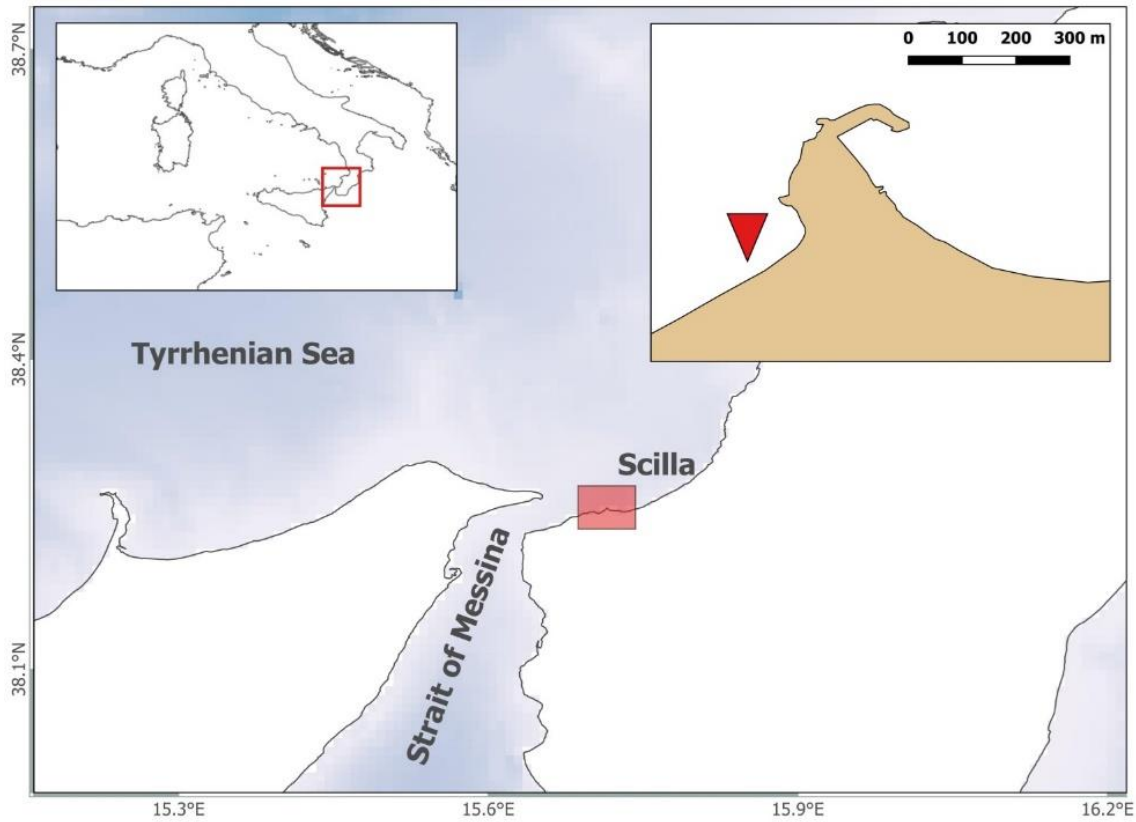

Figure 1. Location of the shallow coastal reproductive area of the common stingray *D. pastinaca* in Scilla Bay in the Central Mediterranean Sea.

2.2. The Common stingray *Dasyatis pastinaca*

Seven species belonging to the Dasyatidae family have been reported in the Mediterranean Sea, including two similar-looking *Dasyatis* species (*D. pastinaca* and *D. tortonesei)* that lead to possible morphological misidentifications (Saadaoui *et al.,* 2016; Serena *et al.,* 2020) despite recently confirmed genetic differences (Vella & Vella, 2021). The common stingray *D. pastinaca* is a large demersal mesopredator that inhabits sandy-muddy bottoms of the North-east Atlantic Ocean and the Mediterranean and Black Seas (Ebert & Stehmann, 2013). The species is considered Vulnerable according to the last IUCN Mediterranean and Black Sea assessment (Dulvy *et al.,* 2016) and is frequently by-caught during artisanal and commercial fishery operations, showing very high fishing mortality rates (Bradaï *et al.,* 2006; Morey *et al.,* 2006; Yeldan *et al.,* 2009; Yemisken *et al.,* 2014), which may be because its venomous caudal sting forces fishers to kill specimens to avoid injuries while freeing them from the nets (Tiralongo *et al.,* 2018). The Tortenese's stingray *Dasyatis tortonesei* (Capapè, 1975) is considered endemic to the Mediterranean Sea (Froese & Pauly, 2019) and has been

assessed as Data Deficient by the global IUCN Red List (Jabado & Derrick, 2020). Its distribution is mostly reported in Maltese and Tunisian waters and along the Levantine basin coast (Vella & Vella, 2021 and references therein), with recent observations in the Balearic Islands (Ramírez-Amaro *et al.,* 2021). In this study our direct observations focused on morphological aspects distinguishable by underwater visual inspection such as the differences in colour of the dorsal part of the disc (from brown to dark grey in the common stingray and brown or ochre yellow in the Tortonese's stingray; Barone *et al.,* 2022), the shape of the head and the snout (which are flatter and shorter in *D. tortonesei* than *D. pastinaca*; Saadaoui *et al.,* 2016) and the coloration of nostrils' and mouth margins (white in *D. pastinaca* and dark in *D. tortonesei*; Barone *et al.,* 2022). The analysis of these characteristics allowed us to attribute the observed individuals in Scilla Bay to the common stingray *D. pastinaca*. In support of our findings, scientific trawl surveys carried out in the same area in the frame of the MEDITS program only reported *D. pastinaca* over several years (Bottari *et al.,* 2014).

2.3. Data collection

Observations were carried out between July 2021 and July 2023 through a combination of structured field surveys and opportunistic reports collected via citizen science contributions. Fieldwork included underwater visual surveys conducted by trained scientific divers along standardized 50-meter linear transects parallel to the shoreline at depths ranging from 8 to 12 meters. Environmental parameters such as water temperature and habitat type were recorded at each survey. Citizen science data were gathered through direct engagement with the local diving community, using a standardized form for reporting sightings of *D. pastinaca*. Reports included date, time, location (GPS or relative position), number of individuals, sex when possible (based on presence of claspers), behavior (e.g., mating, courtship, resting), and any signs of body injury. Data were validated through photo or video documentation, where available and cross-checked with the researcher's field observations.

2.4. Behavioral Assessment

Whenever possible, behavioral interactions such as courtship, mating attempts, and grouping dynamics were noted during both scientific and citizen observations. Individuals were classified as adult or juvenile based on total body size and sexual maturity traits (e.g., claspers

in males). Mating interactions are supported by margin abrasions on the female body, particularly in the posterior half of the disc (Kajiura *et al.,* 2000), while advanced pregnancy can be identified by an evident posterior–dorsal swelling (Chaikin, 2020).

3. Results

A potential reproductive aggregation of the common stingray was documented in Scilla Bay, SoM, through direct underwater observations and citizen science contributions collected between 2021 and 2023. Video footage provided by local recreational scuba divers confirmed the presence of aggregating individuals early in the summers of 2021 and 2022, consistently observed within the same coastal area of the bay. In late June 2023, surveys carried out by scientific divers over three consecutive days recorded a total of 23 stingrays between 8 and 12 meters in depth, with a maximum of 15 individuals counted along a single 50-meter transect. On 25 June 2023, six visibly pregnant females were identified, four resting on *Posidonia oceanica* ((Linnaeus) Delile, 1813) meadows (Fig. 2A) and two concealed among rocky crevices (Fig. 2B, C). The following day, a diver-led visual census recorded 15 pregnant females, 5 on sand, 10 on *P. oceanica*, during a single transect survey. On 27 June, video recordings also documented a mature male with elongated claspers and a large female with clear mating scars, supporting the hypothesis of reproductive activity occurring in the area (Fig. 2C, D). Water temperature ranged between 22–23 °C during all observations. Across all encounters, stingrays exhibited short-range flight responses (~5 meters) to diver approach and showed visible body and opercular contractions. Surveys conducted simultaneously in adjacent coastal areas did not detect any individuals of *D. pastinaca*, suggesting localized habitat use during the aggregation period.

Figure 2. Pregnant females and a mature male of the common stingray *D. pastinaca* sighted in Scilla Bay in the Central Mediterranean Sea. Panels A and B show two pregnant females resting on a Neptune seagrass meadow and algal rocky substrate; panel C shows a mature male with calcified claspers longer than pelvic fins; panel D shows a female with mating scars on her body. Photo credits: Desirée Grancagnolo and Nunzio Bellantoni.

4. Discussion

This study provides the first evidence of a reproductive aggregation of the common stingray in the Central Mediterranean Sea. The repeated seasonal presence of mature individuals, including pregnant females and sexually mature males, coupled with observations of copulation scars, supports the classification of Scilla Bay as a potential reproductive and parturition area for the species. This interpretation aligns with the definition of reproductive sites in elasmobranchs, where mature individuals gather regularly for mating and/or parturition (Hyde et al., 2022). The variety of available habitats in Scilla Bay, including sandy bottoms, rocky areas, and *P. oceanica* meadows, may offer suitable conditions for resting, refuge, and reproductive behavior. The presence of both sexes in the same location, with visible evidence of courtship or post-mating interactions, strengthens this hypothesis. Similar aggregative patterns have been reported for *D. pastinaca* in other parts of the Mediterranean (Morey et al., 2006; Chaikin et al., 2020), though never before in the Central Mediterranean Sea. Although the information on the reproductive behaviors of the common stingray is scant,

females mating within minutes or hours after parturition was previously documented for the congeneric southern stingray *Dasyatis americana* (Chapman *et al.*, 2003). In this regard, our observation of a mixed-sex aggregation with a large female showing evident copulatory scars may suggest that mating events may also occur in Scilla Bay. The involvement of citizen scientists, like trained scuba divers, was key in detecting this short-temporal and spatially restricted phenomenon. Their observations extended the temporal span of data collection and allowed researchers to document reproductive indicators. This reinforces the role of citizen science in monitoring rare, ephemeral biological events that require rapid and repeated observations across seasons (Clapis-Garla et al., 2022; Tiralongo et al., 2019). From a conservation perspective, the congregation of reproductive individuals in a well-frequented coastal area raises concern over potential human-induced disturbances. Recreational diving, boating, and coastal fishing are particularly intense in Scilla Bay during summer months—coinciding with the stingray's presumed breeding period. Unregulated human activity during this sensitive phase may disrupt mating behavior, increase energetic stress, or reduce reproductive success. As shown in other ray species, even short-term human disturbance during parturition or mating may have population-level consequences (Chapman et al., 2003; Jacoby et al., 2012). Given the vulnerability of *D. pastinaca*, characterized by relatively low fecundity (Saadaoui et al., 2015), and the region's overexploitation (Ferretti et al., 2013), targeted management actions are warranted. Ecotourism activities should be promoted and regulated to limit any potential negative effects on the species' behaviours during its congregation (Cattano *et al.,* 2021) and to develop public awareness of their conservation priority (Cattano *et al.,* 2023). We recommend implementing seasonal restrictions on fishing, scuba diving, and boat traffic during peak aggregation periods rather than enforcing year-round closures. This adaptive approach could protect during critical life stages without significantly disrupting local economic activities. This study adds to previous recommendations suggesting that identifying aggregation areas for coastal rays and using non-invasive techniques for the conservation of threatened species and for ensuring their population replenishment (Palacios *et al.,* 2023). Finally, our findings contribute to the designation and management of Important Shark and Ray Areas (ISRAs) in the Mediterranean, including the Strait of Messina (IUCN SSC, 2023). Identifying and protecting reproductive habitats such as Scilla Bay is crucial for ensuring the long-term viability of

threatened elasmobranch populations and promoting coexistence between conservation needs and human use. Engaging local communities, divers, and recreational users in data collection enhances both spatial and temporal coverage, increasing the likelihood of detecting significant phenomena and informing conservation actions.

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Chapter 4: Behavioral Facilitation at Shark Aggregations: Concealment Hunting by Blue Runners behind Sandbar Sharks

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D. Grancagnolo contributed to the conception of the study, field data collection, data analysis, interpretation of the results and manuscript editing.

Abstract

Predators rely on a variety of strategies to approach prey undetected. While camouflage and ambush tactics are well documented, concealment through interspecific associations is less explored. Here, we provide the first documentation of shadowing behavior by the blue runner using sandbar sharks as mobile cover to approach prey shoals in a temperate marine environment. Observations were made over multiple years at a seasonal sandbar shark aggregation site at Lampione Island (Southern Mediterranean Sea), using Baited Remote Underwater Video and Dive-Operated Video techniques. Shadowing blue runners remained near sharks, synchronizing their swimming direction and speed, and left the association only briefly to perform high-speed predation on damselfish and picarels. Compared to unassociated individuals, shadowing runners triggered significantly fewer antipredator responses from prey, indicating delayed detection and potentially enhanced hunting success. These findings suggest that large-bodied shark aggregations serve as ecological facilitators of rare foraging behaviors in other species, expanding their functional role within the community. Protecting shark aggregation sites could preserve emergent behaviors and biodiversity dynamics they support.

1. Introduction

Predation is one of the strongest selective forces in nature and has driven the evolutionary arms race, leading to the development of morpho-behavioural traits in both predators and prey aimed at improving hunting and defense/escape efficacy (Dawkins & Krebs, 1979).

Predator success depends on the ability to approach prey as closely as possible without being detected, with camouflage representing one of the most widespread strategies to enhance hunting success (Stevens & Merilaita 2009; Smith & Ruxton 2018). While camouflage typically involves morphological adaptations or coloration patterns that allow individuals to blend with the environment, alternative concealment tactics may be based on behavioral mechanisms, including interspecific interactions (Lukoschek and McCormick 2000). Among these behavioural tactics, some fish species perform heterospecific associations, generally involving one nuclear and one or more follower individuals (Sazima et al. 2007). These associations may serve to maximize visual occlusion, reduce detection and hinder recognition by prey species. A specific predatory strategy known as 'shadowing' - also referred to as 'riding', 'aligning' or 'hunting by riding' (Ormod 1980; Aronson 1983; Matchette et al. 2023) - consists of a predator swimming closely alongside or behind a larger moving individual to exploit its body as concealment during approach. Shadowing is described as a commensalism interaction, where the follower species benefits, while the nuclear individual -often non-predatory- experiences neither gain nor a loss (Strand 1988; Matchette et al. 2023). Despite its intriguing ecological implications, this behaviour has been poorly documented in scientific literature, and it is mostly reported in tropical coral reef ecosystems, involving piscivorous followers and larger herbivorous nuclear fish (Ormond 1980). In this study, we present the first direct, multi-year observations of a previously undocumented shadowing hunting strategy adopted by the blue runner *Caranx crysos* (Mitchill, 1815), in association with the sandbar shark *Carcharhinus plumbeus* (Nardo, 1827). The blue runner, like other carangid species, is an active pelagic predator capable of performing multiple hunting strategies, with the pursuit hunting -involving acceleration towards prey followed by a return to cruising speed- as the most frequent behavior (Potts, 1983). However, ambush tactics, where predators attack from hidden positions near the sea bottom, have also been reported in congeners (Sancho, 2000). This behavioural plasticity is mirrored in the species' diverse diet, which

primarily consists of small teleost fishes, but may also include crustaceans (Sley et al., 2009) and zooplankton in pelagic zones (Keenan and Benfield, 2003). Predation events observed in this study mostly involved high-speed attacks on schooling prey, such as common damselfish (*Chromis chromis* (Linnaeus, 1758) and picarels (*Spicara* spp.). The shadowing behavior enables blue runners to conceal themselves and approach prey groups without being detected. Teleosts may associate with sharks for various purposes, including increased protection from other predators, enhanced access to food, or to remove parasites from their skin through exfoliation (Andrades et al., 2012; Brunnschweiler, 2006; Fuller & Parsons, 2019; Knochelet al., 2023; Ovegård et al., 2022; Papastamatiou et al., 2007; Williams et al., 2022).To our knowledge, this is the first documented evidence of shadowing behaviour observed in teleosts associated with larger predators, such as a shark, and occurring in a temperate marine environment. Predator aggregation sites, such as seasonal shark gatherings, offer opportunities to observe rare or context-dependent behaviors that are otherwise difficult to detect, such as interactions, predator strategies and community dynamics (Papastamatiou et al., 2022; Sabando et al., 2020; Speed et al., 2011). The seasonal aggregation of sandbar sharks around Lampione Island (Southern Mediterranean Sea) provides a predictable context in which to monitor ecological interactions, such as the shadowing behaviour documented in this study. This aggregation has been recently described and monitored by Cattano et al., 2021 and represents one of the few documented recurring shark aggregations in the Mediterranean basin (Cattano et al., 2023). This remote site falls within a Marine Protected Area (MPA), where tourism activities are regulated through the implementation of a code of conduct and fishing is limited by authorities' restrictions. These management measures have led to a low level of anthropogenic disturbance that allows for observation of natural behaviours and behavioral plasticity in a conservation-oriented, low-impact setting with minimal external interference (Juhel et al., 2029; White et al., 2019). Moreover, the predictable presence of large-bodied predators, like the sandbar shark, may act as a keystone driver structuring ecological networks (Prato et al., 2013). This study aimed to investigate the occurrence, frequency, and functional role of shadowing behaviour performed by the blue runner in association with the sandbar shark around Lampione Island. We hypothesized that (1) blue runners use shadowing as an alternative predatory strategy to reduce detection by prey and increase hunting success; (2) prey groups exhibit delayed or reduced antipredator responses

when approached by shadowing predators compared to unassociated blue runners. Through the combined use of baited and diver-operated video systems, we provided quantitative and qualitative evidence of this association, evaluated its potential adaptive function, and discussed the broader ecological implications of predator-mediated facilitation in marine systems. Our findings suggest that seasonal predator aggregations may not only influence community structure through direct top-down effects (Heupel et al., 2014) but also create ecological windows in which other species adapt their foraging strategies to exploit the presence of dominant predators. This emphasizes the relevance of temporary yet ecologically intense events, which can contribute to the functional diversity of marine communities. In that frame, aggregations like Lampione serve as natural laboratories for uncovering rare or novel ecological processes.

2. Materials and methods

Observations were carried out between 2021 and 2023 around Lampione Island (35°33' N, 12°19' E; Italy, Southern Mediterranean Sea), where a seasonal aggregation of Sandbar sharks occurs each year during the summer months (Cattano et al., 2021; Cattano et al., 2023). Baited Remoted Underwater Video (BRUV) surveys consisted of replicated 1-hour deployments of steel structures equipped with a video camera horizontally oriented towards a metal cage containing ~400g of bait (*Sardinella aurita*; Valenciennes, 1847). BRUVs were deployed around the island in July and September, at depths ranging from 21 to 40 meters over rocky bottoms. To overcome a possible limitation of this technique for behavioral observations (i.e. a fixed field of view that in some cases did not allow predator-prey interactions to be followed throughout their duration), we also conducted Dive Operated Video (DOV) observations in the same periods, between 08:00 am and 05:00 pm and at the same depths (21-40 m). DOVs comprised an aluminum bar holding two GoPro Hero 8 cameras in a single or stereo-view, previously calibrated with CAL software (www.seagis.com.au). In total, we extracted 103 short video clips (54 from BRUV and 49 from DOV) from deployments in which *C. crysos, C. plumbeus*, or both species were detected. All video clips were independently analyzed by two observers using the software BORIS (Friard and Gamba 2016). From BRUV and DOV recordings, we extracted the number of schooling events (coordinated group formations by

prey), the timing of prey response, calculated as the delay (in seconds) between predator approach and the formation of the school. We analysed the BRUV videos to estimate prey group response (in terms of percent number of prey school occurrences) under three different circumstances: I) when single or multiple blue runners approached the prey (n=23 videos); II) when the blue runner approached the prey by swimming close to a shark (i.e. shadowing behaviour)(n=8 videos); III) when a shark approached a prey group (n=20 videos). Additionally, we used DOV observations to calculate the occurrence of prey school formation per time of observation in the presence of hunting blue runners, as well as the average time between the predator approach and the prey school formation. We then compared these measures when the approach was performed by blue runners who were not associated with sandbar sharks and associated with sandbar sharks.

2.1. Statistical analysis

To compare the frequency of schooling events across the three conditions, we used a G-test of goodness-of-fit (Woolf, 1957). To assess pairwise differences, we used a two-sample t-test comparing the number of schooling events under shadowing and non-shadowing conditions. A Welch two-sample t-test was used to compare the response time of prey between the same two conditions (Sakai, 2016). All data were log-transformed to meet the assumptions of normality, which was verified using the Shapiro-Wilk test. All analysis were performed on Rstudio in R environment (R Core Team, 2023)

3. Results

3.1. Predation events and general hunting behavior

A total of 26 predation events by *C. crysos* were recorded throughout the study period (10 from BRUVs and 16 from DOVs). In all cases, predation consisted of high-speed attacks on shoals of small gregarious fishes—primarily *C. chromis* and *Spicara* spp. The most frequently observed hunting strategy consisted of group-based pursuits, with two or more *C. crysos* individuals swimming midwater or close to the seafloor and targeting prey located higher in

the water column. Prey typically responded by forming tightly coordinated schools and darting toward the substrate to seek shelter (Pitcher and Parrish, 1993).

3.2. Shark-blue runner associations

In 34 events (11 from BRUV and 23 from DOV), *C. crysos* was observed in association with a sandbar shark, swimming alongside or beneath the shark while matching its speed and direction (Fig. 1).

Figure 1 – Shadowing behaviour displayed by the blue runner hiding below a sandbar shark before attacking prey groups. The image has been recorded around Lampione island (Pelagie Archipelago, Central Mediterranean Sea) on 11 July 2022.

These associations lasted on average 27 ± 21 seconds in DOV footage and 15 ± 5 seconds in BRUV recordings, although durations are likely underestimated due to camera field-of-view limitations. In 9 out of 34 shadowing events (26%), the blue runner briefly left the shark to attack prey and then returned to its previous position to resume the association. This behaviour was always performed by a single individual in BRUV recordings (100%), and in 87% of DOV cases. Only three DOV recordings showed two or more blue runners competing for the shadowing, with one individual attempting to exclude conspecifics (Fig. 2).

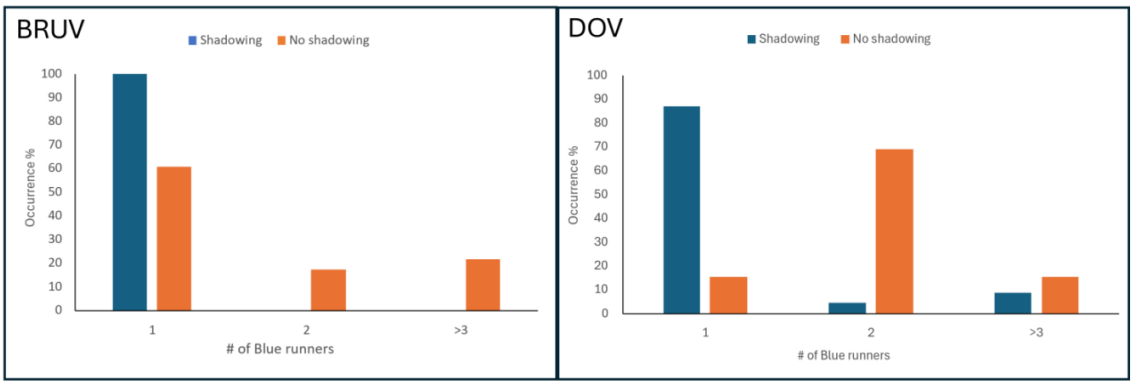

Figure 2 – Percentage occurrence of solitary versus group hunting strategies displayed by the blue runner, whether shadowing or not with sandbar sharks.

3.3. Prey response to predator approaches

School formation by prey occurred in 96% of events with unassociated *C. crysos* (22/23), in 12.5% of shadowing events (1/8) and 15% of shark-only approaches (3/20) (Fig. 3). A G-test confirmed significant differences between the conditions ($G = 30.304$, $df = 2$, $p < 0.001$).

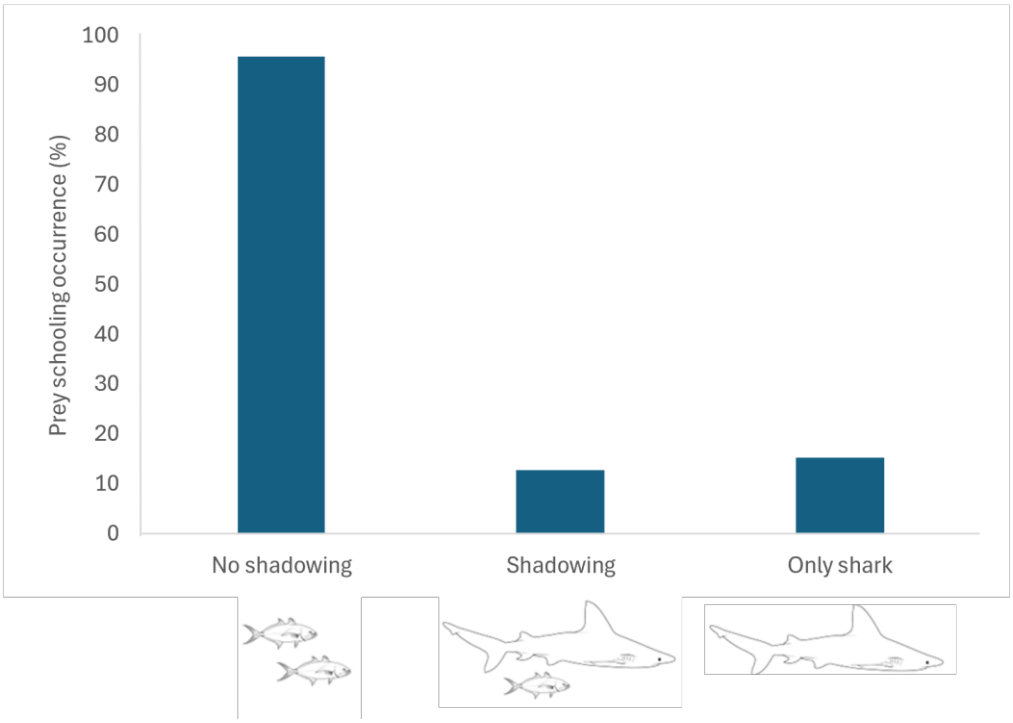

Figure 3 – Occurrence of school formation performed by prey groups in response to approaches of blue runners associated or not with a sandbar shark recorded by BRUVs.

In DOV analyses the number of schooling events was significantly lower in shadowing events ($t = -5.2235$, $p < 0.001$), and the delay in schooling response was significantly greater ($t = -6.9586$, $p < 0.001$), with 0.12 ± 0.03 s mean delay during shadowing events, 6.4 ± 1.6 s mean delay without shadowing (Fig. 4).

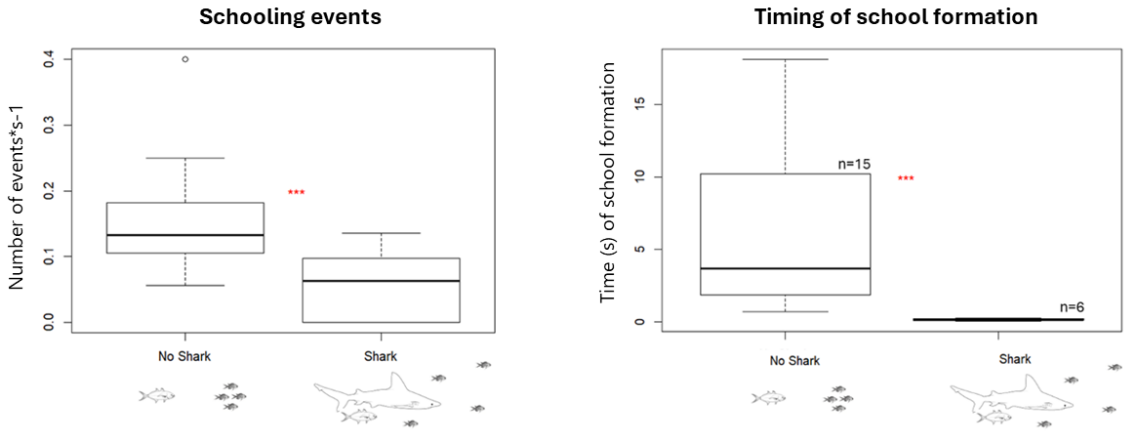

Figure 4 – Response of prey groups (number of schooling formation events and schooling formation delaying time) recorded by DOV to approaches of blue runners associated or not with a sandbar shark.

4. Discussion

This study provides the first documented evidence of shadowing behaviour by a teleost predator (*C. crysos*) associating with a large-bodied elasmobranch (*C. plumbeus*) in a temperate marine ecosystem. Our results indicate that this association enables blue runners to approach prey more closely while remaining undetected, suggesting a predator-concealment function that enhances predator success. This behaviour was observed within a predictable seasonal aggregation of sandbar sharks, highlighting how predator aggregation sites can serve as ecological hotspots where rare or context-dependent interspecific interactions may emerge

and be detected. The shadowing strategy observed here appears to function as a form of dynamic camouflage, with *C. crysos* avoiding triggering early antipredator responses in prey by swimming close to the shark's body. Indeed, our analyses showed that prey groups responded less frequently and more slowly to shadowing predators than to unassociated blue runners. In many cases, prey showed responses similar to those observed when sharks approached alone, indicating that prey likely failed to recognize the presence of the teleost predator while it remained near the shark. Such stealth-based hunting tactics have rarely been described in open-water systems, especially outside coral reef habitats (Peterson et al., 2021). Previous literature has proposed concealment strategies in carangids involving floating objects, jellyfish, or fish aggregating devices (FADs) as anti-predator adaptations (Bonaldo et al., 2004; Carvalho Souza, 2015; Sinopoli et al., 2015), but predator-facilitated ambush strategies have only been hypothesized (Baird, 1993; Ormond, 1980; Mendonça et al., 2011; Sorgon et al., 2021). The observed variability in hunting tactics, from group-based pursuit to solitary shadowing ambushes, highlights the behavioural flexibility of *C. crysos*. Like other carangids, this species is known for its generalist diet and adaptable foraging strategies (Sley et al., 2009; Keenan & Benfield, 2003). By switching tactics depending on context (presence of sharks), *C. crysos* may optimize its foraging efficiency across different ecological scenarios. Shadowing events were performed by solitary individuals, and when multiple conspecifics attempted to shadow the same shark, intraspecific competition was observed. This suggests a potential trade-off, while group hunting may increase attack success in open pursuits, it likely increases prey detection, making stealth-based hunting incompatible with group strategies. Our observations suggest that the association is likely commensal, with blue runners benefiting from concealment while sharks appear unaffected. The absence of clear dietary overlaps (Saidi et al., 2007; Sley et al., 2009) reduces the likelihood of interspecific competition. Moreover, although *C. crysos* is a potential prey item for *C. plumbeus*, the timing and calm nature of the interactions suggest that shadowing likely occurs during periods of low shark foraging activity. In addition to concealment, the association may offer secondary benefits for blue runners, such as hydrodynamic advantage, by reducing swimming effort (Sumikawa & Miyoshi, 2022) and predation risk from other predators while swimming near a large-bodied species (Sheaves et al., 2023). However, these hypotheses require further testing through energetic modelling or tagging approaches. Future research should aim to

quantify foraging success and energetic costs of shadowing vs. other hunting strategies, determine whether shadowing behaviour occurs throughout the day or in specific diel phases, investigate whether and how such associations may influence shark behaviour or movement patterns, assess the role of prey group size, vigilance, and habitat complexity in modulating predator strategy choice (Major, 1978; Polyakov et al., 2022). The rarity of observing such behaviours in the Mediterranean may reflect broader ecological constraints, particularly the scarcity of large-bodied, fast-swimming nuclear species due to historical overfishing of sharks and other top predators (Ferretti et al., 2008). Indeed, *C. plumbeus* populations have experienced regional declines of up to 42% over a decade (Saidi et al., 2020). In this context, seasonal aggregations, such as the sandbar shark around Lampione Island, provide rare ecological windows where heterospecific interactions, like shadowing, can occur. While most facilitative interactions involving sharks reported in the literature involve scavenging, chafing, or risk reduction (Barry et al., 2023; Dove & Robinson, 2021), our study provides novel evidence for their role as active hunting strategies by smaller predators. These findings also illustrate how predator aggregations may foster the diversity of behavioural traits within communities (Cordero-Rivera, 2017), and how aggregation areas can act as natural laboratories for observing rare ecological processes. In summary, BRUVs and DOVs revealed that hunting bluerunners may display an alternative predatory strategy by associating with sharks to conceal themselves and potentially increase their attack success toward prey shoals. Our findings highlight the importance of shark aggregations for studying how the presence of large high-level predators may foster new species interactions and increase different biodiversity facets, thus potentially influencing community structure and function. The sandbar shark aggregation around Lampione Island is a transient phenomenon that may introduce or modify species associations. In this regard, studying communities and species interactions in this location and in other high-level predator aggregation sites may shed light on the ecological importance of species showing spatial and temporal transience, such as many sharks.

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Chapter 5: Exploring aggregation patterns of demersal elasmobranchs in the Mediterranean Sea, using fishery-independent trawl surveys and spatial indices

Abstract

Many sharks and rays form transient aggregations in essential habitats, where individuals of different sexes and maturity stages co-occur to perform key biological functions such as reproduction, nursery, feeding, or social interactions. Understanding the spatial structure of these aggregations is critical to support effective conservation strategies and halt the decline of elasmobranch populations in the heavily exploited Mediterranean Sea. Using 30 years of standardized scientific trawl survey data from the International Bottom Trawl Survey in the Mediterranean program (1994–2023), we investigated sex- and maturity-specific spatial associations in demersal sharks and rays across multiple Geographical Sub-Areas as established by the General Fishery Commission of the Mediterranean and Black Seas. Co-occurrence patterns were quantified using the Half-Weight Index, and spatial dynamics were assessed through patch detection, center of gravity, inertia, and the Global Index of Collocation. Aggregation metrics and multivariate analyses (Between-Class Analysis and PCA) were used to explore intra- and interspecific spatial structure. Our results revealed significant non-random co-occurrences between sex-maturity classes in several species, suggesting that spatial associations may reflect biological functions such as mating or nursery behavior. Immature individuals exhibited more aggregated and spatially heterogeneous distributions than mature individuals, particularly females, who tended to be more dispersed. This clustering of early-life stages suggests the presence of potential nursery areas. Interspecific comparisons highlighted different spatial strategies, with some species (*Scyliorhinus canicula*, *Galeus melastomus*) showing strong aggregation patterns, while others (*Raja clavata*, *Torpedo marmorata*) displayed more uniform distributions. These findings emphasize the importance of identifying persistent aggregation hotspots involving vulnerable life stages and provide a spatial framework to inform targeted conservation planning for deep-sea elasmobranchs in the Mediterranean Sea.

1. Introduction

Many shark and ray species form large conspecific groups known as “aggregations”, which are the co-occurrence of two or more individuals in space and time due to the deliberate use of common drivers (McInturf et al., 2023). These species spend transient phases of their life cycle aggregated in essential habitats, exhibiting site fidelity by seasonally returning to the same locations to perform biological functions such as reproduction, parturition, and feeding (Barnett et al., 2019; Knip et al., 2012; Papastamatiou et al., 2022). For instance, clustered large densities of mature males and females may indicate mating grounds, while dense groups of immature individuals often point to nursery areas with optimal conditions for growth and protection from predators (Heupel et al., 2005; Kinney & Simpfendorfer, 2009).

In the Mediterranean Sea, intense fishery exploitation has driven more than half of the elasmobranch species to be listed as threatened with extinction by the International Union for Conservation of Nature (IUCN) (Walls & Dulvy, 2021). Moreover, the predictable and recurrent presence of a large number of individuals at specific sites may increase their vulnerability to being targeted or by-caught, further intensifying the urgency of implementing conservation actions (Dulvy et al., 2016; Litvinov, 2006; Milazzo et al., 2021). Demersal sharks and rays’ populations are even more susceptible to unregulated fishing due to their K-selected life-history traits - such as slow growth rate and late maturity - exacerbated by deep-sea environmental conditions (low temperature and limited resources) (Simpfendorfer and Kyne 2009; Stevens et al. 2000). Currently, one-third of deep-sea elasmobranchs are already assessed as threatened (Moro et al., 2025) and identifying and protecting hotspots where juvenile or reproductive individuals aggregate is critical to mitigate their ongoing population declines (Heupel, et al., 2018; Kinney & Simpfendorfer, 2009). The effectiveness of conservation and management strategies depends on the spatial structure of aggregation events, which are shaped by a combination of environmental (temperature, depth, substrate) and biological cues (ontogenetic shifts in habitat use, feeding habits, mating, social preferences) (Speed et al., 2010; Guttridge et al., 2009; Mourier et al., 2012).

Understanding the biological mechanisms driving intraspecific aggregations in deep-sea elasmobranchs is challenging due to logistical, technological and financial constraints associated with deep-water research (Finucci et al., 2018). For instance, the depth ranges of

many species exceed the operational limits of most tagging technologies (Rodríguez-Cabello et al., 2016) and baited remote underwater video systems may introduce behavioral biases (McLean et al., 2015). As a result, much of the available knowledge on deep-sea chondrichthyans has historically relied on indirect data sources, such as commercial or scientific trawl surveys (Holt et al., 2013; Moura et al., 2014). In this context, long-term standardized trawl surveys, such as the International Bottom Trawl Survey in the Mediterranean (MEDITS) program, provide a valuable dataset for investigating spatial co-occurrence patterns across broad spatial and temporal scales. By combining 30 years (1994 - 2023) of MEDITS data collected across multiple General Fisheries Commission for the Mediterranean (GFCM) Geographical Sub-Areas (GSAs) with spatial metrics, this study aims to explore the spatial dynamics of sex- and maturity-specific aggregations and evaluate their potential persistence. The aims of this chapter are to: 1) assess whether individuals of different sex–maturity classes within a species co-occur more frequently than expected by chance; 2) identify spatial patches of co-occurrence and assess their temporal persistence; 3) compare intra-specific spatial aggregation patterns across classes and inter-specific strategies using spatial indices and multivariate analysis. I hypothesize that (1) significant co-occurrences between specific sex–maturity classes exist in multiple species and reflect functional biological processes, such as mating, feeding, or nursery-related behaviors; (2) immature individuals exhibit more clustered and aggregated spatial patterns than mature individuals, especially mature females; (3) spatial behaviors differ significantly among species and maturity groups, revealing distinct strategies. By examining both intra- and interspecific aggregation patterns and evaluating their spatial and temporal dynamics, this work contributes to a better understanding of deep-sea elasmobranch potential aggregations and provides insights for conservation planning.

2. Methods

2.1. Data Source and Sub-samplings

Data from the MEDITS program, focusing on elasmobranch catches across the Mediterranean region between 1994 and 2023, were used to analyze intra-class co-occurrences and spatial patterns. The MEDITS program is a standardized, annual scientific trawl survey conducted

since 1994 across multiple GSAs of the Mediterranean Sea. Sampling follows a shared protocol using research vessels equipped with scientific observers who identify, count, and measure all individuals caught. Each haul is carried out during daylight hours using a standardized GOC73 trawl, following a depth-stratified random sampling design (10–800 m), and biological data such as species composition, sex, size, and maturity stage are systematically recorded (MEDITS Working Group, 2017; Spedicato et al., 2019). In cases when a subset of the haul was sampled, the number of individuals was estimated by applying a correction factor derived from the ratio between the total catch weight (*ptot*) and the sampled catch weight (*pechan*). This correction was extended to estimate the number of sexually mature and immature males and females per haul, based on their subsampled proportions.

2.2. Species density and filtering

For each haul, species density was calculated by dividing the number of individuals by the swept area covered in the haul (expressed in squared kilometers and calculated using trawl distance and wing opening information). Only species with a mean catch of ≥ 2 individuals and appearing in ≥ 30 hauls were retained. For these selected species, hauls reporting fewer than two individuals were excluded, according to the definition for elasmobranchs aggregations proposed by McInturf et al. (2023). This density-based screening (≥2 individuals, standardized by swept area) was first used to flag high-density situations, while co-occurrence testing, patch delineation and multi-year persistence were required before interpreting them as biologically meaningful aggregations (see §§ 2.4–2.7).

2.3. Sex and Maturity

Individuals were classified into maturity classes following the criteria proposed by Follesa and Carbonara (2019). Individuals at stages 1 and 2 were considered immature and those at stages 3 and 4 were classified as mature. For records lacking maturity information in the original dataset, classification into mature or immature was inferred using length class data and sex-specific Length at maturity (Lm) values obtained from the scientific literature (Table 1). When Lm values specific to the Mediterranean Sea were unavailable, estimates from other regions were sourced from FishBase (Froese & Pauly, 2010).

Table 1. Sex-length at maturity (Lm) for demersal elasmobranchs obtained from literature research and FishBase.

Species	**Sex**	**Lm**	**References**	**Species**	**Sex**	**Lm**	**References**
Etmopterus spinax	F	34.2	Coelho et al., 2010	*Raja asterias*	F	56.3	Barone et al., 2007; Romanelli et al., 2007
	M	28.3	Coelho et al., 2010		M	51.1	Barone et al., 2007; Romanelli et al., 2007
Galeus melastomus	F	47.98	De Sola et al., 2005; Ragonese et al., 2009; Tursi et al., 1993; Ungaro et al., 1997	*Raja clavata*	F	67.8	Capapé et al., 2007; Demirhan et al., 2005; KrstulovićŠifner et al., 2009
	M	43	De Sola et al., 2005; Ragonese et al., 2009; Tursi et al., 1993; Ungaro et al., 1997		M	61	Capapé et al., 2007; Demirhan et al., 2005; KrstulovićŠifner et al., 2009
Scyliorhinus canicula	F	41.6	Capapé et al.,2008a; Capapé et al., 2008b; Kousteni et al., 2010; Saidi et al., 2004	*Raja miraletus*	F	42.3	Ungaro, 2004
	M	41	Capapé et al.,2008a; Capapé et al.,		M	36.4	Ungaro, 2004

			2008b; Kousteni et al., 2010; Saidi et al., 2004				
Squalus blainville	F	58.6	Cannizzaro et al., 1995; Kousteni & Mehalofonou, 2011; Marouani et al., 2007, 2010	*Raja polystigma*	M	43.6	Ferragut-Perello et al., 2022; Porcu et al., 2020
	M	47.7	Cannizzaro et al., 1995; Kousteni & Mehalofonou, 2011; Marouani et al., 2007, 2010		F	48.8	Ferragut-Perello et al., 2022; Porcu et al., 2020
Dipturus oxyrinchus	F	82.5	Yıgın & Ismen, 2010	*Torpedo marmorata*	F	33.35	Abdel_aziz, 1994; Consalvo et al., 2007
	M	64.5	Yıgın & Ismen, 2010		M	25.3	Abdel_aziz, 1994; Consalvo et al., 2007

2.4. Half-Weight Index (HWI)

The Half-Weight Index (HWI) was calculated to quantify associations between sex-maturity classes (mature males, immature males, mature females, and immature females) within species (Tab. 2). HWI is a metric used in animal social network analysis (Cairns & Schwager, 1987) and accounts for asymmetric sampling efforts and unequal detection frequency across classes.

The HWI is calculated as:

$$HWI = x / (x + 0.5 * (yA + yB))$$

- x: the number of hauls where both classes co-occur,
- yA: the number of hauls where only class 1 is present,
- yB: the number of hauls where only class 2 is present.

This index ranges from 0 (no association) to 1 (high co-occurrence), with values above 0.5 indicating moderate to strong associations (Quintana-Rizzo & Wells, 2001).
For each species and GSA pair, a binary matrix was structured with hauls in rows and the four sex-maturity classes in columns. A random permutation test (n = 1000), shuffling presence/absence values within each class, was performed to assess whether associations were stronger than expected by chance. The HWI for each pairwise combination was compared against the null distribution generated from the permutations. An association was considered statistically significant if its HWI exceeded the 95th percentile of the null distribution ($\alpha = 0.05$), corresponding to a one-tailed test. Only species and class combinations with significant co-occurrence (Monte Carlo Permutations $p<0.05$) were included in further analysis. The HWI and its permutation-based significance test were used to evaluate whether specific sex–maturity classes co-occur more frequently than expected by chance and therefore may indicate biologically meaningful associations.

2.5. Patch Detection

Analyses were conducted on hauls where all classes (i.e., mature males and mature females; immature females and immature males, etc.) with significant HWI co-occurrences were present. For each species, sex–maturity class combination, year and GSA, hauls were grouped into spatial patches based on a threshold distance, calculated as half the mean inter-haul distance within each GSA. Patch detection was performed by assigning hauls to clusters starting from the haul with the highest density. A haul was assigned to an existing patch if its distance from the patch centroid was below the threshold distance, otherwise, a new patch

was initiated (Woillez et al., 2007). Although patch detection provided the framework needed to assess differences in aggregation patterns across maturity groups, it is constrained by the spatial resolution of the MEDITS survey. Neighboring hauls with similar high densities were grouped into patches under the assumption that, given the high mobility of elasmobranchs and the lack of detailed information on their home range in the Mediterranean, these hauls may reflect the same aggregation process.

2.5.1. Operational definition of aggregation for MEDITS data

In this study we adopted the definition of aggregation by McInturf et al. (2023), as a first screening criterion on standardized densities. For consistency with the four-criterion framework developed in Chapter 1 (high abundance, occurrence, site fidelity, seasonality), we defined the aggregation procedure for MEDITS data by setting the following requirements: (i) high local density (screening step), and (ii) evidence of recurrent occurrence and site fidelity (i.e., repeated detections of the same species/sex–maturity combination in the same station/patch across years, see GIC-based persistence in § 2.7). The seasonality criterion could not be applied here because MEDITS surveys are performed in the same season each year.

2.6. Centre of Gravity (CG), Inertia, and Global Index of Collocation (GIC)

For each patch (defined by species, year, GSA, and sex–maturity class combination), the spatial distribution of individuals was characterized by the Centre of Gravity (CG) and Inertia (Tab. 2). The CG represented the density-weighted average position of hauls within the patch, while inertia quantified spatial dispersion around the CG, representing the density-weighted variance (Bez, 1997). The Global Index of Collocation (GIC) was calculated for each patch, GSA, and year to assess spatial overlap between the two co-occurring classes. GIC values range from 0 (complete segregation) to 1 (complete spatial overlap) and were derived from the distance between the CGs of each class, adjusted by their respective inertias (Bez & Rivoirard, 2000).

2.7. Temporal persistence

For patch, GSA and year combinations with a GIC greater than 0.5, interannual spatial persistence was assessed. This analysis was possible because the MEDITS survey follows a standardized and temporally consistent design, with repeated annual trawl hauls conducted at fixed sampling stations using the same gear (GOC73) and protocols across years and subareas (MEDITS Working Group, 2017; Spedicato et al., 2019). The spatial location of co-occurrence was defined as the midpoint between the two associated sex-maturity classes for each year within the same GSA. These yearly midpoints are used solely to track the spatial recurrence of the phenomenon and should not be interpreted as representing the geographic extent of the aggregation.A patch was considered spatially persistent if the CG of the co-occurrence in one year overlapped with or was located within the GSA-specific distance threshold from the CGs of the same species-class combination in other years. This approach allows the identification of recurrent areas potentially associated with aggregation processes, while acknowledging that individual movements and home-range dimensions cannot be fully resolved using the MEDITS sampling design. Only midpoints associated with GIC > 0.5 and exhibiting spatial proximity across multiple years were retained and mapped to visualize patterns of persistent aggregation over time. Only spatially persistent patches were mapped.

Table 2. Summary of spatial and association indices used to quantify intra- and interspecific co-occurrence patterns among sex–maturity classes of demersal elasmobranchs. Metrics provide information on spatial distribution, aggregation, and overlap between groups.

Half-Weight Index (HWI)	$HWI = x / [x + 0.5(y_1 + y_2)]$ (x = shared occurrences; y_1/y_2 = class-specific occurrences) Binary co-occurrence index based on the presence of different **maturity classes** in the same haul.	Measures of **co-occurrence** between life stages: High HWI = strong overlap Low HWI = spatial separation Permutation tests assess significance
	$CG\_lat = \Sigma(lat * weight) / \Sigma(weight)$	

Center of Gravity (CG)	$CG\_lon = \Sigma(lon * weight) / \Sigma(weight)$ Weighted average of haul coordinates using density as weight.	Identifies the **spatial center** of a species distribution for a given year, GSA, and maturity stage.
Inertia (I)	$I = \Sigma(d^2 * weight) / \Sigma(weight)$ (d = distance to CG) Weighted sum of squared distances from each haul to the CG.	Measures **spatial dispersion**: High inertia = widespread distribution Low inertia = localized distribution
Global Index of Collocation (GIC)	$GIC = 1 - [d^2\_CG / (d^2\_CG + I\_m + I\_f)]$ (d^2_CG = distance² between male and female CGs)	Assesses **spatial overlap** between males and females, accounting for their spatial spread. GIC = 1 → complete spatial overlap GIC = 0 → no overlap

2.8. Spatial indices

Spatial indices were calculated to depict intra-specific patterns among classes (immature individuals, mature females, mature males) and inter-specific similarities between species. Indices were calculated only for records with positive densities and defined by the combinations of species, GSA, year and maturity/sex class (Tab. 3).

The Positive Area (PA) represents the total swept area where density is greater than zero, while the Spreading Area (SA) quantifies how uniformly the biomass is distributed across positive hauls (Woillez et al., 2007). The SA/PA ratio quantifies the degree of biomass aggregation across positive hauls (Tab.3). Values close to 1 indicate uniform distribution, whereas values ≤ 0.5 suggest moderate to strong aggregation.

The Equivalent Area (EA) estimates the area that the population would occupy if it were distributed at a constant mean density (Bez et al., 1995; Bez et al., 1997; Matheron, 1971)

(Tab.3). Lower EA values indicate that the biomass is concentrated in fewer hauls, while higher values reflect a balanced distribution.

The Coefficient of Variation (CV) is the ratio between the standard deviation and the mean of density values across hauls and it estimates the heterogeneity of spatial distribution (Tab. 3). Higher values indicate more irregular or patchy patterns (Matheron, 1985). Mean Crowding measures the expected number of individuals co-occurring with a randomly chosen individual from a given class, providing an estimate of local aggregation (Lloyd, 1967) (Tab. 3). These spatial indices (SA/PA, EA, CV, Mean Crowding) were used to test whether immature individuals displayed stronger clustering, higher heterogeneity or more pronounced spatial aggregation than mature classes.

Table 3. Description and interpretation of spatial distribution indices used to assess elasmobranch aggregation patterns. Summary of the spatial indices applied to quantify intraspecific and interspecific aggregation in demersal elasmobranchs.

SA/PA (Spreading Area / Positive Area)	*PA = number of hauls with density > 0* *SA = 2 × Σ(1 - Qcum/Q)* Q = total density Qcum = cumulative density of hauls ranked by density PA: number of hauls with presence SA: rate at which density drops from the most abundant hauls	The ratio SA/PA indicates spatial aggregation: **SA/PA < 0.5 → aggregated** **SA/PA > 0.5 → dispersed**
Equivalent Area (EA)	*EA=(∑w)2/∑w2EA* (w = density or abundance in each haul)	The number of equally abundant hauls needed to account for the observed density distribution **High EA → uniform abundance across hauls** **Low EA → strong dominance of few hauls (high aggregation)**
Coefficient of Variation (CV)	*CV=SD(w)/Mean(w)*	A dimensionless index of variability in density or abundance **High CV → patchy or clumped distribution** **Low CV → more homogeneous density**
Mean Crowding (MC)	*MC=∑zi(zi−1) /∑ziMC* zi= density in each haul	The average number of companions experienced by an individual **High MC → individuals clumped** **Low MC → more uniform distribution**

2.9. Data analysis

2.9.1. Intraspecific spatial patterns

For intraspecific analysis, spatial indices were calculated for each maturity group (immature, mature males, mature females) per species, GSA, and year. For species with sufficient data, a Between-Class Analysis (BCA) was performed using the ade4 package to test for differences in spatial patterns between groups (Chessel et al., 2004). BCA was used to account for group structure while assessing differences in spatial distribution among maturity stages.

Data suitability for BCA was assessed through Bartlett's test to confirm correlation among variables, and the Kaiser-Meyer-Olkin (KMO) measure to evaluate sampling suitability (Nkansah, 2018). Only variables with overall KMO values ≥ 0.6 were retained for analysis (Nkansah, 2018). A Monte Carlo permutation test with 999 permutations was used to evaluate statistical significance ($\alpha = 0.05$). The contribution of each index to spatial variance was assessed using eigenvalues and correlation circles. The BCA addressed Hypothesis 2 by quantifying spatial differences between immature individuals, mature females and mature males and identifying whether specific life stages exhibited more aggregated or more dispersed patterns.

2.9.2. Inter-specific spatial patterns

For interspecific comparisons, mean values of the indices were aggregated per species and maturity group (mature vs immature) and then standardized. Two separate Principal Component Analyses (PCA) were conducted to explore patterns in spatial distribution. The first PCA considered maturity groups separately (mature vs. immature) for each species, allowing the assessment of ontogenetic differences in spatial patterns across taxa. The second PCA considered maturity classes together for each species, providing an overview of interspecific variability. PCA assumptions were verified using the Kaiser-Meyer-Olkin (KMO) test (threshold > 0.6) and Bartlett's test (significance at $p < 0.05$). The EA index was excluded from this analysis due to its high correlation with the CV, which could lead to multicollinearity. To assess significant differences in spatial distribution across species, a PERMANOVA was applied to the full dataset of spatial indices using 999 permutations and Euclidean distance. The Cluster analysis was performed on species centroids (averaged spatial indices per species and maturity group), using K-means clustering. The optimal number of clusters was selected using the elbow method, and groupings were visualized over the PCA space. This analysis was used to evaluate Hypothesis 3, which proposed that species differ in their spatial strategies and aggregation tendencies, through the structure of spatial indices and maturity-group patterns.

3. Results

3.1. Species and hauls filtering

A total of 20711 hauls and 30 elasmobranch species were present in the MEDITS dataset (1994–2023) and considered for this study. After applying filtering criteria, 17703 hauls and 21 species were retained for analysis. Among sharks, out of the 16 species present in the original dataset, 11 were retained after filtering. The species with the highest retention rates were *Galeus melastomus* (Rafinesque, 1810) (94.1%), *Etmopterus spinax* (Linnaeus, 1758) (86.7%), and *Scyliorhinus canicula* (Linnaeus, 1758) (84.5%). Intermediate retention rates were observed in *Squalus blainville* (Risso, 1827) (76.2%) and *Squalus acanthias* (Linnaeus, 1758) (64.6%), while the remaining species showed lower proportions. Seven species (*Galeorhinus galeus* (Linnaeus, 1758), *Hexanchus griseus* (Bonnaterre, 1788), *Dalatias licha* (Bonnaterre, 1788), *Oxynotus centrina* (Linnaeus, 1758), *Squatina aculeata* (Cuvier, 1829), *Squatina oculata* (Bonaparte, 1840), *Squatina squatina* (Linnaeus, 1758)) did not retain any valid haul after applying the filtering criteria. Among the 12 batoid species, 10 were retained. The most represented were *Raja clavata* (Linnaeus, 1758) and *Raja miraletus* (Linnaeus, 1758), with 68.7% and 68.3% of hauls retained, respectively. Other species like *Leucoraja melitensis* (Clark, 1926) and *Raja polystigma* (Regan, 1923) had moderate retention (around 60%). *Torpedo marmorata* (Risso, 1810), *Leucoraja circularis* (Couch, 1838) and *Rostroraja alba* (Lacepède, 1810) showed lower retention (23–27%). Two species (*Dipturus batis* (Linnaeus, 1758) and *Raja undulata* (Lacepède, 1802)) were excluded from further analyses due to not meeting the filtering thresholds.

3.2. Sex-Maturity Class Co-occurrence Patterns

The HWI analysis revealed significant intra-specific co-occurrence patterns between sex-maturity classes. Among the 21 elasmobranch species analyzed, 11 showed statistically significant association among classes ($p < 0.05$), suggesting a non-random spatial aggregation of individuals based on sex and maturity (Fig. 1).

High HWI values (> 0.6) were observed among immature males and females in *S. canicula* (HWI: 0.86), *S. blainville* (HWI: 0.81), and *E. spinax* (HWI: 0.87), indicating potential shared use of habitats at early life stages. *G. melastomus* has strong associations across multiple class combinations, suggesting limited spatial segregation by sex or maturity (HWI for immature males and females: 0.9; HWI for immature females and mature females: 0.62; HWI for immature males and mature males: 0.7; HWI for mature males and mature females: 0.7).

Batoid species such as *R. polystigma* and *R. miraletus* showed weak but significant associations between mature males and females (HWI: 0.2; HWI: 0.3). Mature males and females of *R. clavata* and *R. asterias* have moderate HWI values (HWI: 0.4; HWI: 0.5) but lacked statistical significance in other pairings, suggesting variable spatial overlap depending on sex-maturity composition. *L. melitensis*, *R. asterias*, *D. oxyrinchus*, and *T. marmorata* showed significant class pairings among mature males and females (HWI: 0.6; HWI: 0.5; HWI: 0.5; HWI: 0.4). Other combinations (some mature pairings) had non-significant permutation-tested associations ($p \geq 0.05$) (Tab. 4). These significant associations among sex–maturity classes in 11 out of 21 species support Hypothesis 1 and indicate that co-occurrence patterns are not randomly structured.

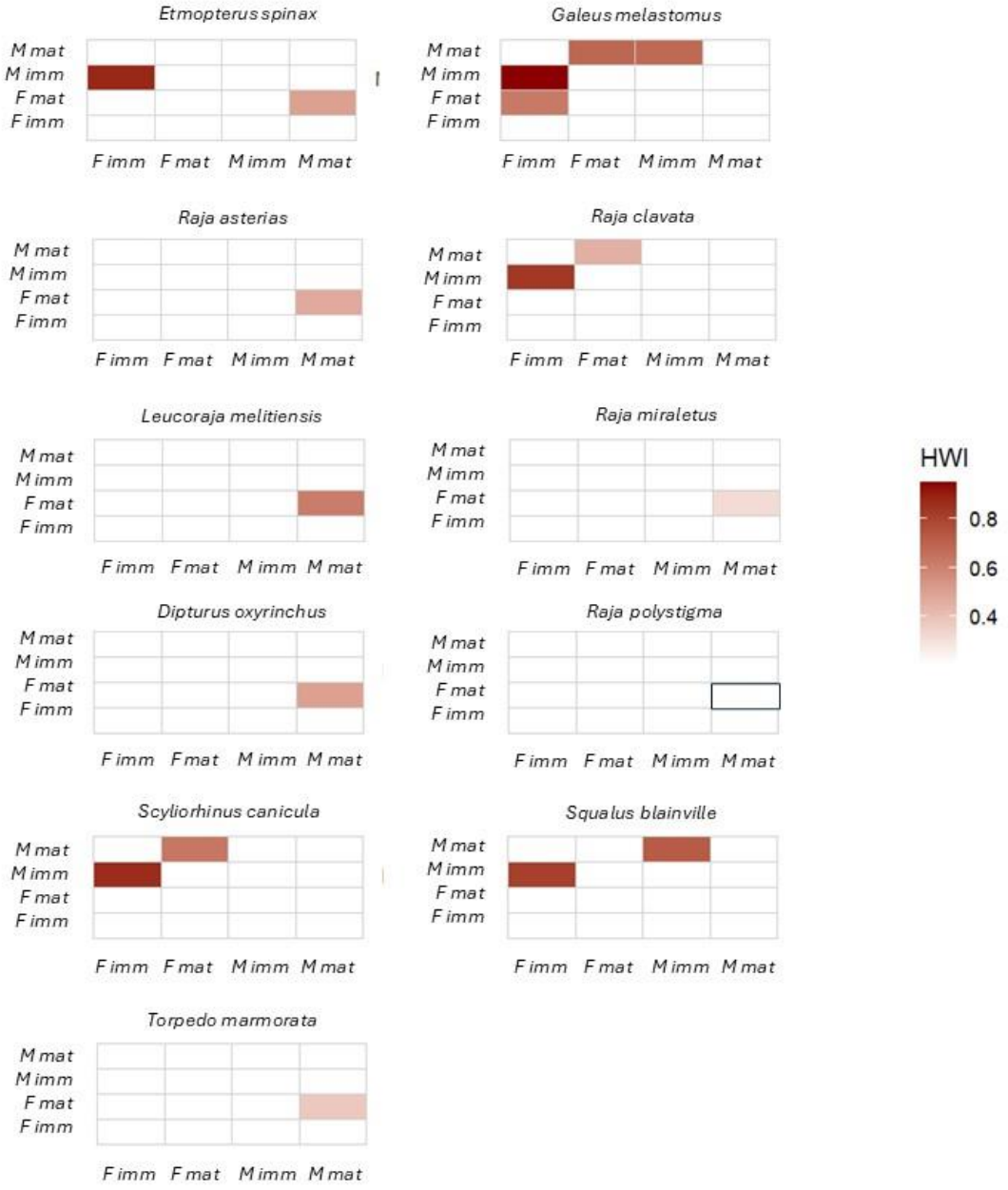


Figure 1. Heatmaps of pairwise Half-Weight Index (HWI) values between sex–maturity classes. Color intensity reflects the strength of the association, with darker shades indicating stronger co-occurrence. Only significant pairwise associations ($p < 0.05$) are shown. F imm: immature females; F mat: mature females; M imm: immature males; M mat: mature males.

Table 4. Results of the permutation on the Half-weight index. Raja cla: *Raja calvata*; Scyo can: *Scyliorhinus canicula*, Galu mel: *Galeus melastomus*; Raja pol: *Raja polystigma*; Etmo spi: *Etmopterus spinax*; Raja mir: *Raja miraletus*; Squa bla: *Squalus blainville*; Torp mar: *Torpedo marmorata*; Raja ast: *Raja asterias*; Raja mel: *Leucoraja melitiensis*; Raja oxy: *Dipturus oxyrinchus*

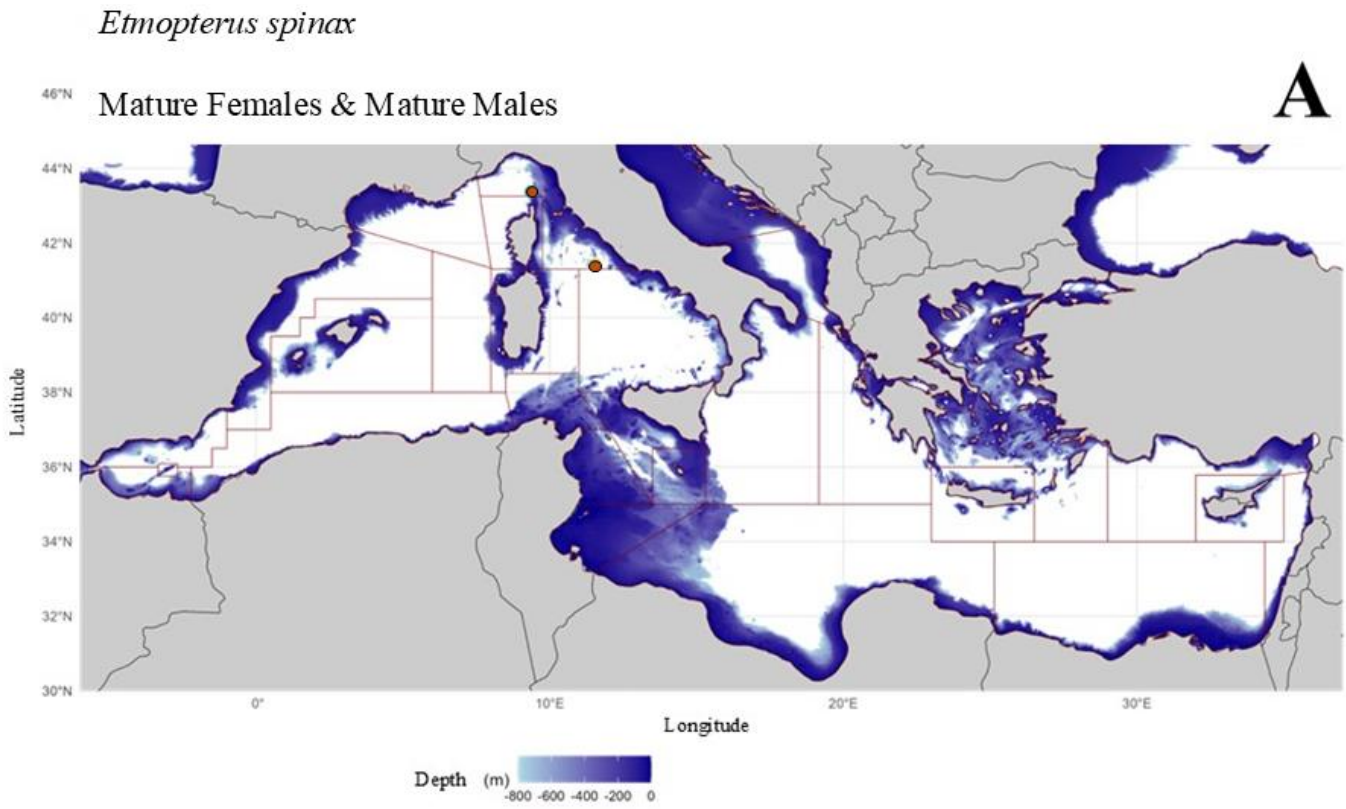
Species	Class 1	Class 2	HWI	*p perm*	*p value*
Raja cla	F imm	M imm	0.835	0.81	0
	F mat	M mat	0.453	0.292	0
Scyo can	F imm	M imm	0.869	0.8	0
	F mat	M mat	0.636	0.563	0
Galu mel	F imm	M imm	0.95	0.942	0
	F imm	F mat	0.627	0.62	0
	M imm	M mat	0.68	0.671	0
	F mat	F mat	0.689	0.49	0
Raja pol	M mat	M imm	0.205	0.129	0.02
Etmo spi	F imm	F mat	0.879	0.874	0.033
	M mat	F mat	0.501	0-273	0
Raja mir	M mat	M imm	0.316	0.253	0
Squa bla	F imm	M mat	0.812	0.758	0
	M imm	F mat	0.723	0.679	0
Torp mar	M mat	F mat	0.375	0.149	0
Raja ast	M mat	F mat	0.471	0.282	0
Raja mel	M mat	F mat	0.615	0.516	0,023
Raja oxy	M mat	F mat	0.501	0.423	0

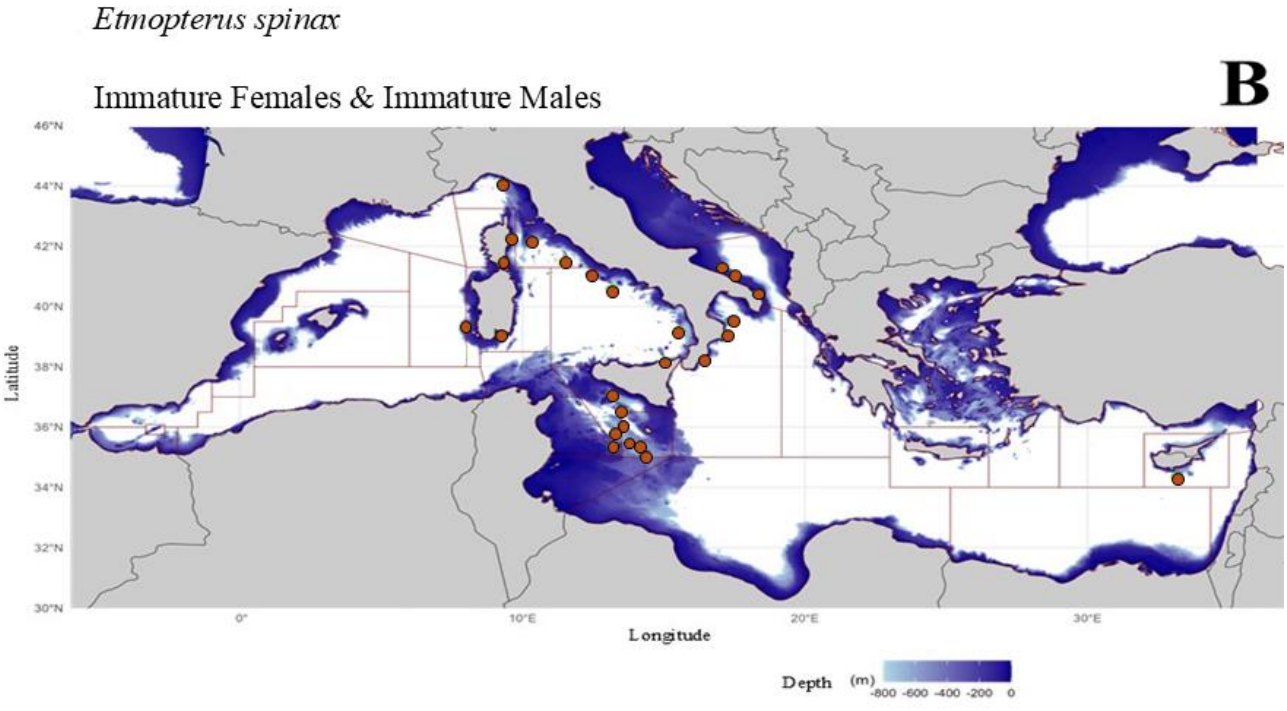
3.3. Persistent Co-occurrence Patches

Persistent spatial co-occurrence patterns across sex and maturity classes were detected for all four shark species, although with different degrees of spatial extent and temporal stability. The recurrence of these patches over multiple years indicates that many of the detected co-occurrences correspond to stable spatial features, further supporting Hypothesis 1.

For *E. spinax*, spatial analysis revealed multiple persistent co-occurrence patches both for immature and mature individuals, highlighting the species' tendency to form dense aggregations at different stages (Fig. 4A-B). Persistent co-occurrence of mature males and females was limited in space, with stable aggregations detected only in the northern Tyrrhenian Sea (GSA 9) and a maximum recurrence of 4 years (Fig. 4A). Persistent patches of immature individuals were more widespread and mainly located in the southern Tyrrhenian Sea (GSA 10), the southern Adriatic (GSA 18) and the Sicilian Channel (GSA 16), with some sites used for up to nine years (Fig. 4B). *S. canicula* showed a high degree of spatial fidelity over time, with persistent co-occurrence patches between sex-maturity classes (immature females vs immature males; mature females vs mature males) across multiple GSAs (Fig. 4C-D). The co-occurrence of mature males and females (Fig. 4C) was localized along the Spanish coasts (GSA 5 and 6), in the Gulf of Lion (GSA 7), in the northern Tyrrhenian Sea (GSA 9) and in the Strait of Sicily (GSA 16 and GSA 15). Juvenile aggregations (immature females and immature males) are more widely distributed, showing recurrent patches in the Balearic Islands (GSA 5), Gulf of Lion (GSA 7), Tyrrhenian Sea (GSA 9), and the Strait of Sicily (GSA 16 and GSA 15). *S. blainville* showed more spatially restricted co-occurrence patterns compared to other shark species (Fig. 4E–F). Only male associations were limited in number and mainly occurred in the central Mediterranean, particularly the Strait of Sicily (GSA 16) and Aegean Sea (GSA 22) (Fig. 4E). Juvenile associations (immature males and immature females) were more numerous, with persistent patches distributed across the Strait of Sicily (GSA 16), Aegean Sea (GSA 22), southern Tyrrhenian, and western Sardinia (Fig. 4F). *G. melastomus* has the most spatially extensive and temporally persistent co-occurrence patterns among all combinations (Fig. 5A–D). Female-only and male-only associations (immature vs mature) were widespread, with stable patches detected in the western Sardinian Sea (GSA 11), Gulf of Lion (GSA 7), Spain (GSA 6), southern Tyrrhenian (GSA 10), Strait of Sicily (GSA 16), and northern Ionian Sea (GSA 19). Additional recurrent aggregations occurred in the southern Adriatic (GSA 18) and Aegean Sea (GSA 22) (Fig. 5A–B). The co-occurrence of mature males and females was more limited, with recurrent patches identified in the northern Ionian, Gulf of Lion, southern Tyrrhenian and Strait of Sicily (Fig. 5C). Juvenile aggregations (immature females and males) were the most spatially widespread, recurring in

the Ligurian Sea, Tyrrhenian Sea, Sardinian Channel, Strait of Sicily, Ionian Sea, and Aegean margins (Fig. 4D).

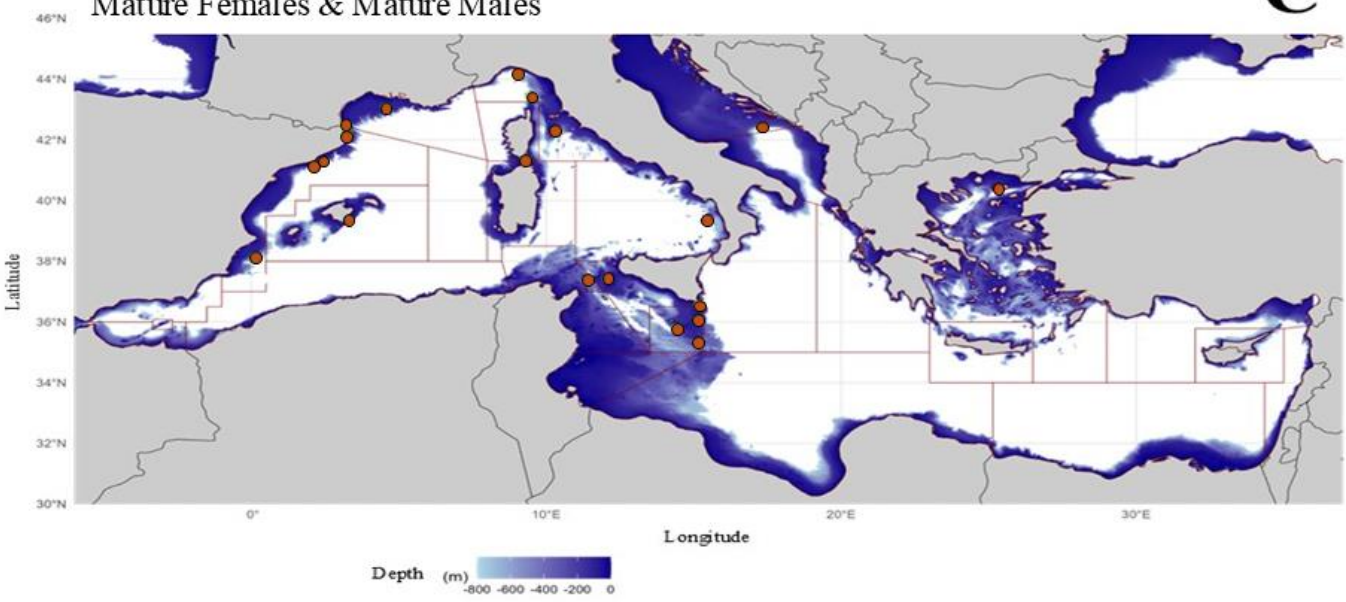




Scyliorhinus canicula

Mature Females & Mature Males

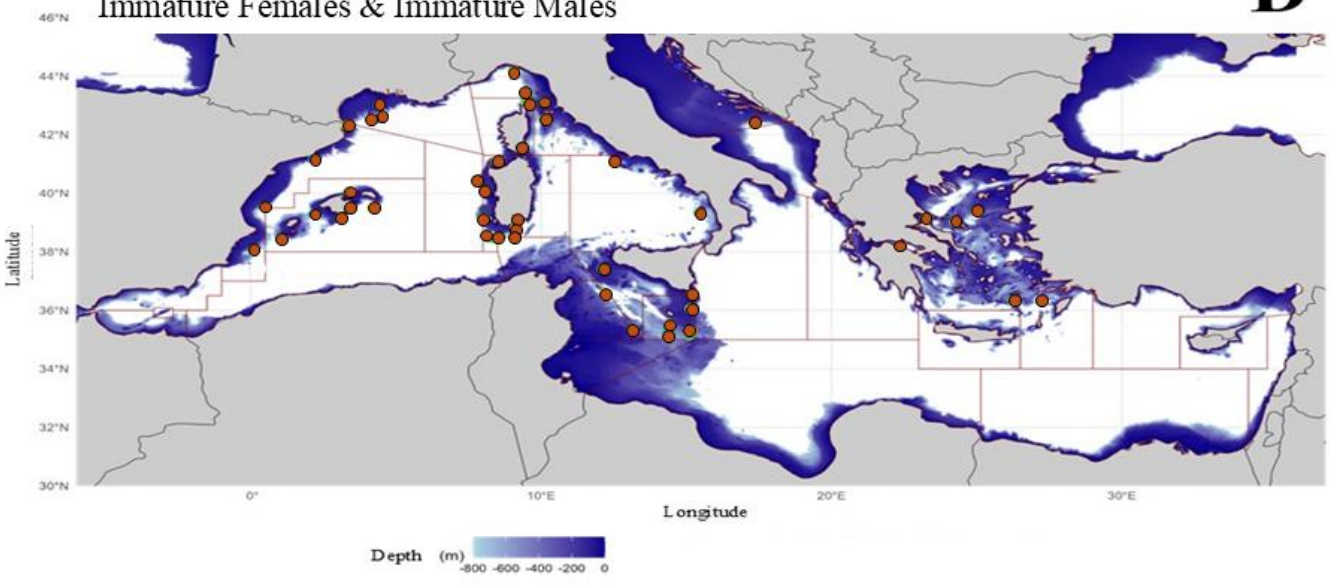
C



Scyliorhinus canicula

Immature Females & Immature Males

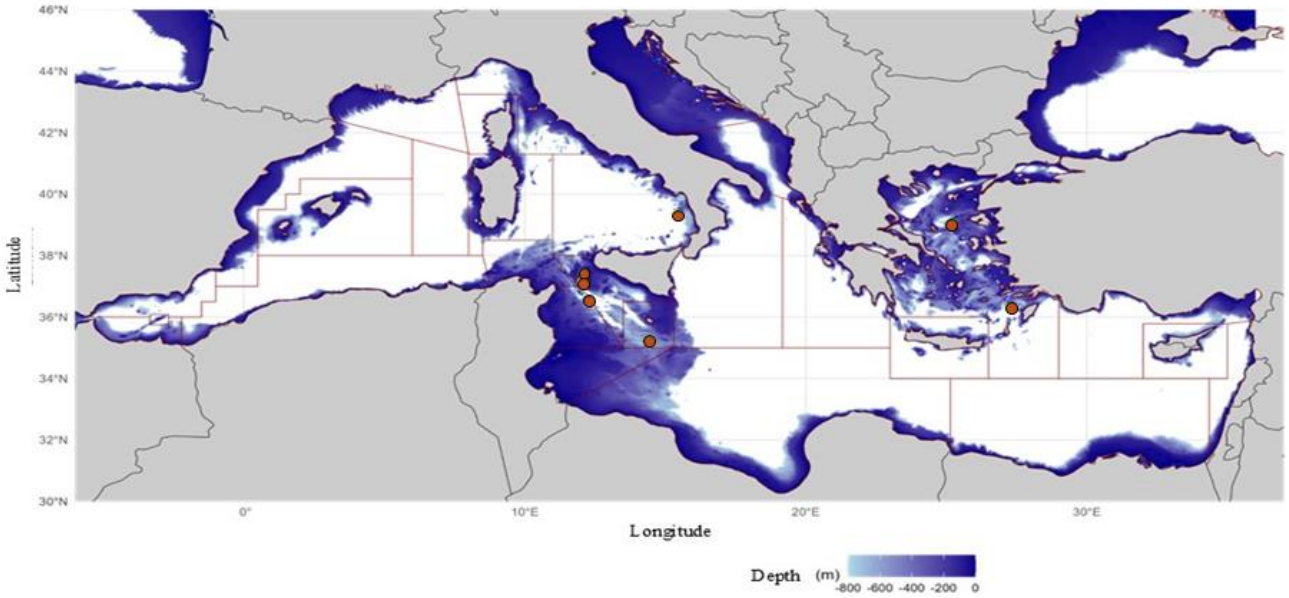
D



Squalus blainville

Immature Males & Mature Males

E



Squalus blainville

Immature Females & Immature Males

F

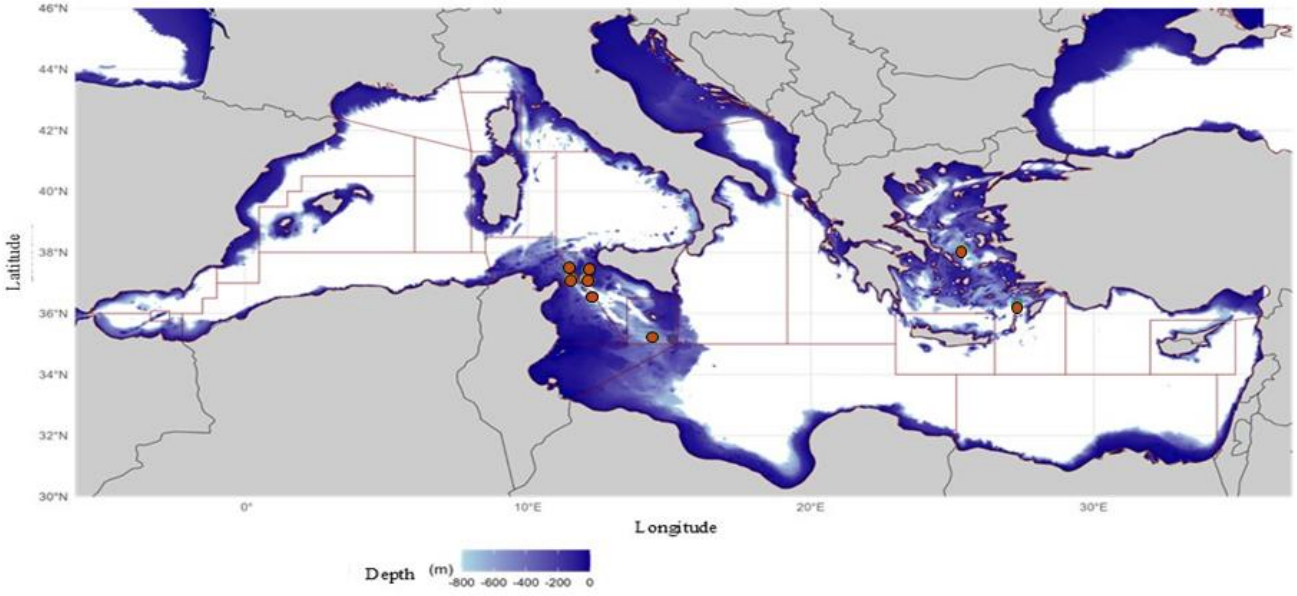
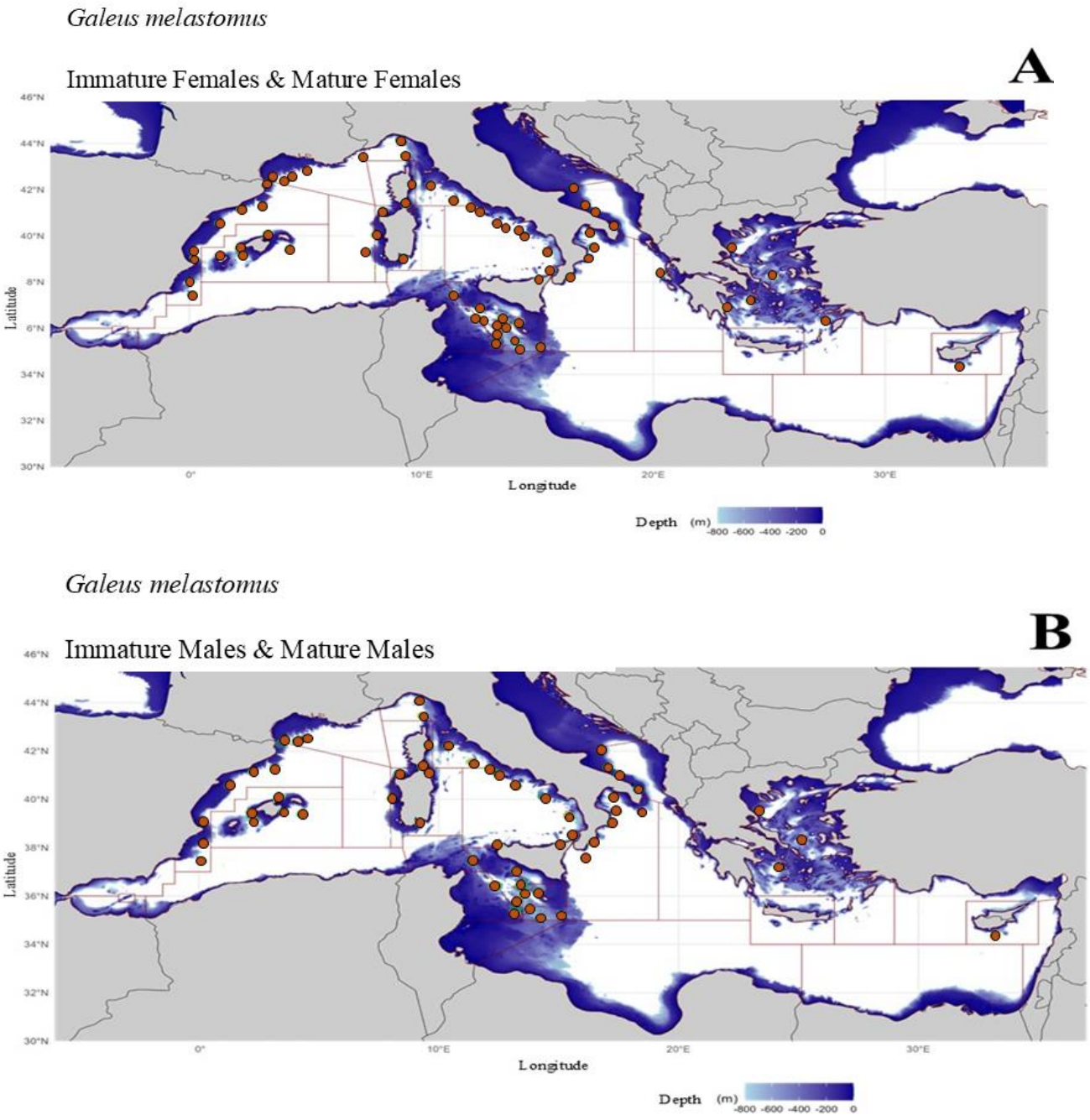


Figure 4. Panels show the location of patches where significant sex–maturity class co-occurrences were detected across multiple years: (A–B) *Etmopterus spinax*: (A) mature males vs. mature females, (B) immature males vs. immature females; (C–D) *Scyliorhinus canicula*: (C) mature males vs. mature females, (D) immature males vs. immature females; (E–F) *Squalus blainville*: (E) mature males vs. immature males, (F) immature males vs. immature females. Only spatially persistent patches (i.e., recurring across years with GIC > 0.5) are shown. White areas indicate depths > 800 m, which are not sampled by MEDITS surveys.



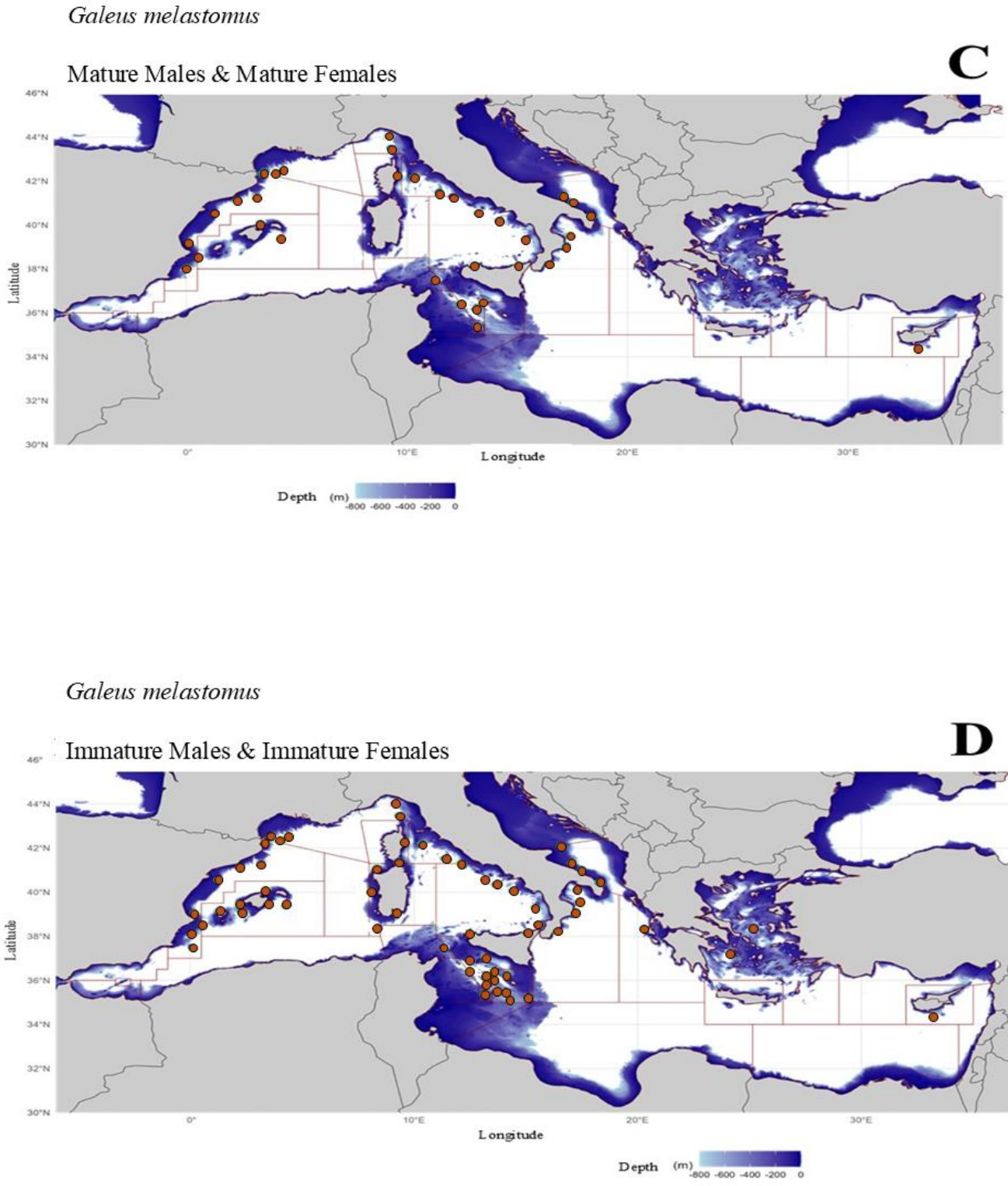
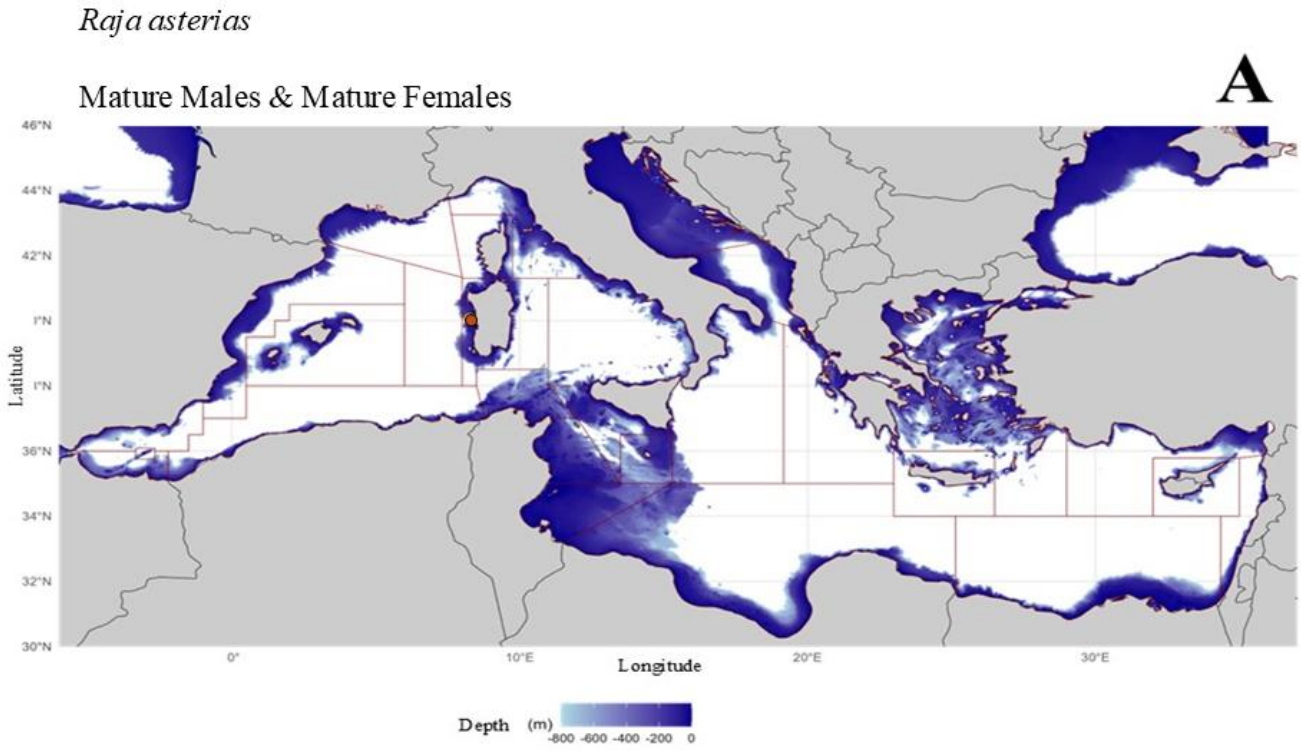


Figure 5. Panels show the location of patches where significant sex–maturity class co-occurrences were detected across multiple years: *Galeus melastomus* A) immature females vs mature females, B) immature males vs mature males, C) mature males vs mature females, D) immature males vs immature females. Only spatially persistent patches (i.e., recurring

across years with GIC > 0.5) are shown. White areas indicate depths > 800 m, which are not sampled by MEDITS surveys.

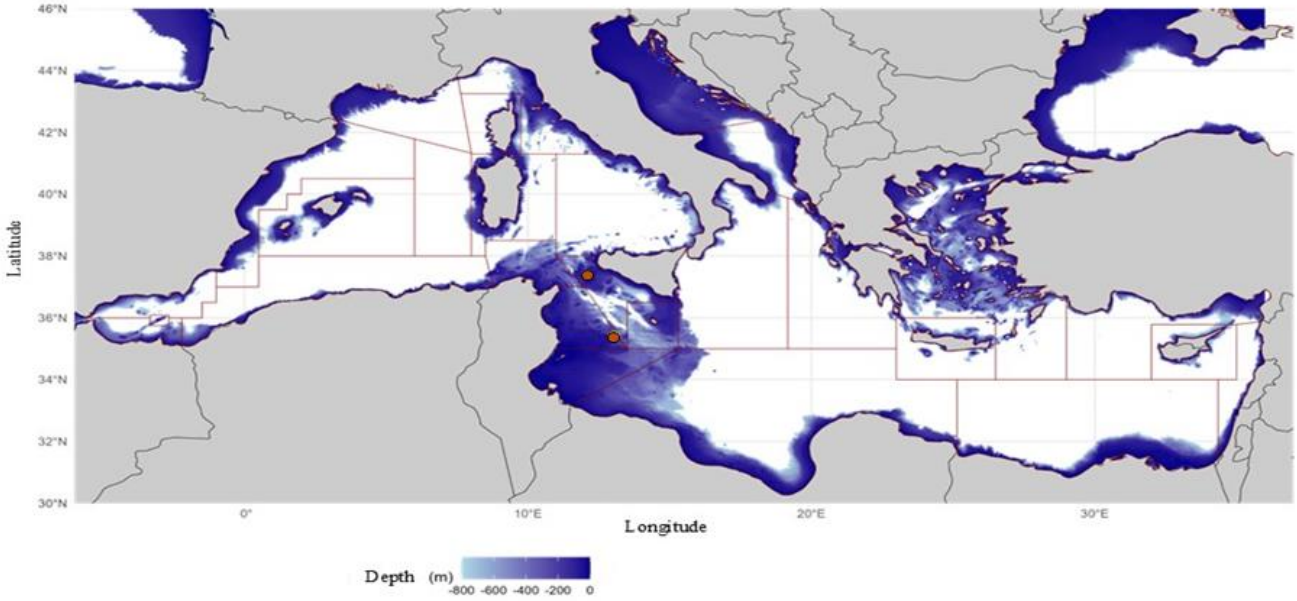
Persistent spatial co-occurrence patterns between sex and maturity classes were also detected for batoid species, though with a generally more localized distribution compared to sharks. For *R. asterias*, a stable aggregation of mature males and females (Fig. 6A) were only detected in the Sardinian Sea (GSA 11). *L. melitensis* displayed persistent co-occurrence of mature males and females (Fig. 6B) in the Strait of Sicily (GSA 16), showing stability over several years. For *Raja clavata*, persistent co-occurrence of mature males and females (Fig. 6C) was recorded in the Sardinian Sea (GSA 11), the Strait of Sicily (GSA 16), the Maltese waters (GSA 15) and the Balearic Sea (GSA 5). Immature male–female associations (Fig. 6D) were more spatially widespread, including the Balearic Islands (GSA 5), the Corsica waters (GSA 8), the western Sardinian Sea (GSA 11), the Strait of Sicily (GSA 16), the Maltese waters (GSA 15), and the northern and central Adriatic (GSA 17 and 18) and the Aegean Sea (GSA 22). Some of these juvenile patches persisted for up to eight years, suggesting repeated use as potential nursery grounds.



Leucoraja melitiensis

B

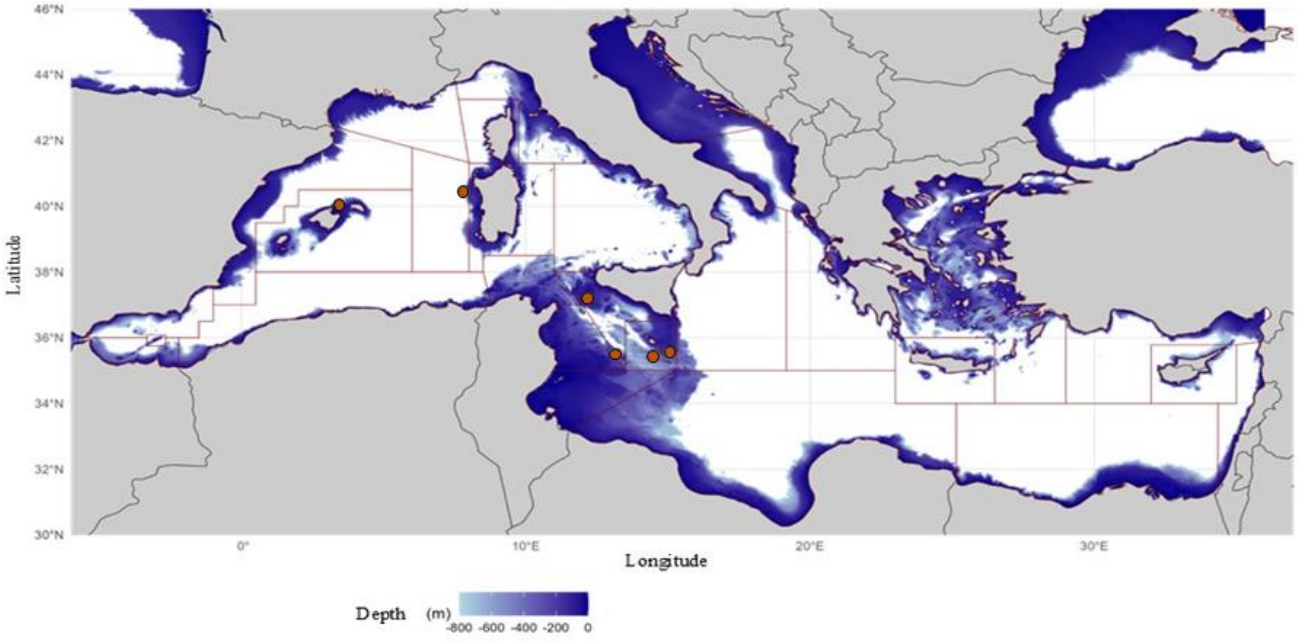
Mature Males & Mature Females



Raja clavata

C

Mature Males & Mature Females



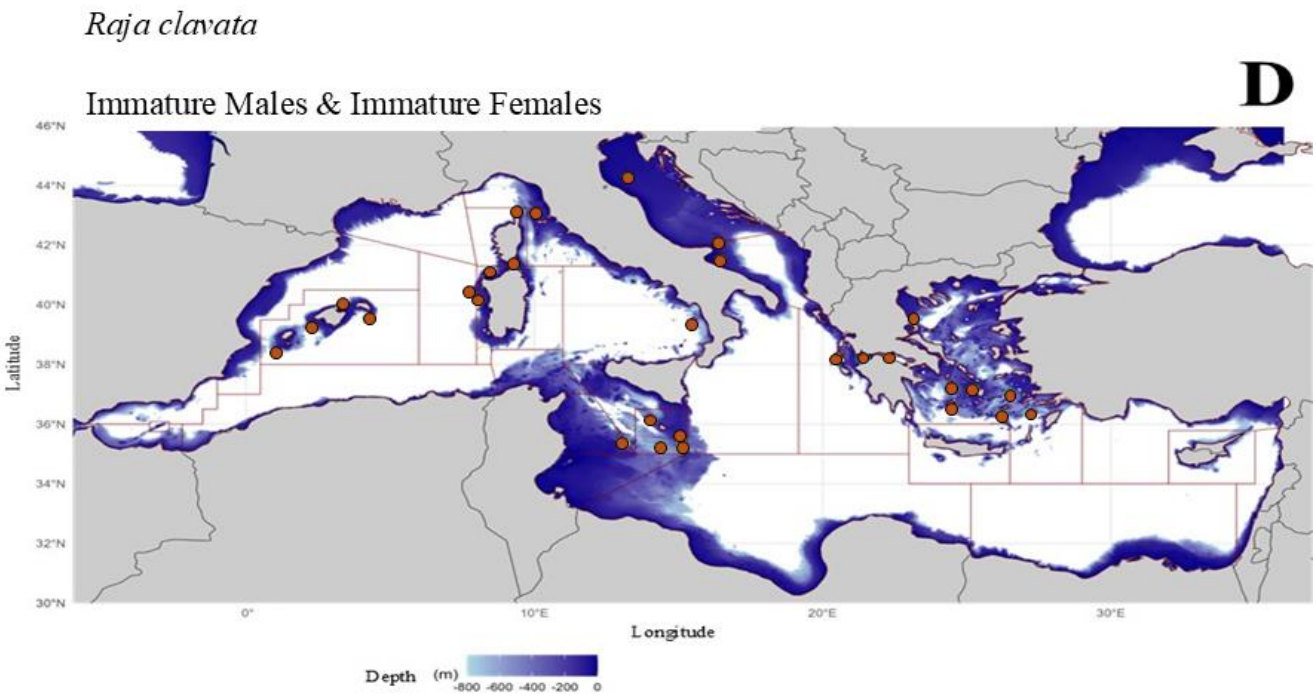


Figure 6. Panels show the location of patches where significant sex–maturity class co-occurrences were detected across multiple years: (A) *R. asterias* – mature males vs mature females; (B) *L. melitensis* – mature males vs mature females; (C) *R. clavata* – mature males vs mature females; (D) *R. clavata* – immature males vs immature females. Only spatially persistent patches (i.e., recurring across years with GIC > 0.5) are shown. White areas indicate depths > 800 m, which are not sampled by MEDITS surveys.

3.4. Intraspecific spatial patterns

Overall, both sharks and batoids exhibit higher aggregation in immature stages and more uniform spatial distributions in adults, particularly mature females. However, a few species (*R. asterias*, *L. melitensis*, *T. marmorata*) showed no significant maturity-related differences, indicating either homogeneous spatial patterns or that such differences were not detectable with the available data. These intraspecific patterns support Hypothesis 2, indicating that immature stages tend to be more clustered and spatially heterogeneous than mature individuals, while adults, especially females, are more dispersed.

The BCA performed for *E. spinax* using SA/PA, CV and Mean crowding indices, revealing a spatial differentiation among maturity stages. EA was excluded because of its KMO value below the suitability levels admitted for multivariate analysis (KMO < 0.6). The first axis

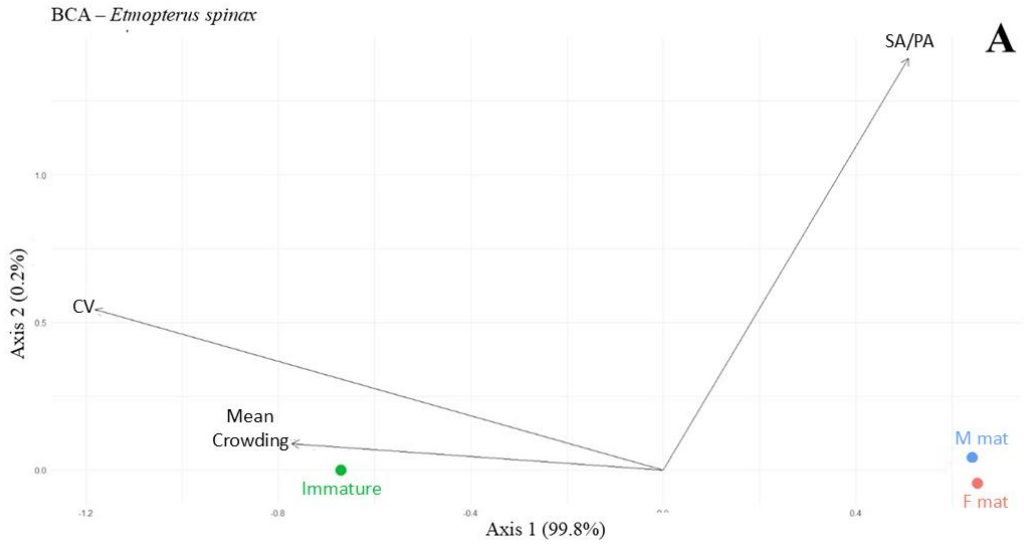
explained 99.8% of the variance and divided immature and mature individuals, while the second accounted for 0.2%. A Monte Carlo permutation test confirmed the significance of the observed differences (Observed: 0.145, $p < 0.001$). Immature individuals have the lowest spatial uniformity and the highest levels of crowding and variability, with a mean SA/PA of 0.42 (± 0.14), CV of 0.90 (± 0.40), and Mean Crowding of 211.7 (± 372.0). Mature males have a more balanced distribution and are less aggregated, showing a SA/PA of 0.50 (± 0.15), CV of 0.48 (± 0.30), and Mean Crowding of 25.2 (± 27.6). Similarly, mature females exhibited a SA/PA of 0.48 (± 0.13), CV of 0.46 (± 0.31), and Mean Crowding of 22.3 (± 15.9) (Fig. 7A).

The BCA conducted to explore spatial distribution patterns among maturity stages of *S. canicula*, was based on three spatial indices: SA/PA, CV, and Mean Crowding. The Equivalent Area (EA) was excluded from the analysis due to its low Kaiser-Meyer-Olkin (KMO) value (0.24), indicating insufficient shared variance with the other indices and poor suitability for dimension reduction. The BCA revealed differences between maturity groups along the first axis, which explained 99.4% of the between-class variance. A Monte Carlo test confirmed the significant differences (observed: 0.147; $p < 0.001$). Immature individuals were associated with higher spatial aggregation, characterized by lower spatial uniformity (SA/PA = 0.36 ± 0.12) and higher values of aggregation indices (CV = 1.17 ± 0.45, Mean Crowding = 588.1 ± 880.6). Mature females displayed a uniform spatial distribution (SA/PA = 0.49 ± 0.12) and lower aggregation (CV = 0.72 ± 0.39, Mean Crowding = 77.2 ± 102.7). Mature males showed intermediate values (SA/PA = 0.44 ± 0.12; CV = 0.91 ± 0.40; Mean Crowding = 210.7 ± 295.4) (Fig. 7B).

The BCA for *S. blainville* showed differences among maturity stages, with the first axis explaining 98% of the total variance and the second axis explaining 2%. The Monte Carlo test confirmed the significance of the grouping (Observed: 0.147, $p < 0.001$). SA/PA, CV and Mean crowding were the indices used to perform BCA, while EA was excluded (KMO <0.6). Immature individuals had lower spatial uniformity in dispersion and higher crowding, with a mean SA/PA of 0.33 (± 0.15), CV of 0.99 (± 0.44), and Mean Crowding of 258.3 (± 283.6). Mature males showed higher dispersion and lower variability, with SA/PA of 0.41 (± 0.13), CV of 0.73 (± 0.32), and Mean Crowding of 67.1 (± 46.0). Mature females had similar spatial

evenness to males but lower crowding, with SA/PA of 0.40 (± 0.14), CV of 0.62 (± 0.34), and Mean Crowding of 50.6 (± 50.4) (Fig. 7C).

The BCA conducted to assess spatial distribution differences among maturity stages of *G. melastomus* was based on SA/PA, Coefficient of Variation (CV), and Mean Crowding. The Equivalent Area (EA) was excluded due to its low score in preliminary tests (KMO < 0.6). The first axis of the BCA explained 99.4% of the between-group variance, and the associated Monte Carlo test confirmed the significant differences (observed: 0.134; $p < 0.001$). Immature individuals were associated with the most aggregated spatial pattern, with the lowest spatial dispersion (SA/PA = 0.37 ± 0.12) and the highest levels of aggregation (CV = 1.18 ± 0.41, Mean Crowding = 801.1 ± 1344.8). Mature females showed the most homogeneous distribution (SA/PA = 0.46 ± 0.13) and the lowest aggregation (CV = 0.81 ± 0.36, Mean Crowding = 96.9 ± 135.2), while mature males had intermediate values (SA/PA = 0.43 ± 0.12; CV = 0.85 ± 0.35; Mean Crowding = 133.8 ± 167.0) (Fig. 7D).



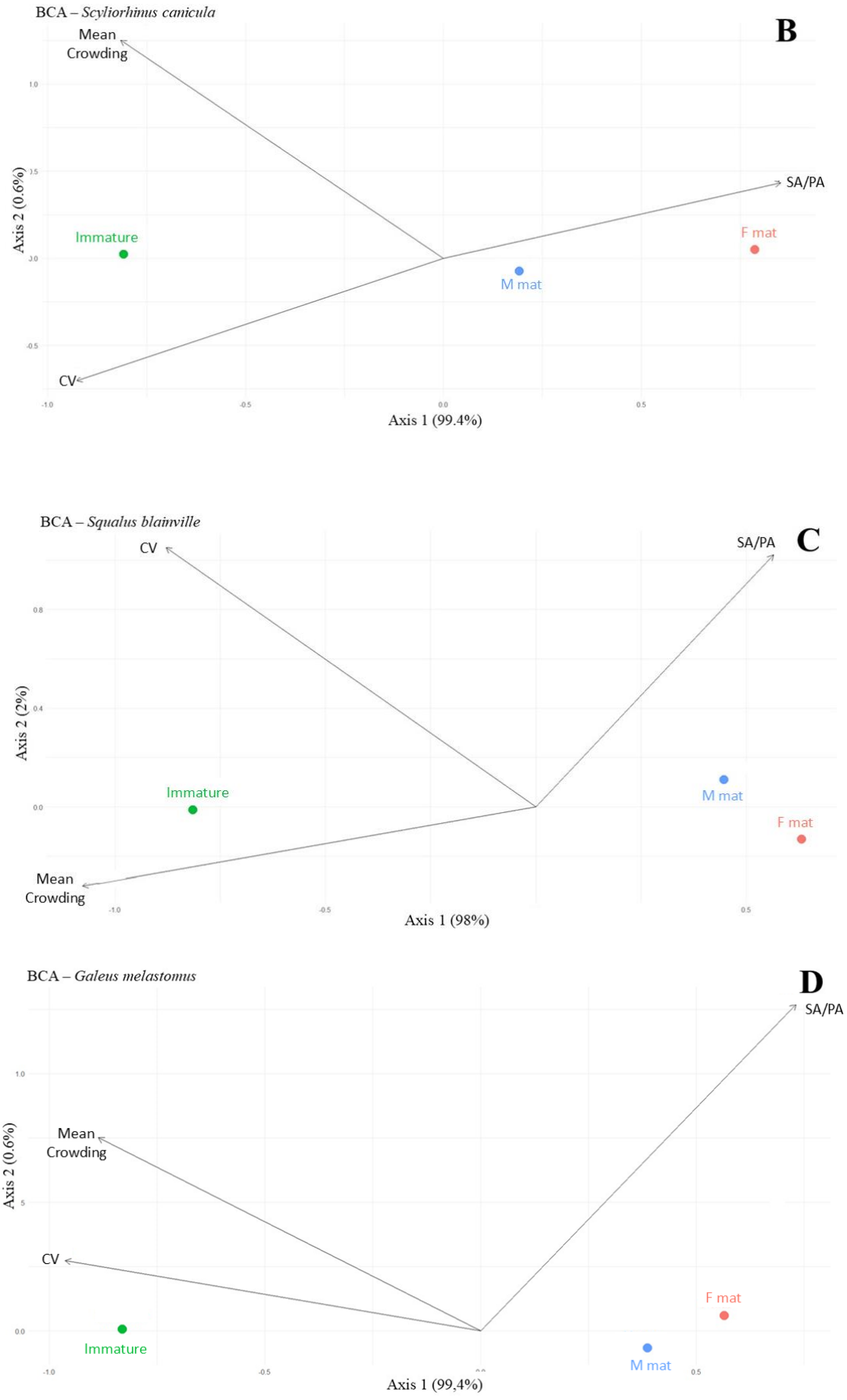


Figure 7. BCA plots of spatial distribution differences among maturity stages for four shark

species: (A) *E. spinax*, (B) *S. canicula*, (C) *S. blainville*, and (D) *G. melastomus*. Each plot is based on three spatial indices (SA/PA, Coefficient of Variation, and Mean Crowding).

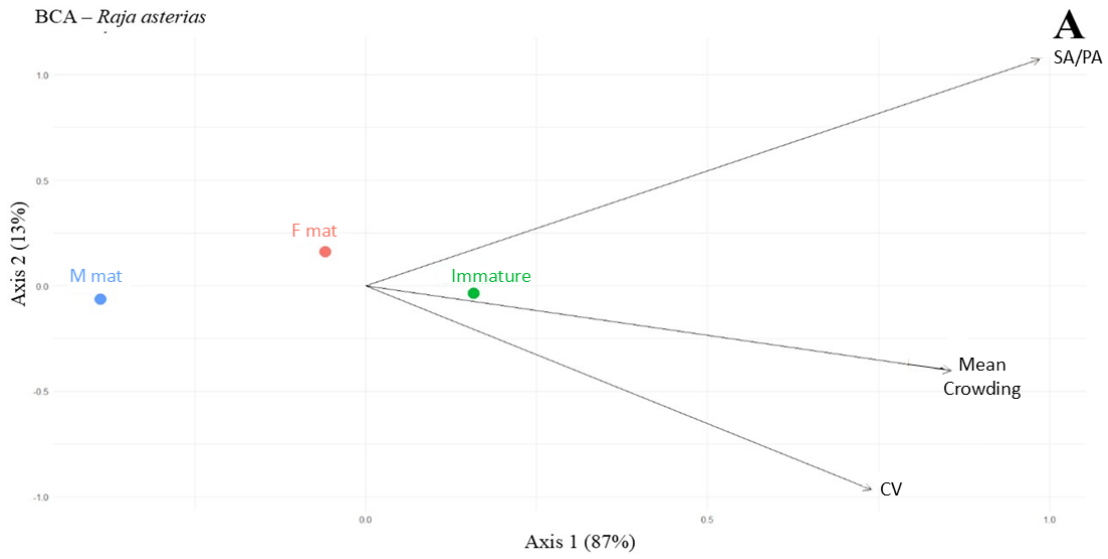
A BCA was conducted for *R. asterias* using SA/PA, Coefficient of Variation (CV), and Mean Crowding to evaluate spatial distribution differences among maturity stages and sexes. The first two BCA axes explained 87% and 13% of the variance, respectively. The Monte Carlo permutation test was not statistically significant (observed: 0.018; $p < 0.188$), indicating weak between-group structure in the spatial indices. This result indicates a homogeneous spatial structure across maturity stages and sexes in *R. asterias*, with no clear ontogenetic shift or sexual differences in distribution patterns (Fig. 8A).

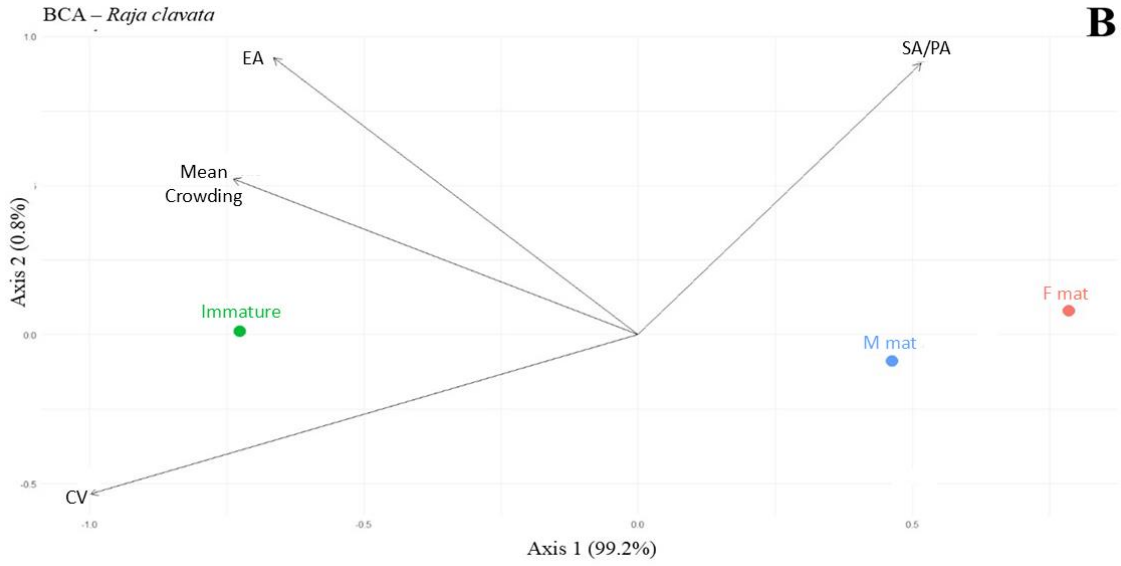
The BCA for *R. clavata* showed a separation among maturity stages based on spatial indices. Axis 1, explaining 99.2% of the between-group variance, divided immature individuals from both mature males and females. Statistical differences were supported by a Monte Carlo test (observed: 0.1, $p< 0.001$). Immature individuals were associated with higher spatial aggregation, as indicated by the highest mean CV (0.93 ± 0.4) and Mean Crowding (136 ± 195), along with the lowest spatial dispersion (SA/PA = 0.43 ± 0.14). Mature females showed the most uniform spatial distribution (SA/PA = 0.50 ± 0.13) and the lowest aggregation metrics (CV = 0.46 ± 0.26; Mean Crowding = 33 ± 28). Mature males have intermediate values (SA/PA = 0.48 ± 0.11; CV = 0.58 ± 0.34; Mean Crowding = 47 ± 48). These results suggest a shift from spatial aggregation in immature individuals to more evenly dispersed distributions in mature stages, particularly among females (Fig. 8B).

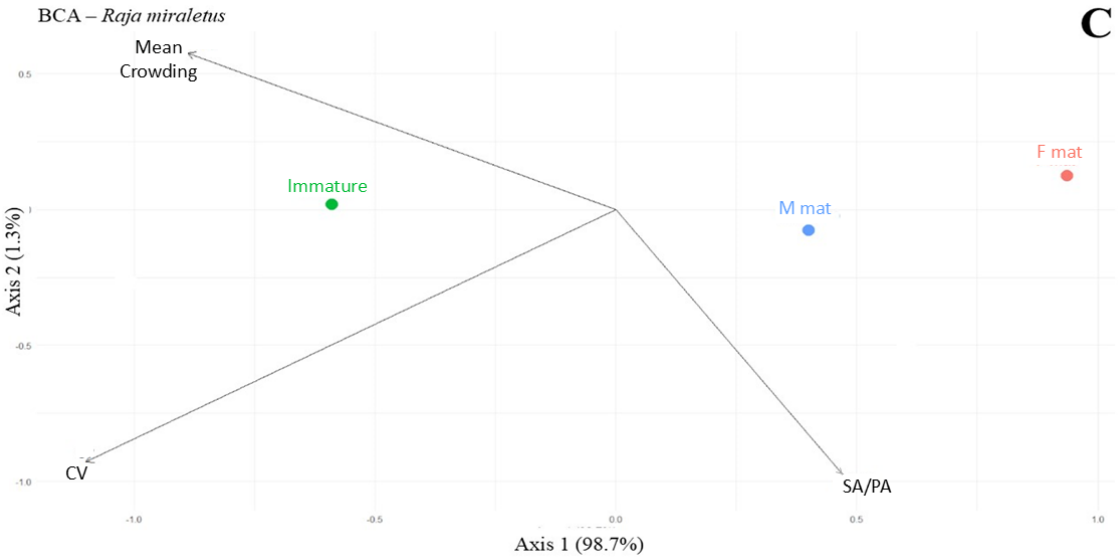
The BCA was performed on *R. miraletus* using three spatial indices: SA/PA, Coefficient of Variation (CV), and Mean Crowding. The Equivalent Area (EA) was excluded due to a low KMO value (<0.6), suggesting a low suitability for multivariate analysis. The BCA divided the maturity groups along the first axis, which accounted for 98.7% of the between-group variance. The Monte Carlo test confirmed statistically significant differences (Observed: 0.120; $p < 0.001$). Immature individuals have the most aggregated spatial distribution, with lower SA/PA (0.42 ± 0.14) and higher aggregation metrics (CV = 0.81 ± 0.40, Mean Crowding = 147.5 ± 153.2). Mature females were the most widely distributed group, characterized by the highest SA/PA (0.48 ± 0.10) and the lowest CV (0.34 ± 0.29) and Mean

Crowding (41.8 ± 32.9). Mature males showed intermediate spatial structure (SA/PA = 0.48 ± 0.13; CV = 0.55 ± 0.32; Mean Crowding = 71.2 ± 66.6) (Fig. 8C).

The BCA was conducted for *R. polystigma* to examine spatial distribution differences among maturity stages, using three indices: SA/PA, Coefficient of Variation (CV), and Mean Crowding. The Equivalent Area (EA) was excluded due to its low sampling suitability (KMO < 0.6). The first BCA axis explained 99.8% of the between-group variance, with the Monte Carlo test confirming statistical significance (observed: 0.073; $p < 0.001$). Immature individuals were characterized by higher aggregation, showing lower spatial uniformity (SA/PA = 0.44 ± 0.15) and higher variability (CV = 0.78 ± 0.51) and crowding levels (Mean Crowding = 478.2 ± 3597.3). Mature females and males have more homogeneous spatial patterns. Mature females had the lowest CV (0.32 ± 0.25) and low crowding (34.5 ± 16.1), with relatively high spatial balance (SA/PA = 0.47 ± 0.11). Mature males showed similar values (SA/PA = 0.47 ± 0.12; CV = 0.36 ± 0.25; Mean Crowding = 31.1 ± 17.2) (Fig. 8D).




BCA – Raja clavata
B
EA
SA/PA
Mean
Crowding
Immature
F mat
M mat
CV
Axis 2 (0.8%)
Axis 1 (99.2%)


BCA – Raja miraletus
C
Mean
Crowding
Immature
F mat
M mat
CV
SA/PA
Axis 2 (1.3%)
Axis 1 (98.7%)

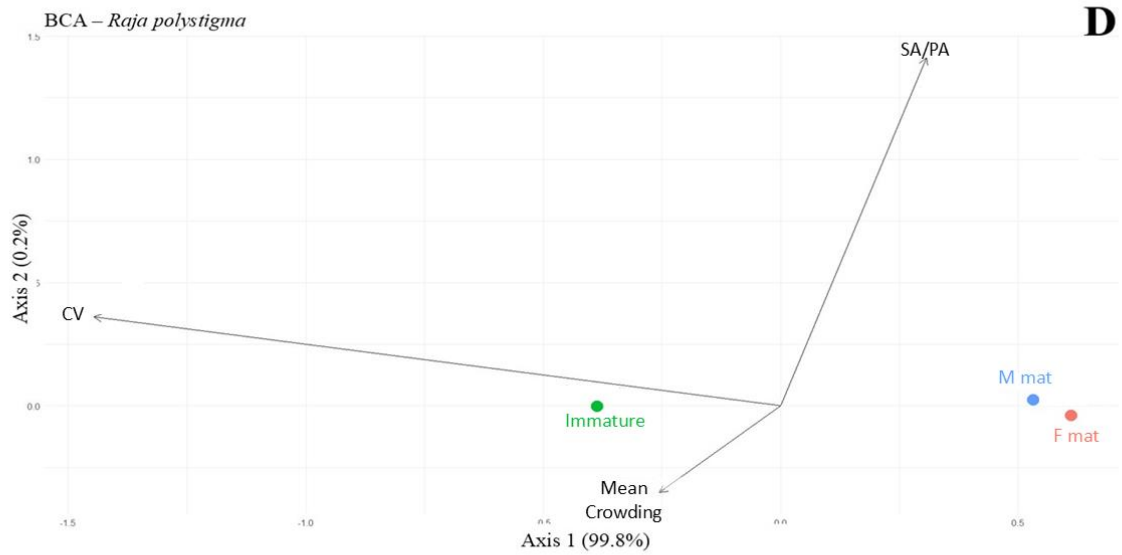


Figure 8. BCA plots of spatial distribution differences among maturity stages for four shark species: (A) *R. asterias*, (B) *R. clavata*, (C) *R. miraletus*, and (D) *R. polystigma*. Each plot is based on three spatial indices (SA/PA, Coefficient of Variation, and Mean Crowding).

The BCA for *D. oxyrinchus* was performed on SA/PA, CV and Mean crowding indices and highlighted a differentiation in spatial distribution patterns among maturity stages. EA was excluded because of its unsuitability for multivariate analysis (KMO > 0.6). The first axis explained 93% of the variance, while the second accounted for 7%. The Monte Carlo permutation test revealed a statistically significant difference between groups (observed = 0.046; p = 0.002). Immature individuals exhibited a mean SA/PA of 0.47 (± 0.16), a CV of 0.65 (± 0.56), and a Mean Crowding value of 69.2 (± 149.7). Mature males showed more uniform distribution and lower aggregation (SA/PA = 0.48 ± 0.13, CV = 0.45 ± 0.35, Mean Crowding = 29.4 ± 69.7). Mature females have the most homogenous and dispersed spatial pattern among all groups (SA/PA = 0.54 ± 0.12, CV = 0.38 ± 0.23, Mean Crowding = 17.6 ± 9.2) (Fig. 9).

For *L. melitensis*, the BCA did not show significant separation among maturity stages,

the Monte Carlo permutation test showed a non-significant result (Observed: 0.034, p = 0.457), indicating that the differences in spatial distribution indices among maturity groups and sexes are not statistically relevant.

The BCA for *T. marmorata* did not show statistical differences among maturity stages and sexes (Monte Carlo test, observed: 0.037, p = 0.089). The EA index was excluded from the analysis due to insufficient sampling suitability (KMO < 0.6).

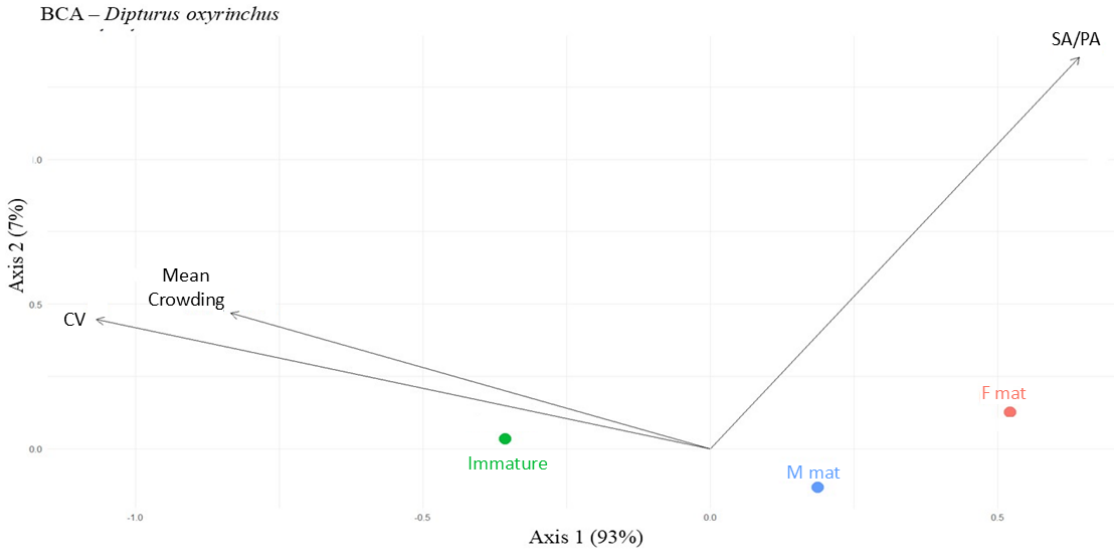


Figure 9. BCA plots of spatial distribution differences among maturity stages for *D. oxyrinchus* based on three spatial indices (SA/PA, Coefficient of Variation, and Mean Crowding).

3.5. Interspecific patterns in spatial distribution

The first PCA performed on species-level centroids (mean spatial indices per species and maturity group) revealed patterns of spatial distribution associated with maturity status and species identity. The first two principal components explained 88.4% of the total variance (PC1: 65.5%, PC2: 22.9%). PC1 was driven by the SA/PA index, indicating the degree of spatial uniformity, while PC2 was associated with the Mean Crowding. The Coefficient of Variation (CV) had a lower explanatory contribution and was orthogonal to both main gradients. K-means clustering on the PCA centroids identified three clusters, separating species–maturity group combinations based on their spatial distribution characteristics:

Cluster 1 grouped mature and immature individuals of most batoid species (e.g., *R. clavata*, *R. miraletus*, *R. polystigma*, *T. marmorata*), characterized by higher SA/PA values and lower crowding that suggest more uniform and dispersed spatial distributions (Fig. 10).

Cluster 2 included immature stages of *S. canicula*, *S. blainvillei*, *E. spinax*, *R. clavata*, *R. miraletus*, and mature stages of *S. canicular* and *G. melastomus*, which were all associated with higher spatial aggregation (higher Mean Crowding and CV, lower SA/PA).

Cluster 3 grouped a small subset composed by immature individuals of *G. melastomus* and mature individuals of *S. blainvillei* and *L. melitiensis.* Those species and maturity groups are segregated along PC2, indicating high crowding and patchiness in their spatial distribution.

The PERMANOVA conducted using Euclidean distances on standardized spatial indices revealed a significant effect of species on the multivariate structure of spatial indices (R^2 = 0.09, F = 36.72, $p < 0.001$, 999 permutations). These interspecific differences in spatial crowding and dispersion support Hypothesis 3, showing that species and maturity groups adopt different aggregation strategies.

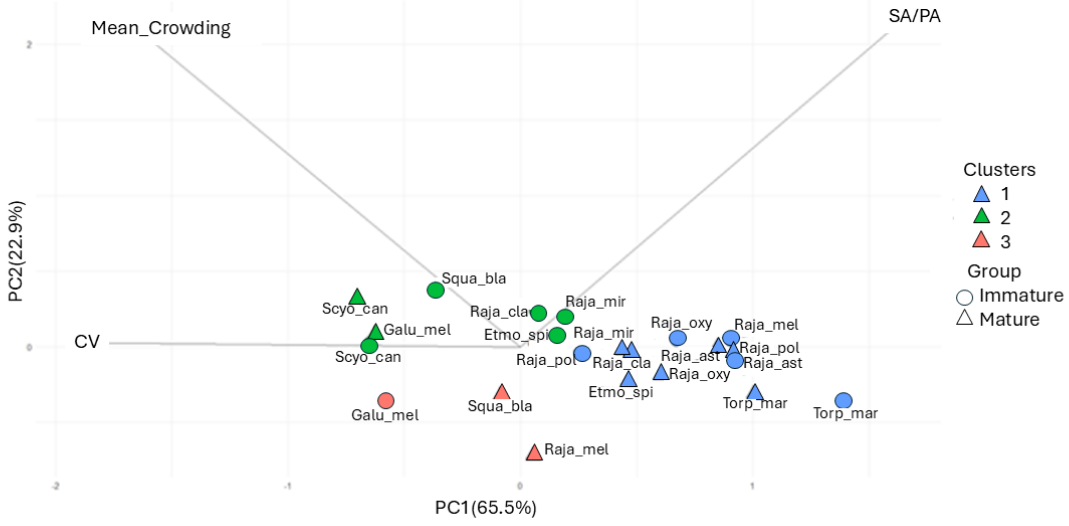


Figure 10. PCA biplot based on the contribution and direction of the spatial indices (SA/PA, CV, Mean Crowding) to the PCA axes. Symbols represent species–maturity group combinations, colours identify the three clusters obtained through K-means clustering.

The second PCA was performed on species-level mean spatial indices, considering maturity classes jointly for each species to explore interspecific differences in spatial strategies. The first principal components explained a proportion of variance of 68.2%, while PC2 the 17.9%). The PCA biplot reveals interspecific differences in spatial dispersion, *S. canicula* and *G. melastomus* belong to the same cluster and appear to be spatially segregated from other species, mainly along PC1, which is associated with high values of crowding and CV in mature individuals. *T. marmorata* and *L. melitensis* belong to the second cluster and are separated along PC2, indicating distinct spatial dynamics, driven by lower SA/PA and more homogeneous distributions. The other species, belonging to the third cluster, are similar in their spatial characteristics (Fig. 11). The PERMANOVA conducted using Euclidean distances on standardized spatial indices, revealed a significant effect of species on the multivariate structure of spatial indices ($R^2 = 0.09$, $F = 36.72$, $p < 0.001$, 999 permutations).

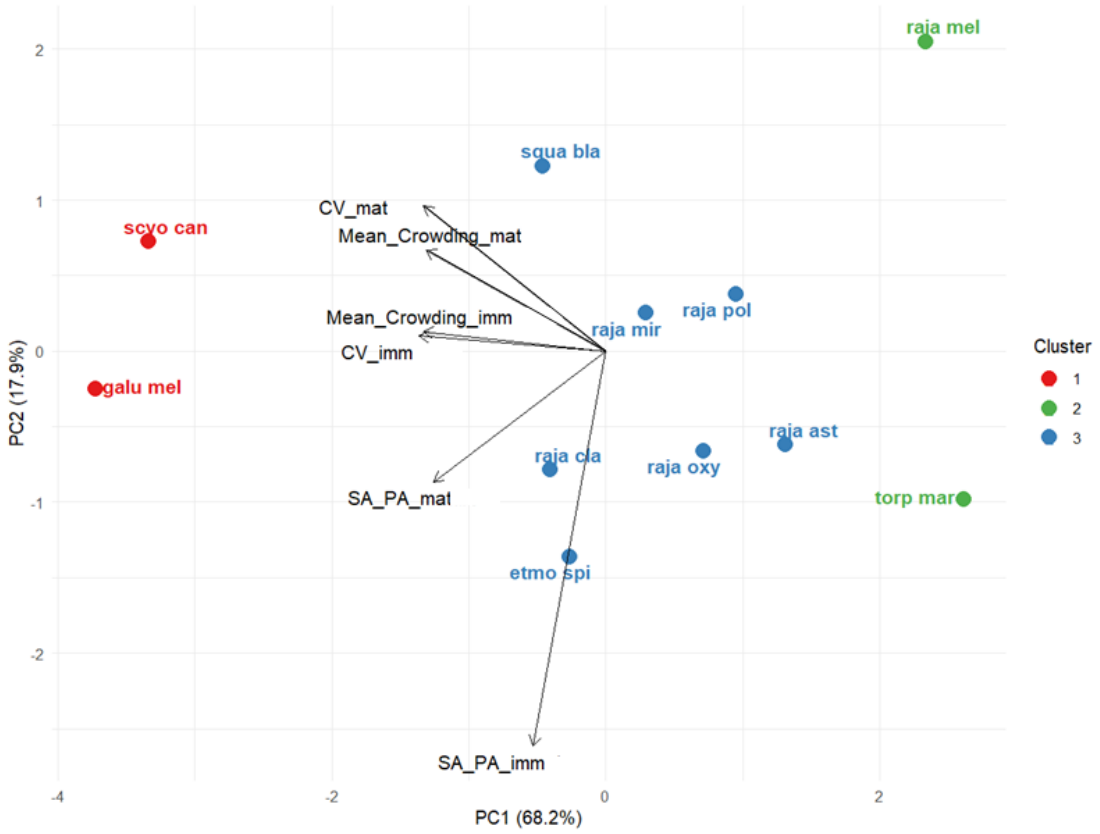


Figure 11. PCA biplot representing the contribution of each spatial index—Coefficient of Variation (CV), Mean Crowding, and SA/PA—calculated separately for immature (*imm*) and mature (*mat*) individuals. Points correspond to species, coloured according to the three clusters identified via K-means clustering. CV_mat: Coefficient of variation of mature individuals; CV_imm: Coefficient of variation of immature individuals; Mean_Crowding_mat: Mean Crowding mature individuals; Mean_crowding_imm: Mean Crowding immature individuals; SA/PA_mat: Spreading area/ Positive area of mature individuals; SA/PA_imm: Spreading Area/Positive Area of immature individuals.

4. Discussion

This study assessed sex– and maturity-specific spatial aggregation patterns in demersal elasmobranchs, based on 30 years of standardized fishery-independent data. By combining association metrics, persistence analyses and spatial indices, we identified intra- and interspecific differences in aggregation dynamics, offering new insights into the gregarious behaviors of Mediterranean deep-sea elasmobranchs. Our findings support the three working hypotheses: (i) Hypothesis 1 was supported by the detection of significant, non-random co-

occurrences among sex–maturity classes and their recurrent spatial patches; (ii) Hypothesis 2 was supported by the ontogenetic shift from aggregated juvenile distributions to more homogeneous adult patterns in several species; (iii) Hypothesis 3 was supported by the interspecific differences in spatial indices and clustering of species–maturity groups revealed by the multivariate analyses.

4.1. Sex-Maturity Class Co-occurrence Patterns

Our results show significant co-occurrences between sex–maturity classes for multiple species. However, for others that usually occur at higher abundances, identifying aggregation patterns was more challenging, as it requires disentangling the biological processes behind high densities. For instance, in *G. melastomus,* significant co-occurrences across multiple sex-maturity combinations may result from the absence of strong spatial segregation by sex or maturity throughout the life cycle (Karampetsis et al., 2022; Rinelli et al., 2005). Moreover, the ubiquity of *G. melastomus* MEDITS catches (D'Iglio et al., 2021; Ramirez-Amaro et al., 2015) could lead to high association values due to overlapping distributions.

The results of the association index for *S. canicula* showed patterns consistent with previous evidence of size-based grouping (Rodríguez-Cabello et al., 2007; Sims et al., 2001). Juveniles were frequently detected in high co-occurrence and density, suggesting nursery use similar to that reported for the central Adriatic Sea (De Santis et al., 2024). In adults, previous studies showed the existence of a sexual segregation driven by female avoidance of male sexual harassment (Finotto et al., 2015; Sims et al., 2001; Wearmouth et al., 2012). However, the significant co-occurrences observed in our dataset could indicate reproductive dynamics and familiarity-driven associations, as supported by evidence of spatial structure shaped by sociality among individuals (Jacoby et al., 2012).

For *S. blainville*, *E. spinax* and *R. clavata*, significant co-occurrences of immature males and females suggest a shared use of nursery-like habitats, in agreement with previous evidence for juvenile aggregations for these species (Ellis et al., 2024; Mattína et al., 2024; Turan et al., 2024). In *E. spinax*, mature individuals aggregated, potentially as a reproductive strategy to increase mate encounters and female mate choice (Duchatelet et al., 2020), consistent with schooling behaviors described for the species (Claes & Mallefet, 2009, 2010; Springer, 1967). In addition to the expected patterns, we detected a significant co-occurrence between mature

and immature males of *S. blainville*, potentially resulting from trophic overlap when prey availability is high (Kousteni et al., 2017) and from sexual segregation for competition, reproduction purposes or to reduce cannibalism (Anastasopoulou et al., 2018; Stenberg, 2005). In batoids, significant associations were detected only among mature individuals, which may reflect seasonal reproductive aggregations for increased mating opportunities (Di Crescenzo et al., 2024; Holden, 1975; Perier et al., 2011; Siskey et al., 2019). The consistency of our findings with previous studies across different species and regions supports our interpretations. The lack of bathymetric segregation reported for *R. clavata* in the Mediterranean (Massutí & Moranta, 2003) strengthens the hypothesis that adult co-occurrences could not be due solely to habitat overlap but potentially reflect reproductive strategies. Although information on the gregarious behaviour of *S. blainville* is limited, evidence from its congener *S. acanthias* indicates that spiny dogfish may form large schools of immature individuals in their nursery areas (Bradai et al., 2012; Marino et al., 2015), further validating our results.

4.2. Persistent Co-occurrence Patches

The persistence analyses showed that several species repeatedly use the same areas across years, with patches of immature individuals more numerous and temporally stable than those of mature individuals. This pattern indicates a recurrent use of specific sites, which could reflect stable environmental conditions, habitat suitability or reproductive behaviors possibly associated with philopatric tendencies (Bester-van der Merwe et al., 2022; Flowers et al., 2016). Juvenile persistence was evident for *S. canicula*, *G. melastomus*, *E. spinax, S. blainville* and *R. clavata*, where aggregations lasted up to 8–9 years, supporting the concept of long-term nursery ground use (Coelho & Erzini, 2010; Jacoby et al., 2012; McAllister et al., 2024; Treude et al., 2011; Turan et al., 2024). Although the repeated use of nursery (Bangley et al., 2018; Gruber et al., 1988) and gestation areas (Hight & Lowe, 2007; Nosal et al., 2021) have been documented for several shark species, few breeding grounds have been identified (Chapman et al., 2015; b. Jacoby et al., 2012). As in our study, most sharks' breeding sites are inferred from indirect evidence (Jorgensen et al., 2012; Pratt & Carrier, 2001). The persistence of juvenile patches across nearly a decade provides strong evidence of site fidelity or repeated use of specific areas in several Mediterranean demersal

elasmobranchs, as also reported in other regions (Chapman et al., 2015). Moreover, the presence of persistent patches in species with different life-history traits (small benthic sharks, deep-sea sharks, and skates) suggests that spatial fidelity is a common feature across taxa. It is important to note that the patches identified in this study should not be interpreted as precise delineations of aggregation sites. They represent areas where MEDITS hauls with similar high densities and significant co-occurrences cluster in space and time. Given the spacing of trawl stations and the high mobility of elasmobranchs, the true spatial extent and internal structure of aggregation events cannot be resolved with survey data alone. Fine-scale tracking or tagging studies would be required to map the actual extent of reproductive or nursery aggregations. In this sense, our patches are best viewed as spatial proxies of aggregation processes, highlighting broader zones of recurrent co-occurrence and potential biological importance.

4.3. Intraspecific spatial patterns

Our analyses revealed ontogenetic differences in spatial patterns across several shark and skate species. Overall, both sharks and batoids have higher aggregation in immature stages, reflected in lower spatial uniformity and higher crowding values, while adults, especially mature females, have more homogeneous distributions. This general trend was evident in *E. spinax*, *S. canicula*, *S. blainville*, *G. melastomus*, *R. clavata*, *R. miraletus*, *R. polystigma* and *D. oxyrinchus*. *R. asterias*, *L. melitensis* and *T. marmorata* did not show significant maturity-related differences. This may indicate homogeneous spatial structures across life stages or limitations in detecting fine-scale segregation with the available data. The observed ontogenetic shift from aggregated juveniles to more uniform distributions in adults aligns with biological expectations. Juveniles of many elasmobranchs tend to concentrate in nursery areas that provide suitable environmental conditions, prey availability, and refuge from predation (McMillan et al., 2021). Mature individuals, especially females, often adopt more dispersed distributions, likely reflecting different energetic requirements and reproductive strategies (Rodríguez-Cabello et al., 2007; Simpson et al., 2021; Sims et al., 2001). Our analyses show that *E. spinax*, *S. canicula*, *S. blainville*, *G. melastomus*, and *R. clavata* exhibit a shift from aggregated immature stages to more uniform adult distributions. While previous research has highlighted nursery use in juveniles for some of these species (Ellis et al., 2008;

McAllister et al., 2024), our study, to our knowledge, provides the first quantitative evidence of such ontogenetic differentiation in spatial crowding patterns across multiple taxa. These findings provide new insight into life-stage-specific association patterns and strengthen the hypothesis that ontogenetic spatial differentiation is a widespread trait among demersal elasmobranchs. Despite these identified patterns, some limits should be discussed. Species such as *R. asterias*, *L. melitensis* and *T. marmorata* showed no significant spatial differentiation among maturity stages. These results may reflect homogeneous spatial distributions, insufficient sampling resolution in trawl surveys to capture fine-scale segregation or low sample sizes for rarer species. Our results indicate that ontogenetic differences in spatial structure are common in Mediterranean demersal elasmobranchs.

4.4. Interspecific spatial patterns

The multivariate analyses revealed differences in spatial distribution linked to both species identity and maturity stage. The first PCA separated three main clusters, (i) batoid species, which exhibited more uniform and dispersed spatial distributions; (ii) immature sharks belonging to *S. canicula*, *S. blainvillei*, and *E. spinax*, as well as some mature groups associated with higher spatial aggregation, and (iii) a subset of species–maturity groups (*G. melastomus* immatures, *S. blainvillei* adults, *L. melitensis* adults) characterized by high crowding and patchiness. The second PCA highlighted interspecific differences, with *S. canicula* and *G. melastomus* showing greater aggregation relative to other taxa, while *T. marmorata* and *L. melitensis* had more homogeneous distributions. *G. melastomus* and *S. canicula* share oviparous and continuous reproductive mode, high abundance in catches and overlapping diets (Kousteni et al. 2010; Capapé et al. 2014; Valls, 2017), supporting the hypothesis that ecologically similar small sharks may have convergent aggregation pattern. Batoids clustered together with dispersed distributions, which is consistent with their benthic lifestyle, low mobility, oviparous reproductive strategies and habitat requirements (Schlaff et al., 2014). The tendency of immature sharks such as *S. canicula*, *S. blainvillei* and *E. spinax* to form more aggregated distributions supports their use of nursery habitats, consistent with previous observations of juveniles concentrating on specific areas offering favorable conditions for growth and protection (Cau et al., 2017; D'Onghia et al., 2010; Kabasakal et al., 2024). *S. blainvillei* mature individuals clustered with highly aggregated immature *G.*

melastomus, a pattern that could be linked to reproductive aggregations, as reported for its congener *S. acanthias*, which is known to form large schools of mature individuals during the mating season (Bonanomi et al., 2018; Marino et al., 2015). Large aggregations of *G. melastomus* have also been documented in deep-sea environments (Sisma-Ventura et al., 2024), although the observed similarity in our analysis likely reflects comparable spatial concentration patterns rather than shared ecological or biological drivers.

These findings indicate that spatial aggregation patterns are shaped by both ontogeny and species-specific ecology.

4.5. Conservation and Management

This study identified intra- and interspecific differences in aggregation dynamics, offering new insights into the gregarious behaviors of Mediterranean deep-sea elasmobranchs, and their implications for conservation and management. Persistent juvenile aggregations indicate long-term nursery use and should be prioritized for spatial protection and adaptive management. Measures such as temporal closures or gear restrictions could reduce juvenile mortality and support early life-stage survival (Carrier & Pratt, 1998). However, conservation efforts that focus exclusively on nursery areas without protecting adult populations may fail to achieve population recovery. The collapse of *G. galeus* populations, despite 30 years of nursery-focused protection, demonstrated that unregulated fishing of adults can undermine recruitment and population persistence (Kinney & Simpfendorfer, 2009; Punt et al., 2000). Adult aggregation sites may represent potential reproductive hotspots, the protection of thich is crucial to sustaining population recruitment (Barnett et al., 2019). The more uniform distribution of mature individuals, especially females, suggests that reproductive critical habitats are less spatially predictable than nurseries, posing challenges for conservation and management (Finotto et al., 2021; Jirik & Lowe, 2012). Male-only aggregations, such as those observed in *S. blainville*, may correspond to feeding grounds where resource availability is high, pointing to areas where fishing pressure should be regulated (Jacoby et al., 2012; Sims, 2003). Our results show that long-term persistent aggregation patches are common among Mediterranean demersal elasmobranchs, especially for juveniles. Conservation strategies should therefore integrate nursery protection with broader measures that also include adult populations, avoiding the risk of decline observed in other species. Depending on each

species' conservation status, persistent areas should be prioritized for spatial management to reduce exposure to both targeted and incidental fishing, which is increased by the repeated use and predictability of specific sites (McAllister et al., 2017). The aggregation of juvenile sharks reinforces the importance of nursery grounds, while the broader dispersion of batoids suggests that different conservation actions are needed. Highly aggregated groups, such as immature *G. melastomus* or mature *S. blainvillei*, may indicate potential critical habitats that may be vulnerable to localized fishing pressure. The more homogeneous distributions of species like *L. melitensis* and *T. marmorata* underscore the need for finer-scale monitoring and the importance of considering additional factors, such as phylogeny and ecology, when interpreting interspecific differences in spatial aggregation. From a management perspective, these findings provide a baseline to guide the development of spatially explicit conservation measures that account for both interspecific and ontogenetic differences in aggregation patterns.

5. Limitations and future perspectives

Because MEDITS is a fishery-independent trawl survey conducted once per year and always in the same season, the four aggregation criteria applied in Chapter 1 (high abundance, high occurrence, site fidelity, and seasonality) cannot be fully implemented here. In particular, seasonality cannot be assessed, and density-based thresholds would artificially inflate the detection of aggregations in species that naturally occur at high densities. The analyses rely on trawl survey data, which, despite being standardized across decades, are limited in spatial and temporal resolution and may underestimate fine-scale dynamics and seasonality of aggregation. The absence of direct behavioral observations (e.g. mating events) means that interpretations of reproductive aggregations are inferred indirectly from patterns of co-occurrence supported by literature evidence. In species occurring at high abundances, the interpretation of co-occurrence patterns requires particular caution. For *S. canicula*, despite its high density in MEDITS hauls, the structure of co-occurrences showed that immature individuals form recurrent patches and adults display class-specific non-random associations potentially linked with nurseries and potential reproductive or familiarity-driven grouping. *G. melastomus* exhibited significant associations across all sex–maturity combinations and widespread co-occurrence patches. This pattern may reflect an artefact of its ubiquitous

distribution and high catchability in trawl surveys, potentially inflating association indices and masking biologically meaningful aggregations. Therefore, while co-occurrence in *S. canicula* may indicate functional aggregation processes, the pervasive overlap observed in *G. melastomus* should be interpreted with greater caution. Our framework was designed to detect temporary, spatially restricted aggregation events showing site fidelity across years, which naturally emerge more clearly in species with heterogeneous distributions. As a consequence, the co-occurrence patterns observed in *G. melastomus* may indicate that trawl-survey data may be insufficient to resolve fine-scale aggregation in highly ubiquitous species, for which higher-resolution data may be required. We did not modify the aggregation threshold (≥2 individuals), which follows the definition proposed by McInturf et al. (2023) to ensure consistency across species. In highly abundant and ubiquitous species such as G. melastomus, widespread co-occurrences may reflect the species' continuous distribution and high catchability in MEDITS surveys rather than true biologically driven aggregations. Some species (*T. marmorata*, *R. polystigma*, *D. oxyrinchus*, *R. miraletus*) did not exhibit persistent patches. Possible explanations are (i) use of habitats not well covered by trawl surveys, such as rocky coastal areas (Tiralongo et al., 2019); (ii) species-specific life-history traits, such as range-restricted endemics (Porcu et al., 2020); and (iii) differences in catchability that may bias survey data (Damalas & Vassilopoulou, 2011). The multivariate model showed a significant species effect, indicating that species identity contributes to differences in spatial structure. However, local environmental conditions may play a role in shaping aggregation patterns, but these factors were not considered in the present study**.** Although reproductive strategies might influence aggregation patterns, our analyses did not reveal a clear separation between oviparous and ovoviviparous species. The multivariate analysis did not cluster species according to their reproductive strategy, indicating that this factor was not a major driver of spatial differentiation in our dataset. For this reason, no statistical test was performed to assess differences between reproductive modes. Future research should integrate environmental modeling, and finer-scale sampling to quantify the spatial extent, ecological drivers and temporal dynamics of aggregation processes.

6.1 Conclusions

This study provides an assessment of sex- and maturity-specific aggregation patterns in Mediterranean demersal elasmobranchs, revealing intra- and interspecific differences in spatial organization. These patterns reflect the combined influence of life stage, species-specific ecology, and behavioral strategies, offering new insights into the gregarious dynamics of deep-sea and benthic sharks and rays. By providing a quantitative baseline for the aggregation of these taxa, our findings highlight that effective conservation strategies must consider the gregarious dynamics of different life stages and sexes, ensuring that both juvenile nurseries and adult permanent aggregation sites are integrated into spatial management plans.

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Concluding remarks and thesis impact

This thesis provided an analysis of elasmobranch aggregations in the Mediterranean Sea, addressing the topic from multiple perspectives and across different spatial and temporal scales, to fill knowledge gaps and contribute to the development of management actions and targeted conservation strategies. Through an integrated approach -combining a systematic literature review, citizen science data, direct field observations, and long-term fishery-independent datasets- it was possible to design a comprehensive picture of shark and ray aggregation dynamics, highlighting recurring ecological patterns and species/area-specific features. The first part of the thesis (Chapter 1) systematized the available information on Mediterranean aggregations through a replicable classification protocol that distinguishes between confirmed, potential, and single occurrences. This methodology, which can also be applied in data-poor contexts, allowed the identification of recurring spatial and seasonal distributions: aggregations are mostly concentrated in spring and summer, in epipelagic waters and coastal habitats such as rocky bottoms, sandy/muddy substrates, and brackish areas, with biological functions primarily linked to reproduction and feeding. However, a high proportion of events could not be assigned to a clear biological purpose, underscoring the need for targeted behavioral and ecological studies on the species involved. Another emerging aspect is the uneven distribution of research efforts among different Mediterranean sub-areas. Some regions with high potential biodiversity and reported aggregations remain poorly investigated, risking an underestimation of their importance and a lack of protective measures. In this context, the integration of alternative data sources (citizen science, local ecological knowledge, opportunistic monitoring) has proven to be a valuable resource to expand spatial and temporal coverage, if data collection and dissemination are managed not to increase human pressure on sensitive sites. The second and third parts (Chapter 2 & Chapter 3) focused on species-specific case studies-such as those on the common eagle ray *M. aquila* and the common stingray *D. pastinaca*- which highlighted the importance of coastal sites and MPAs as reproductive aggregation grounds, but also their vulnerability to both direct and indirect anthropogenic pressures. These findings reinforce the need for site-specific management measures able to balance sustainable resource use with the protection of the most sensitive life stages. The fourth part (Chapter 4) focused on the study of the sandbar shark *C. plumbeus* aggregation at Lampione Island in the Pelagie Archipelago (Southern Sicily, Italy), revealing

the role of such events in facilitating interspecific interactions, opening novel research perspectives at least at regional level on the ecological functions of such aggregations beyond single-species contexts. The fifth part (Chapter 5) presented a basin-wide analysis based on 30 years of standardized trawl survey data from the MEDITS program, revealing patterns of co-occurrence among demersal species, sexes, and life stages. The results suggest that aggregations may have complex and diversified patterns. These results may support the adoption of spatial and temporal management tools that consider the demographic structure of aggregations.

Overall, the results of this thesis emphasize that elasmobranch aggregations in the Mediterranean represent critical phases in the life cycle of sharks and rays and that their conservation requires adaptive and dynamic management strategies. Such strategies should be grounded in updated, integrated scientific knowledge and include measures such as seasonal closures, gear-specific regulations, and the protection of key habitats, while also promoting sustainable, non-extractive economic alternatives, including responsible forms of marine ecotourism. Beyond its ecological contributions, this thesis provides both a methodological framework and a conceptual foundation to guide future research and conservation planning in the Mediterranean. The multi-scale and multi-source approach adopted here has proven effective in filling significant knowledge gaps and in generating operational guidance. Finally, this work highlights how a deeper understanding of aggregation dynamics can serve as a strategic lever for the protection of elasmobranch populations, especially within highly anthropized and vulnerable marine ecosystems such as the Mediterranean Sea.

This thesis has generated a series of significant outcomes in terms of ecological insights on aggregation of Mediterranean sharks and rays, supporting conservation strategies and scientific outreach. From a conservation perspective, the results presented guided the establishment of two Important Shark and Ray Areas (ISRA - https://sharkrayareas.org/) (Fig. 1).

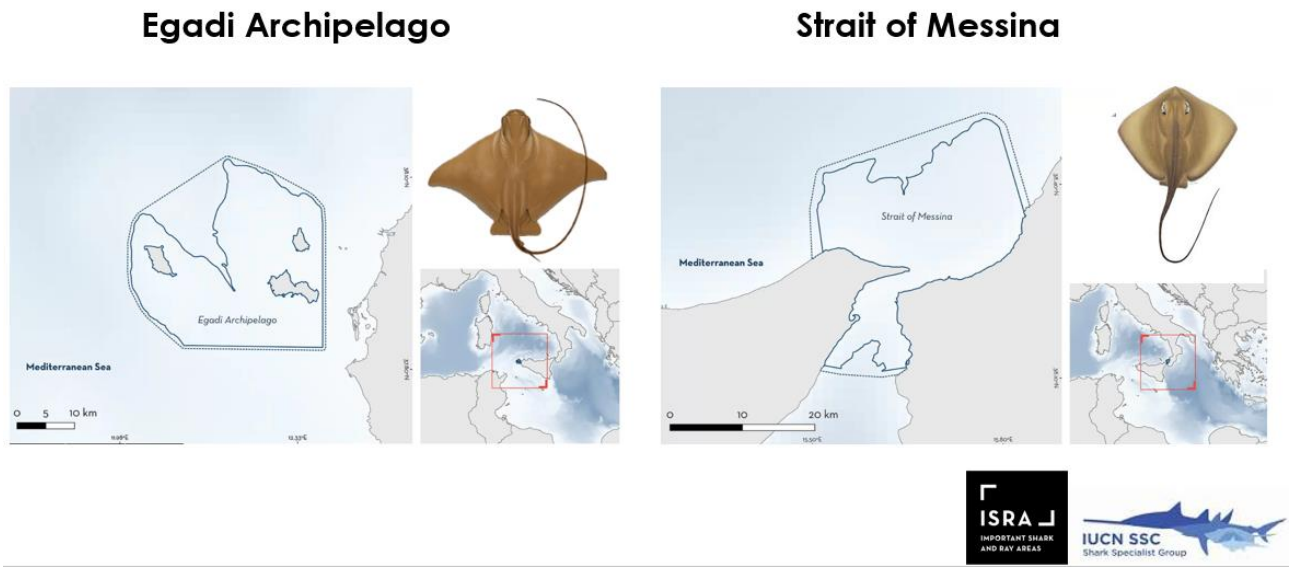

Figure 1. Site location of the two ISRAs (https://sharkrayareas.org/) established with the results of this thesis.

This thesis also contributed to the outreach article of the *Science Shot* magazine (https://www.science.org/content/article/watch-fish-hunt-hiding-behind-shark), highlighting the international relevance and ecological importance of the research topic. In terms of scientific outreach and communication, the findings have been shared with the public through various initiatives, including the Biodiversity Exhibition in Rome (hosted from 27th of November 2024 to 30th of March 2025) and an episode of the program "*Le Sentinelle della Biodiversità*". These events provided opportunities to raise public awareness about the conservation of elasmobranchs.

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