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# PAST AND RECENT DEMOGRAPHIC HISTORIES OF WESTERN MEDITERRANEAN DEMERSAL CHONDRICHTHYANS

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Doctor by the Universitat de les Illes Balears

*A la nueva integrante de la familia “Melanie”*

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# List of Manuscripts

The four chapters containing original research (Chapter I-IV) are present as a collection of five papers, two already published, two others currently submitted, and the last one in preparation for its submission, preceded by a General Introduction, Objectives, and Material and Methods, and followed by Conclusions and References. For this reason, each chapter can be read separately, because it has its own Summary, Keywords, Introduction, Material and Methods, Results and Discussion. The following scientific papers are partially or totally reflected in the Thesis.

Ramírez-Amaro S., Ordines F., Terrasa B., Esteban A., García C., Guijarro B., Massutí E. (2015). Demersal chondrichthyans in the western Mediterranean: assemblages and biological parameters of their main species. *Marine and Freshwater Research* 67: 636–652 (Chapter I).

Ramírez-Amaro S., Picornell A., Arenas M., Castro J.A., Massutí E., Ramon C., Terrasa B. (submitted). Contrasting evolutionary patterns in populations of demersal sharks sampled at a fine scale throughout the western Mediterranean (Chapter II).

Ramírez-Amaro S., Ordines F., Puerto M.A., García C., Ramon C., Terrasa B., Massutí E. (2017). New morphological and molecular evidence confirm the presence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea and extend its distribution to the western basin. *Mediterranean Marine Science* 18/2: 253–261 (Chapter III).

Ramírez-Amaro S., Ordines F., Picornell A., Castro J.A., Ramon C., Massutí E., Terrasa B. (submitted). The evolutionary history of Mediterranean Batoidea (Chondrichthyes: Neoselachii): a molecular phylogenetic approach (Chapter III).

Ramírez-Amaro S., Ordines F., Esteban A., García C., Guijarro B., Salmerón F., Terrasa B., Massutí E. (in preparation). Spatio-temporal trends in demersal Chondrichthyans exploited by bottom trawling in the western Mediterranean: some resist, but others do not. (Chapter IV).

## Summary

Chondrichthyans (sharks, rays and chimaeras) play a key role in marine ecosystems, since they are top predators. Because of their life-history traits (slow growth, late maturity and low fecundity), they are especially vulnerable to fishing. In the Mediterranean, which hosts 7% of living chondrichthyans, a notable change in marine ecosystems has occurred due to anthropogenic impacts, with overfishing as one of the most important. For this reason, and because these species can be an important by-catch of fisheries, the International Union for Conservation of Nature (IUCN) considers this region a key hotspot of extinction risk for chondrichthyans.

This Thesis contributes to improve the scientific knowledge of chondrichthyans on the circalittoral and bathyal soft bottoms along the western Mediterranean. Methods traditionally used to study population dynamics and biological communities, jointly with molecular tools, were applied to assess the past and present status of these vulnerable species. Data and samples were obtained during the MEDITS bottom trawl research surveys, developed annually in spring-summer since 1994 throughout the four geographic sub-areas (GSAs) established by the General Fisheries Commission for the Mediterranean (GFCM) in the study area: Northern Alboran Sea (GSA01) and Alboran Island (GSA02) considered jointly as GSA01, Balearic Islands (GSA05) and Northern Spain (GSA06).

A total of 33 species of demersal chondrichthyans, belonging to 13 families were identified, of which 26, 28, and 19 species were caught in GSA01, GSA05 and GSA06, respectively. Two different assemblages were detected in each GSA, corresponding to the continental shelf and slope. The highest values of diversity, abundance, and biomass were recorded on the continental shelf of GSA05 and the slope of GSA01, while GSA06 showed the lowest values at both depth strata. These results can be explained by differences in trawl fishing exploitation and types of bottom between GSAs. Biological parameters such as optimum depth, length at first maturity, and size structure are provided for the most abundant species: *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax*, and *Raja clavata*. Spatio-temporal trends show fairly stable populations over the last 20 years regarding richness, abundance and biomass, even with an increment in density of species dwelling on the continental shelf and the most abundant on the slope, but decreasing trends in some other deep water species. This can be related to the reduction of trawl fishing effort on the continental shelf over the last decades, because fishing fleet has been reduced and displaced towards deeper waters.

Genetic analyses revealed contrasting population patterns in *S. canicula* and *G. melastomus*, the two most abundant chondrichthyans on the continental shelf and slope, respectively. The first species showed genetic differences and low connectivity between Alboran Sea (GSA01-GSA02) and Balearic Sea (GSA05-GSA06), while the second one did not show geographic differences. Distinct demographic histories were also detected for both species. While *G. melastomus* exhibited a recent population expansion, *S. canicula* showed stability at present, but signs of past bottleneck events. Phylogenetic results revealed a parallel evolutionary history between batoids and the Mediterranean, with two important speciation events: i) during the Eocene-Oligocene transition, when almost all genera were originated; and ii) during the Miocene, when almost all current species appeared. Depth and benthic habitats were shown as the main drivers of speciation in *Raja* genera, both being of equal importance alongside physical distance and geographical barriers. Molecular analysis showed the presence of 18 batoids in the western Mediterranean and revealed misidentifications based only on morphological characters, including the confirmation of *Dasyatis* cf. *tortonesei* as a valid species and the presence of *Dipturus nidarosiensis* in the Mediterranean.

**Keywords**: Chondrichthyans, batoids, *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax*, *Raja clavata*, *Raja* spp., *Dasyatis* cf. *tortonesei*, *Dipturus nidarosiensis*, vulnerable species, demersal, assemblages, diversity, population dynamics, abundance, biomass, length at first maturity, length frequency, population genetic structure, connectivity, demographic history, phylogeny, speciation, mtDNA, STRs, DNA barcoding, temporal trends, assessment, conservation status, management, recovery, fisheries, bottom trawl, research survey, GFCM, Mediterranean.

## Título

Historias demográficas pasadas y recientes de los condrictios demersales del Mediterráneo Occidental

## Resumen

Los condrictios (tiburones, rayas y quimeras) desempeñan un papel clave en los ecosistemas marinos, dado que son depredadores apicales. Debido a las características de su ciclo vital (crecimiento lento, madurez tardía y baja fecundidad), son especialmente vulnerables a la pesca. El Mediterráneo, que alberga alrededor del 7% de los condrictios actuales, ha sufrido cambios importantes en sus ecosistemas marinos, causados por los impactos antropogénicos, entre los cuales la sobrepesca es uno de los más importantes. Por esta razón, y debido a que estas especies son capturadas frecuentemente de forma accidental en las pesquerías, la Unión Internacional para la Conservación de la Naturaleza (UICN) considera esta región como un punto clave de riesgo de extinción para los condrictios.

Esta Tesis contribuye a mejorar el conocimiento científico de los condrictios en los fondos circalitorales y batiales del Mediterráneo Occidental. Se han aplicado métodos tradicionales de estudio de dinámica de poblaciones y comunidades biológicas, así como herramientas moleculares, con el propósito de evaluar la situación pasada y presente de estas especies vulnerables. Los datos y muestras se obtuvieron durante las campañas de investigación MEDITS, con arte de arrastre de fondo, realizadas anualmente desde 1994 en primavera-verano, a lo largo de cuatro sub-áreas geográficas (GSAs), establecidas por la Comisión General de Pesca para el Mediterráneo (CGPM) en el área de estudio: Norte del Mar de Alborán (GSA01) e Isla de Alborán (GSA02), considerados conjuntamente como GSA01, Islas Baleares (GSA05) y Norte de España (GSA06).

Se han identificado un total de 33 especies de condrictios demersales, pertenecientes a 13 familias, de los cuales 26, 28 y 19 especies fueron capturados en la GSA01, GSA05 y GSA06, respectivamente. Se han detectado dos comunidades diferentes en cada GSA, que corresponden a la plataforma continental y al talud. Los valores más altos de diversidad, abundancia y biomasa se han obtenido en la plataforma continental de la GSA05 y en el talud de la GSA01, mientras que la GSA06 ha mostrado los valores más bajos en ambos estratos batimétricos. Estos resultados se pueden explicar por las diferencias en el esfuerzo de la pesca de arrastre y los tipos de fondo entre estas GSAs. Se han estimado parámetros biológicos (profundidad optima, talla de primera madurez y frecuencia de tallas) de las especies más abundantes: *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax* y *Raja clavata*. Las tendencias espacio-temporales muestran poblaciones bastante estables durante los últimos 20 años con respecto a la riqueza de especies, abundancia y biomasa, incluso con un aumento de la densidad de especies que habitan la plataforma continental y en la especie más abundante en el talud, pero con tendencias decrecientes en algunas otras especies de gran profundidad. Esto se puede relacionar con la reducción del esfuerzo de la pesca de arrastre en la plataforma continental durante las últimas décadas, a causa de la disminución de la flota y de su desplazamiento hacia aguas más profundas.

Los análisis genéticos han mostrado que *S. canicula* y *G. melastomus*, las dos especies de condrictios más abundantes de la plataforma continental y el talud, respectivamente, tienen patrones poblacionales distintos. La primera ha presentado diferencias genéticas y baja conectividad entre el Mar de Alborán (GSA01-GSA02) y el Mar Balear (GSA05-GSA06), pero la segunda especie no ha mostrado estas diferencias geográficas. También se ha detectado una historia demográfica distinta entre ambas especies. Mientras que *G. melastomus* ha tenido una expansión poblacional reciente, *S. canicula* muestra mayor estabilidad, pero con evidencias de haber sufrido eventos de cuello de botella en el pasado. Los resultados filogenéticos han mostrado una historia evolutiva paralela de los batoideos y el Mediterráneo, con dos importantes eventos de especiación: i) durante la transición Eoceno-Oligoceno, cuando se originaron casi todos los géneros; y ii) durante el Mioceno, cuando aparecieron casi todas las especies actuales. Se ha demostrado que la profundidad y los hábitats bentónicos son los principales factores de especiación en el género *Raja*, siendo de igual importancia que las barreras geográficas y la distancia física. El análisis molecular ha demostrado la presencia de 18 especies de batoideos en el Mediterráneo Occidental y ha evidenciado identificaciones erróneas basadas sólo en caracteres morfológicos. Se ha confirmado *Dasyatis* cf. *tortonesei* como especie válida y la presencia de *Dipturus nidarosiensis* en el Mediterráneo.

**Palabras clave**: Condrictios, batoideos, *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax*, *Raja clavata*, *Raja* spp., *Dasyatis* cf. *tortonesei*, *Dipturus nidarosiensis*, especies vulnerables, demersales, comunidades, diversidad, dinámica de poblaciones, abundancia, biomasa, talla de primera madurez, frecuencia de tallas, estructura genética poblacional, conectividad, historia demográfica, filogenia, especiación, ADNmt, STRs, código de barras de ADN, tendencias temporales, evaluación, estado de conservación, gestión, recuperación, pesquerías, arrastre de fondo, campaña de investigación, CGPM, Mediterráneo.

## Títol

Històries demogràfiques passades i recents dels condrictis demersals de la Mediterrània Occidental

## Resum

Els condrictis (taurons, rajades i quimeres) tenen un paper clau en els ecosistemes marins, ja que són depredadors apicals. Per les característiques del seu cicle vital (creixement lent, maduresa tardana i baixa fecunditat), són especialment vulnerables a la pesca. La Mediterrània, on hi ha al voltant del 7% dels condrictis actuals, ha sofert canvis importants en els seus ecosistemes marins, causats pels impactes antropogènics, entre els quals la sobrepesca és un dels més importants. Per aquesta raó, i pel fet que aquestes espècies són capturades freqüentment de forma accidental a les pesqueries, la Unió Internacional per a la Conservació de la Naturalesa (IUCN) considera aquesta regió com un punt clau de risc d'extinció per als condrictis.

Aquesta Tesi contribueix a millorar el coneixement científic dels condrictis en els fons circalitorals i batials de la Mediterrània Occidental. S'han aplicat mètodes d'estudi tradicionals de dinàmica de poblacions i comunitats biològiques, així com eines moleculars amb el propòsit d'avaluar la situació passada i present d'aquestes espècies vulnerables. Les dades i mostres s'han obtingut durant les campanyes d'investigació MEDITS, amb art de ròssec de fons, realitzades anualment des del 1994 a la primavera-estiu, al llarg de quatre subàrees geogràfiques (GSAs) establertes per la Comissió General de Pesca per a la Mediterrània (CGPM) en l'àrea d'estudi: Nord de la Mar d'Alborán (GSA01) i Illa d'Alborán (GSA02), considerats conjuntament com GSA01, Illes Balears (GSA05) i Nord d'Espanya (GSA06).

S'han identificat un total de 33 espècies de condrictis demersals, que pertanyen a 13 famílies, de les quals 26, 28 i 19 espècies van ser capturades a les GSA01, GSA05 i GSA06, respectivament. A cada GSA s'han detectat dues comunitats diferents, que corresponen a la plataforma continental i al talús. Els valors més alts de diversitat, abundància i biomassa s'han obtingut en la plataforma continental de la GSA05 i en el talús de la GSA01, mentre que la GSA06 ha mostrat els valors més baixos en ambdós estrats batimètrics. Aquests resultats es poden explicar per les diferències en l'esforç de la pesca de ròssec i els tipus de fons entre aquestes GSAs. S'han estimat paràmetres biològics (profunditat òptima, talla de primera maduresa i freqüència de talles) de les espècies més abundants: *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax* i *Raja clavata*. Les tendències espai-temporals mostren poblacions bastant estables durant els últims 20 anys pel que fa a la riquesa d'espècies, abundància i biomassa, fins i tot amb un augment de la densitat d'espècies que habiten la plataforma continental i l'espècie més abundant al talús, però amb tendències decreixents en algunes altres espècies de gran profunditat. Això es pot relacionar amb la reducció de l'esforç de la pesca de ròssec en la plataforma continental durant les últimes dècades, a causa de la disminució de la flota i del seu desplaçament cap a aigües més profundes.

Les anàlisis genètiques han mostrat que *S. canicula* i *G. melastomus*, les dues espècies de condrictis més abundants en la plataforma continental i el talús, respectivament, tenen patrons poblacionals diferents. La primera ha presentat diferències genètiques i baixa connectivitat entre la Mar d'Alborán (GSA01-GSA02) i la Mar Balear (GSA05-GSA06), però la segona espècie no ha mostrat aquestes diferències geogràfiques. També s'ha detectat una història demogràfica diferent entre ambdues espècies. Mentre que *G. melastomus* ha tingut una expansió poblacional recent, *S. canicula* mostra major estabilitat, però amb evidències d'haver sofert esdeveniments de coll de botella en el passat. Els resultats filogenètics han mostrat una història evolutiva paral·lela dels batoïdeus i la Mediterrània, amb dos importants esdeveniments d'especiació: i) durant la transició Eocè-Oligocè, quan es van originar gairebé tots els gèneres; i ii) durant el Miocè, quan van aparèixer la majoria de les espècies actuals. S'ha demostrat que la profunditat i els hàbitats bentònics són els principals factors d'especiació en el gènere *Raja*, sent d'igual importància que les barreres geogràfiques i la distància física. L'anàlisi molecular ha demostrat la presència de 18 espècies de batoïdeus a la Mediterrània Occidental i ha evidenciat identificacions errònies basades només en caràcters morfològics. S'ha confirmat *Dasyatis* cf. *tortonesei* com a espècie vàlida i la presència de *Dipturus nidarosiensis* a la Mediterrània.

**Paraules clau**: Condrictis, batoïdeus, *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax*, *Raja clavata*, *Raja* spp., *Dasyatis* cf. *tortonesei*, *Dipturus nidarosiensis*, espècies vulnerables, demersals, comunitats, diversitat, dinàmica de poblacions, abundància, biomassa, talla de primera maduresa, freqüència de talles, estructura genètica poblacional, connectivitat, història demogràfica, filogènia, especiació, ADNmt, STRs, codi de barres d'ADN, tendències temporals, avaluació, estat de conservació, gestió, recuperació, pesqueries, ròssec de fondària, campanya de recerca, CGPM, Mediterrani.

# Abbreviations and Acronyms

| | |
|---|---|
| AIC | Akaike Information Criterion |
| AICc | Corrected Akaike Information Criterion |
| AMOVA | Analyses of Molecular Variance |
| ANOSIM | Analysis of Similitude |
| AW | Atlantic Water |
| BOLD | Barcode of Life Data System |
| BIC | Bayesian Information Criteria |
| Bm | Bathymetric distribution models |
| BPEC | Bayesian Phylogeographic and Ecological Clustering |
| CB | Crinoid Beds |
| COI | Cytochrome Oxidase Subunit I |
| CR | Critical Endangered |
| CR | Control Region |
| CS | Continental Shelf |
| Cytb | Cytochrome b |
| DD | Data Deficient |
| DFA | Dynamic Factor Analysis |
| DSM | Deep Shelf Sandy-Mud Bottom |
| EBSP | Extended Bayesian Skyline Plot |
| EMDW | Eastern Mediterranean Deep Water |
| EN | Endangered |
| FAO | Food and Agriculture Organization of the United Nations |
| GFCM | General Fisheries Commission for the Mediterranean |
| GLM | Generalized Linear Model |
| GMYC | General Mixed Yule Coalescent |
| GSA | Geographic Sub-Area |
| GSA01 | Northern Alboran Sea |
| GSA02 | Alboran Island |
| GSA05 | Balearic Islands |
| GSA06 | Northern Spain |
| GW | Garza-Williamson Index |
| *h* | Haplotype Diversity |
| H’ | Shannon-Wiener Diversity |
| HWE | Hardy-Weinberg Equilibrium |
| IUCN | International Union for the Conservation of the Nature |
| J | Evenness |
| *k* | Haplotype Differences |
| LAMARC | Likelihood Analysis with Metropolis Algorithm using Random Coalescence |
| LC | Least Concern |
| LD | Linkage Disequilibrium |

| | |
|---|---|
| LFd | Length-Frequency distribution |
| LIW | Levantine Intermediate Water |
| LWp | Length-Weigh Relationship |
| $L_{50}$ | Length at First Maturity |
| MB | Maërl Beds |
| MCMC | Markov Chain Monte Carlo |
| MCO | Miocene-Climatic Optimum |
| MDS | Multidimensional Scaling |
| MEDITS | Mediterranean International Trawl Survey |
| MSA | Multiple Sequence Alignment |
| MSC | Messinian Salinity Crisis |
| mtDNA | Mitochondrial DNA |
| MYA | Million Years Ago |
| Na | Allelic Frequencies |
| NC | Northern Current |
| ND2 | Dehydrogenase Subunit 2 |
| NT | Near Threatened |
| PCR | Polymerase Chain Reaction |
| PB | Peyssonnelia Beds |
| PTP | Poisson Tree Process |
| Rs | Allelic Richness |
| RDA | Redundancy Analysis |
| $\pi$ | Nucleotide Diversity |
| S | Richness |
| SC | Somatic Condition |
| SIMPER | Similarity Percentage Analysis |
| SNPs | Single Nucleotide Polymorphisms |
| SSM | Shelf Sandy-Mud Bottom |
| STR | Short Tandem Repeat Loci |
| UNEP | United Nations Environment Programme |
| VU | Vulnerable |
| WIW | Western Intermediate Water |
| WMDW | Western Mediterranean Deep Water |

# TABLE OF CONTENT

# 1. GENERAL INTRODUCTION

## 1.1 The Mediterranean Sea

### *1.1.1 Geological history*

The Mediterranean is what remains of an ancient ocean named Tethys (during the Cretaceous; 145-65 million years ago “Mya”), a mass of equatorial water open to the east that divided the two mega-continents, Laurasia and Gondwana (Figure 1.1) that had emerged when Pangaea broke up (Golonoka, 2007; Meynard *et al.*, 2012). At this time, Africa, Europe and the Adriatic plates were coming closer together, causing the ancestral Mediterranean to become smaller and its shape and connectivity to change drastically (Meynard *et al.*, 2012).

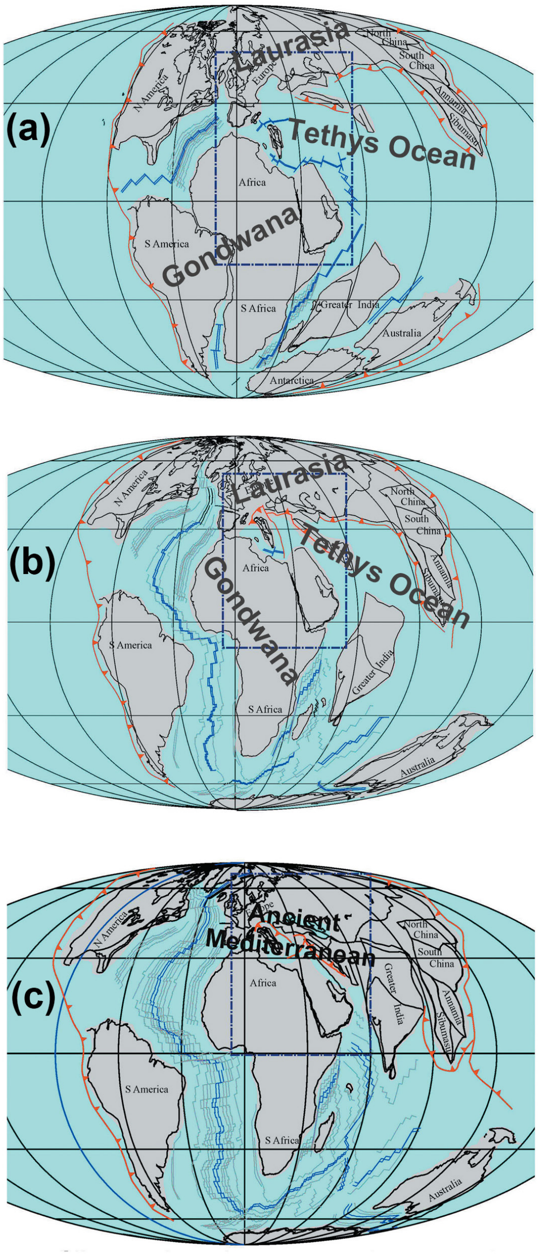


Figure 1.1. Global paleogeography maps: (a) Early Cretaceous (around 120 Mya); (b) Cretaceous-Paleocene boundary (around 65.5 Mya); (c) Eocene-Oligocene boundary (around 34 Mya). Modified from Berra and Angiolini (2014).

During the Miocene (23-5 Mya), the Mediterranean was isolated from the Indo-Pacific, and was subsequently isolated from the Atlantic Ocean (7-5 Mya) as well, causing a period of environmental stress, characterised by high desiccation and low sea level, which is known as the Messinian Salinity Crisis (MSC; 5.9-5.3 Mya; Ivanovic *et al.*, 2014). In this period, the Mediterranean was reduced to a series of small lakes, causing a rise in water salinity and an important extinction crisis among marine fauna (Marsili, 2008; Meynard *et al.*, 2012). After that, about 5 Mya, the connection with the Atlantic reopened through the Strait of Gibraltar, allowing the colonization of new species into the Mediterranean (Lejeusne *et al.*, 2010).

### *1.1.2 Geomorphology*

At present, the Mediterranean is the largest (2,969,000 Km$^2$) and deepest (average 1,460 m, maximum 5,267 m) enclosed sea on Earth. It is a relatively small yet deep sea, whose basin constitutes 0.81% of the total water surface of the planet (Coll *et al.*, 2010). It occupies an area around 3800 Km wide from east to west and has a maximum north-south distance of 900 km. Its western limit, the Strait of Gibraltar, is a shallow (320 m depth) and narrow (14 Km wide) channel, and is the only (natural) connection with the Atlantic Ocean. Its north-eastern limit, the shallow (average depth of 55 m) and narrow (maximum width of 7 Km) Strait of Dardanelles, connects the Mediterranean to the Black Sea. At its south-eastern limit, the Mediterranean has also been connected since 1869 to the Red Sea and the Indian Ocean (Figure 1.2) by the Suez Canal.

The Mediterranean is a semi-enclosed microtidal sea, which is over-supplied from a sedimentary point of view. It can be divided into two main basins: western (area: 0.85 million km2) and eastern (area: 1.65 million km2), separated by the Strait of Sicily which is 150 km wide and has a maximum depth of 400 m. At the same time, these basins have different geological evolution and physiographic characteristics, and as such are also divided into main sub-basins (e.g. MediMap Group, 2005). The western basin is divided into five sub-basins (Alboran, Algeria, Balearic, Liguro-Provençal and Tyrrhenian), and the eastern basin into four sub-basins (Adriatic, Ionian, Levantine and Aegean) (Figure 1.2).

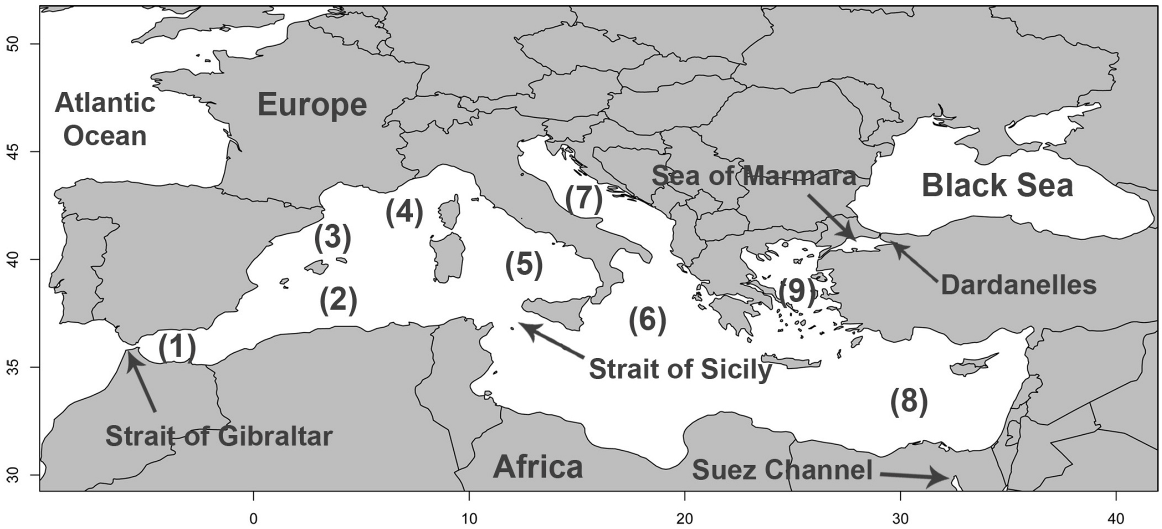


Figure 1.2. Map of the Mediterranean Sea. The sub-basins are indicated: (1) Alboran; (2) Algerian; (3) Balearic; (4) Liguro- Provençal; (5) Tyrrhenian; (6) Ionian; (7) Adriatic; (8) Levantine; (9) Aegean.

The continental shelf of the Mediterranean, which constitutes the main habitat for living marine resources, is narrow and represents less than 25% of the total area (Figure 1.3). The only exceptions are the Tunisian Plateau and some areas related to the sediment supply from the main Mediterranean rivers (Nile, Po, Rhone and Ebro; Figure 1.3). By contrast, the slope extends to 60% of the whole basin, with large areas of open sea. It is a relatively narrow zone, in which the change from 200 m to around 2500 m occurs within a few tens of km (Coll *et al*., 2010). The abyssal plains cover about 15% of the bottoms and are defined by several isobaths in the western and eastern basins, where they reach a maximum depth of 2,855 and 5,121 m, respectively (Würtz, 2012). In this zone, almost 518 large submarine canyons have been identified across the whole area (Harris and Whiteway, 2011; Würtz, 2012).

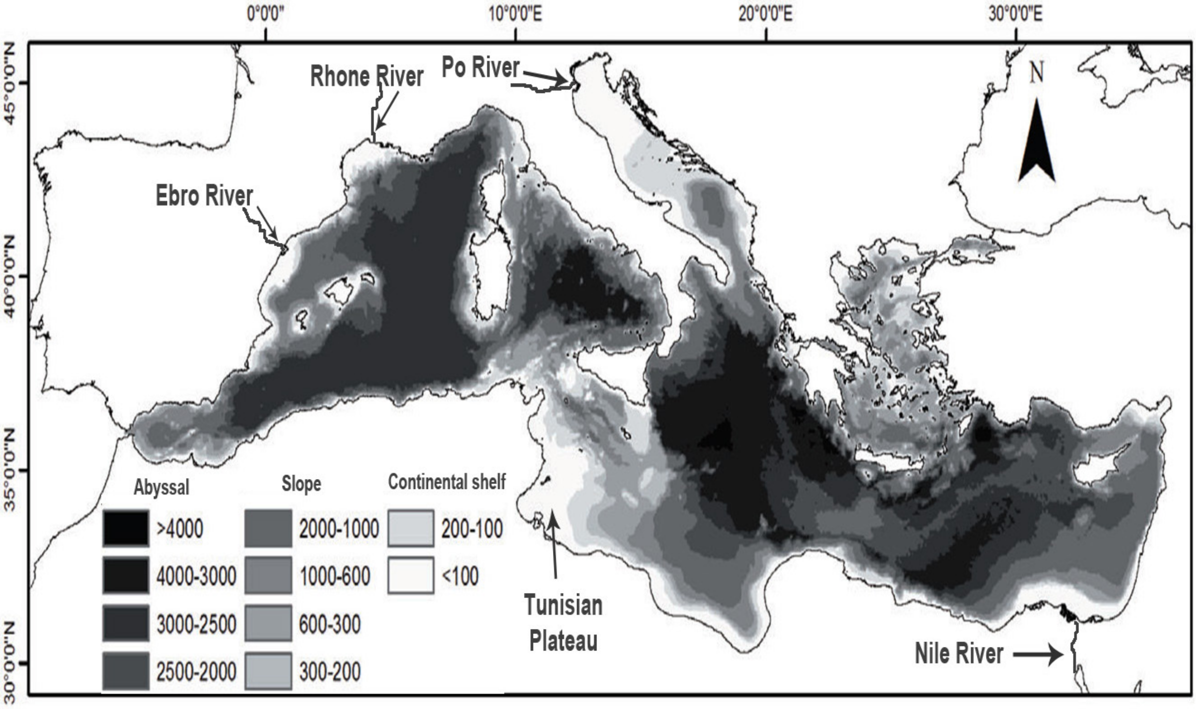


Figure 1.3. Map of the Mediterranean Sea showing its bathymetric characteristics according to continental shelf, slope and abyssal plans. The arrows indicate the main rivers. Modified from Coll *et al*. (2012).

### *1.1.3 Oceanography*

The climate in the Mediterranean is characterized by hot, dry and summers and cool, wet winters. The annual mean sea surface temperature shows high seasonality and important gradients from west to east and from north to south (Hopkins, 1985). This sea is generally oligotrophic, but regional features enrich coastal areas through changing wind conditions, temporal thermoclines, currents and river discharges (Estrada, 1996; Zavatarelli *et al*., 1998). Productivity is characterized by a strong environmental gradient, where the easternmost part of the eastern basin is more oligotrophic than the western one (Danovaro *et al*., 1999). Biological production decreases from north to south and from west to east and is inversely related to the increase in temperature and salinity (Coll *et al*., 2010).

The general circulation in the Mediterranean is complex and is composed of three predominant, interacting spatial scales: basin scale (including the thermohaline circulation), sub-basin scale and mesoscale (Robinson *et al*., 2001). Its circulation follows a cyclonic path along the continental slope (Millot, 1985). Complexity and scales arise from multiple driving forces, from strong topographic and coastal influences, and from the internal dynamic process (Robinson *et al*., 2001). The Mediterranean is an evaporation basin (mean evaporation exceeds precipitation), which has important implications in its water masses circulation. The key factor in the water balance of the Mediterranean is the exchange with the Atlantic Ocean through the Strait of Gibraltar. The inflow of Atlantic Water (AW; T: 15-16°C, S: 36-37, d: 1.028-1.27) is continuously modified, due to interaction with the atmosphere and to mixing with older AW remaining at the surface and with the waters underneath (Millot and Taupier-Letage, 2005;

Tanhua *et al*., 2013). AW circulating in the Mediterranean increases in density, because evaporation exceeds precipitation and then new water masses originate via convection events driven by intense local cooling due to winter storms (Robinson *et al*., 2001; Figure 1.4).

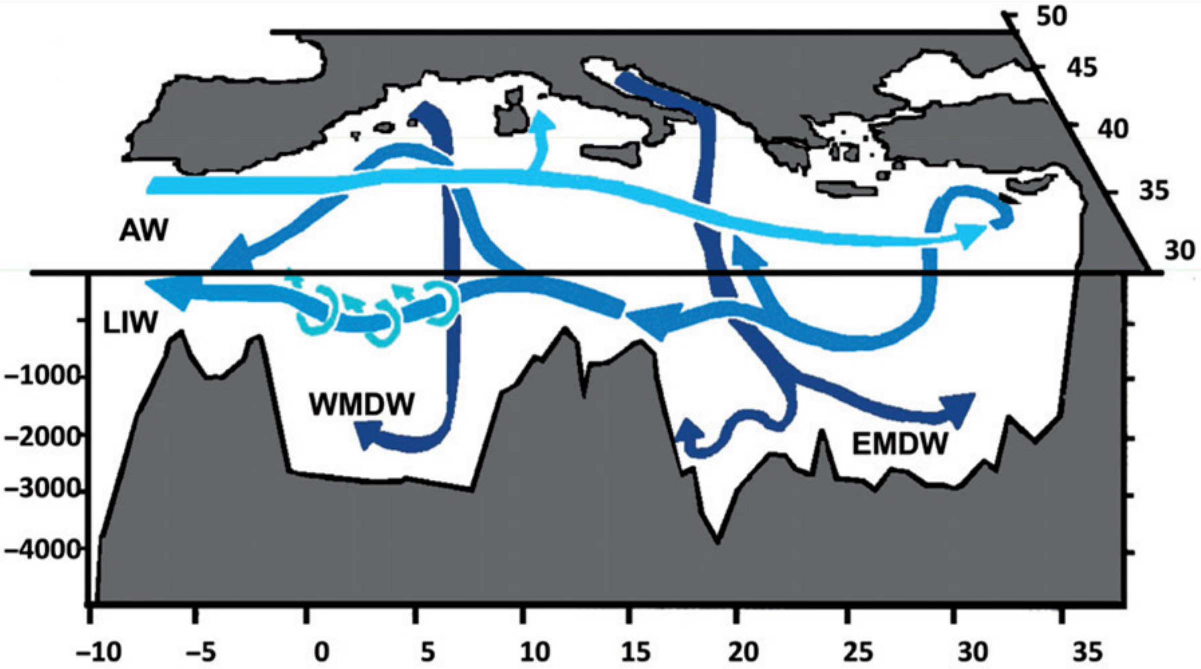


Figure 1.4. General circulation in the Mediterranean Sea: WMDM, Western Mediterranean Deep Water; EMDW, Eastern Mediterranean Deep Water; LIW, Levantine Intermediate Water; AW, Atlantic Water. Modified from Bergamasco and Malanotte-Rizzoli (2010).

Mediterranean bottom water is produced in the western basin (WMDW: Western Mediterranean Deep Water; Figure 1.4) and in the eastern basin in the southern Adriatic (EMDW: Eastern Mediterranean Deep Water; Figure 1.4). WMDW is formed during deep winter convection and by cascading events in the Gulf of Lions and the Ligurian Sea (Canals *et al*., 2006; Puig *et al*., 2013). EMDW is formed in the Adriatic or the Aegean sub-basins and, through the narrow and sallow straits, sinks into deeper parts of the EMDW (Roether *et al*., 1996).

Two water masses are found at intermediate depths in the Mediterranean: Levantine Intermediate Water (LIW) formed in the Eastern Mediterranean; and Western Intermediate Water (WIW) formed in winter over the continental shelf and slope (Vargas- Yáñez *et al*., 2012). LIW flows through the Sicilian channel at depths from 250 m to the bottom and spreads into the western basin (Manzella *et al*., 1988). The main part of this water mass flows from the continental slope of the Tyrrhenian Sea, to the Sardinian channel and reaches the north-western region through the continental slope west of Corsica. LIW is an important water mass which circulates through both the eastern and western basins and contributes to the efflux from Gibraltar to the Atlantic, mixed with both bottom water masses (Robinson *et al*., 2001). The WIW is transported by the Northern Current (NC) into the Gulf of Valencia and the Ibiza Channel between the end of winter and beginning of spring, but is not found in the Balearic channels every year (Lopéz-Jurado *et al*., 2008). The NC flows south-westward along the Gulf of Lions slope from the Lingurian Sea to the Catalan Sea in the north-western Mediterranean (Millot, 1999).

### *1.1.4 Flora and fauna*

Although representing only 0.8% of the area and less than 0.25% of the volume of the world's oceans, the Mediterranean is a region of high biodiversity that includes about 7% of known world marine fauna and 18% of world marine flora, 28% of which are endemic (Bianchi and Morri, 2000; Coll *et al*., 2010). Despite its small dimensions, the Mediterranean coastal waters are a hot spot of biodiversity hosting a high percentage of endemic species (Coll *et al*., 2010). The degree of endemism is especially high around the Mediterranean islands, where species have evolved in very specific habitats (Blonde and Aronson, 1999).

Mediterranean biodiversity ranks among the best known in the world. Currently, it is known to host more than 17,000 described marine species at least 26% of which are marine microbes (Bacteria, Archaea, Protists). Within the Animalia Kingdom, the greatest proportion of species has been recorded for Crustacea (13.2%; 2239 species), Mollusca (12.4%; 2113 species), Annelida (6.6%; 1172 species), Plathyhelminthes (5.9%; 800-1000 species), Cnidaria (4.5%; 757 species), Vertebrata (4.1%; 693 species), Porifera (4.0%; 681 species), Bryozoa (2.3%; 388 species), Tunicata (1.3%; 229 species) and Echinodermata (0.9%; 154 species) (Coll *et al*., 2010). Mediterranean fauna and flora are differently distributed between its basins, where the western Mediterranean displays the highest values of species richness, likely owing to the influx of Atlantic species and the wide range of physiochemical conditions. The central Mediterranean (Adriatic and Aegean seas) has the second highest species richness, while the Levantine basin and south-eastern side have in general the lowest species richness, which is due to the unfavourable conditions, such as the high salinity prevailing in the area (Por and Dimentman, 2004; Boudouresque, 2004; Coll *et al*., 2010). Moreover, diversity in the eastern end is being influenced by species introductions, which are entering through the Suez Canal (Lessepsian migrants), and subsequently moving northwards and westwards (UNEP, 2010). At present, habitat loss and degradation, followed by fishing impacts, pollution, climate change, eutrophication, and the establishment of alien species are the most important threats affecting Mediterranean biodiversity (Coll *et al*., 2010).

### *1.1.5 Fisheries*

The Mediterranean is one of the oldest and most intensively exploited marine systems (Ferretti *et al*., 2005; Lotze *et al*., 2011). The Mediterranean fisheries are diverse, with many fleets (total number of fishing boats have been estimated around 100000) based all along the coast in many ports (e.g. CIHEAM, 2002). Its fisheries are commonly classified as the trawl gears (bottom trawls fishing on the slope and on the continental shelf and pelagic trawl), towed gears and dredges, purse seine, small-scale gear (e.g. gillinets, bottom lines) and gear for large pelagic species (loglines, tuna seines and driftnets), each specialising in the exploitation of different species or groups of species (CIHEAM, 2002). Concerning fleets, except for the pelagic fleets fishing large pelagic species in the open sea, most Mediterranean fleets are small mow capital venture fishing vessels. Small-scale fishery exploits a large number of benthic and demersal species mainly distributed over the continental shelf. Total landings of Mediterranean fisheries have increased steadily since 1950, remaining around one million and a half tons since the 1990s. Although Mediterranean fisheries only represent a small proportion of the world production, the mean prices of landings (which are mainly sold fresh) are well above the average prices of world markets. In the European Union, Mediterranean fisheries represent about 20% of the total catch but 35% of the total value of landings (Lleonart and Maynou, 2003). From a socio-economic point of view, fishing activities in the Mediterranean employ several hundreds of thousands of persons (Lleonart and Maynou, 2003).

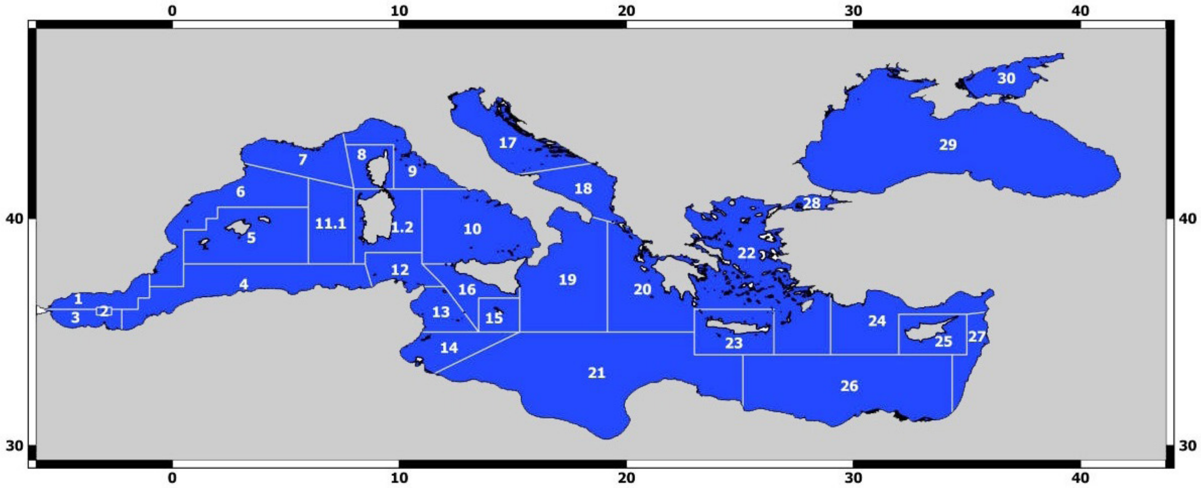


Figure 1.5. Map of the 30 geographical sub-areas (GSA) established by the General Fisheries Commission for the Mediterranean: (01) Northern Alboran Sea; (02) Alboran Island; (03) Southern Alboran Sea; (04) Algeria; (05) Balearic Islands; (06) Northern Spain; (07) Gulf of Lions; (08) Corsica Islands; (09) Ligurian and North Tyrrhenian Sea; (10) South and Central Tyrrhenian Sea; (11.1) Sardinia West; (11.2) Sardinia East; (13) Gulf of Hammamet; (14) Gulf of Gabes; (15) Malta Island; (16) South of Sicily; (17) Northern Adriatic; (18) Southern Adriatic Sea; (19) western Ionian Sea; (20) eastern Ionian Sea; (21) Southern Ionian Sea; (22) Aegean Sea; (23) Crete; (24) North Levant; (25) Cyprus Island; (26) South Levant; (27) Levant; (28) Marmara Sea; (29) Black Sea; (30) Azov Sea. Modified from http://www.sibm.it/MEDITS%202011/principalegeo.htm.

Catch estimation is difficult in the Mediterranean because of the multi-specific and multi-gear features of most fisheries, the dispersed landing sites and the high fraction of unreported catches (Abella *et al.*, 2012). In this sense, fishery scientists have to adopt compromises to delineate entities for monitoring harvest stocks, such as management units or geographic areas (Quetglas *et al.*, 2012). In the case of the Mediterranean, the General Fisheries Commission for the Mediterranean (GFCM; http://www.fao.org/gfcm/es/), an international organism comprising 25 Mediterranean countries and linked to the Food and Agriculture Organization of the United Nations (FAO), has established 30 geographical sub-areas (GSA) throughout the Mediterranean for assessment and management purposes (Figure 1.5). These divisions are based on political and statistical considerations rather than biological or economic factors (Lleonart and Maynou, 2003). Recent assessments suggest that most commercially viable demersal and pelagic species are overexploited in the entire Mediterranean (Palomera *et al.*, 2007; Colloca *et al.*, 2013; Quetglas *et al*., 2013; Tsikliras *et al*., 2015). The pattern of exploitation and the state of stocks differs between the western and eastern Mediterranean subareas, with the eastern fisheries found to be in worse shape (Tsikliras *et al*., 2015).

## 1.2 The study subjects: Chondrichthyans

### *1.2.1 Biology*

The Class Chondrichthyes (usually called chondrichthyans), which include sharks, rays, chimaeras, and their relatives, make up one of the oldest and most ecologically diverse vertebrate lineages. They arose at least 420 million years ago and rapidly radiated out to occupy the upper tiers of aquatic food webs (Compagno, 1990). Chondrichthyans are essentially defined by the following features: prismatic endoskeletal calcification (tesserae); dermal skeleton consisting of denticles (placoid scales); teeth are usually not fused to jaws and are replaced serially; biting edge of upper jaw formed by palatoquadrate and lower jaw by Meckel's cartilage; and internal fertilization in at least all know taxa by means of claspers (of males, derived from pelvic axis and termed myxopterigia) (Grogan and Lund, 2004; Nelson, 2006). It is generally accepted that the Class Chondrichthyes is a monophyletic group divisible into two sister taxa, the Elasmobranchii and Holocephali. Since Weigmann (2016) Chondrichthyes have been represented by 16 orders, 61 families and 199 genera and more than 1188 species (Figure 1.6). In particular, sharks include nine orders, 34 families, 105 genera and 509 species of sharks; six orders, 24 families, 88 genera and 630 species of batoids; and one order, three families, six genera and 49 species of holocephalans.

Most chondrichthyans are marine organisms of relatively shallow temperate and particularly tropical waters, although all oceans have one or more species. Their habitats include continental and insular shelf waters from the interdital zone out to 200 m depth and even abyssal habitats (>2000 m) (Fowler *et al*., 2005). In some areas, some species have even penetrated into estuaries which act as nurseries and some enter, or are endemic of fresh water (Ebert and Stehmann, 2013). Some species are strictly benthic, like skates (Rajoidei) and angel sharks (Squatinidae), whereas others like the white shark *Carcharodon carcharias* (Lamnidae) are pelagic, restricting most of their activities to the upper layers of the ocean (Fowler *et al*., 2005).

It is widely recognized that chondrichthyans often play a key role in the transfer of energy between upper trophic levels within marine ecosystems (Wetherbee and Cortés, 2004). Most chondrichthyan species are carnivores with body sizes from 20 cm to >10 m and feeding types ranging from filter-feeding (e.g. *Carcharodon carcharias*) to suction crushing (e.g. *Raja asterias*) and opportunistic scavengers (e.g. *Scyliorhinus canicula*). While larger species (>3m total length) function as top predators, there is a high diversity of mesopredatory chondrichthyans (<1.5 m total length) that are prey for larger species (Ferretti *et al*., 2010). This discloses the high connectivity of chondrichthyans in food web models (Bascompte *et al*., 2005) and the likely limited effects on any particular prey species (Ellis and Musick, 2007) (Figure 1.7). Ecosystem models predict that, in some situations, cartilaginous fishes will exert considerable top-down impacts on their prey (Stevens *et al*., 2000), so their loss could result in complex community changes, including trophic cascades, mesodepredator release and consequent declines in some commercial fishes (Ferretti *et al*., 2010).

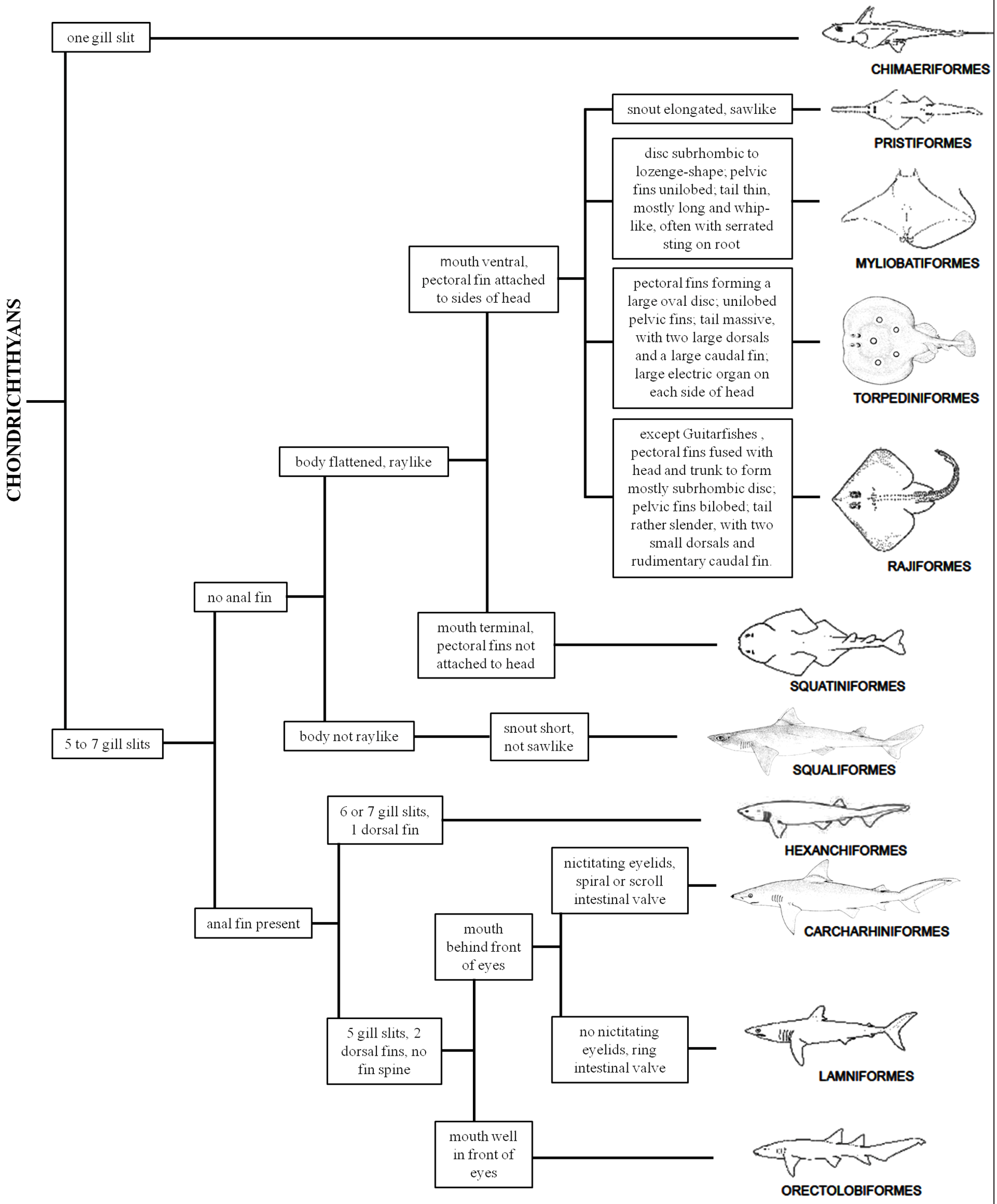


Figure 1.6. Principal diagnostic features of higher classification of sharks, batoids and chimaeras (Orders). Modified from Ebert and Stehmann (2013).

Life-history traits of chondrichthyans vary widely, particularly depending on their reproductive traits: gestation period (from 2 to 42 moths), reproductive mode (egg-laying, live-bearing), egg hatching period (from 1 to 27 months), maternal investment (yolk-only, uterine milk, oophagosity, uterine cannibalism, placentation), fecundity (from 1 to 400 offspring), offspring size (from 20 to 1800 cm long), age at maturity (1.5 to >30 years) and longevity (5 to >50 years) (Compagno, 1990, Cortés, 2000; Dulvy, and Reynolds, 2002). Such life-history traits can result in the vulnerability of chondrichthyan populations to fishing exploitation (Dulvy and Forrest, 2010). Cartilaginous fishes are characterized by conservative life-history traits. Thus, almost all chondrichthyans are considered K-selected species compared to teleosts (Stevens *et al*., 2000; Cailliet *et al*., 2005). They display slow growth, long gestation periods, late maturity, low fecundity and productivity and long life cycle (Camhi *et al*., 1998; Cortés, 2000; Cailliet *et al*., 2005). These biological traits result in low reproductive potential and low capacity to increase population for many species. This has serious implications for chondrichthyan populations, limiting their capacity to sustain fisheries and to recover from declines (Cailliet *et al*., 2005; Cavanagh and Gibson, 2007).

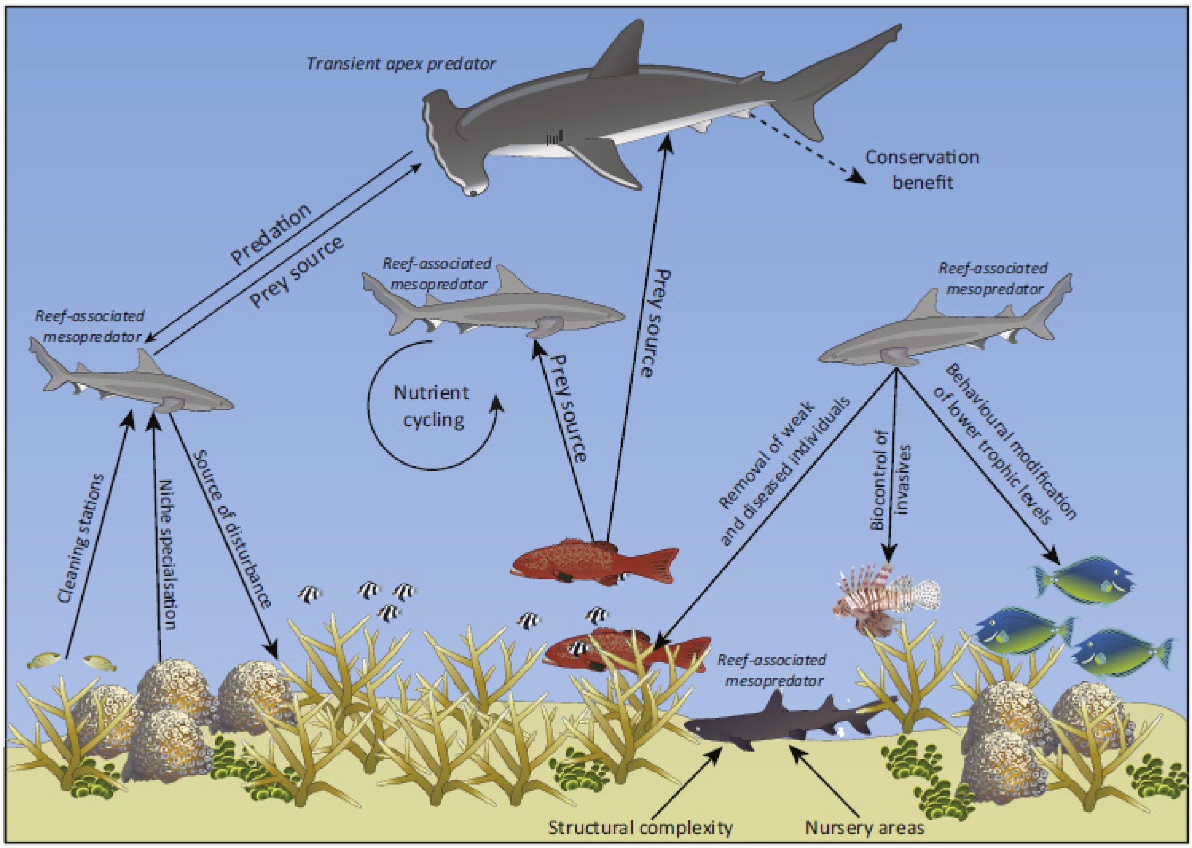


Figure 1.7. Example of ecological role of sharks on coral reefs. From Roff *et al*. (2016).

### *1.2.2 Mediterranean chondrichthyans*

Mediterranean chondrichthyans include one chimaera, 59 sharks belonging to 17 families and 27 genera, and 39 batoids including 9 families and 16 genera. However, due to the similarity in morphology or body plan, some of these species such as *Squalus megalops*, *Dasyatis marmo-*

*rata* and *Dasyatis* cf. *tortonosei*, have a doubtful taxonomic status, being considered as invalid or synonym species (Serena, 2005; Bradai *et al*., 2012) (Figure 1.8). The number of endemisms of Mediterranean chondrichthyans is low, with only five batoid species: *Leucorara melitensis*, *Raja asterias*, *Raja polystigma*, *Raja radula* and *Mubula mobular* (Serena, 2005; Bradai *et al*., 2012).

Around three chondrichthyans are considered lessepsian species *Himantura uarnak*, *Glaucostegus halavi* and *Torpedo sinuspersici* (Golani *et al*., 2002; Saad *et al*., 2004). Further, in the Mediterranean it is possible to consider the following species as vagrants from Atlantic regions: *Carcharhinus altimus*, *Carcharhinus falciformis*, *Galeocerdo cuvier*, *Rhizoprionodon acutus* and *Sphyrna mokarran*. The first two species are recognized as established in the area, and the second ones as alien species (Golani *et al*., 2002; Ibrahim *et al*., 2016).

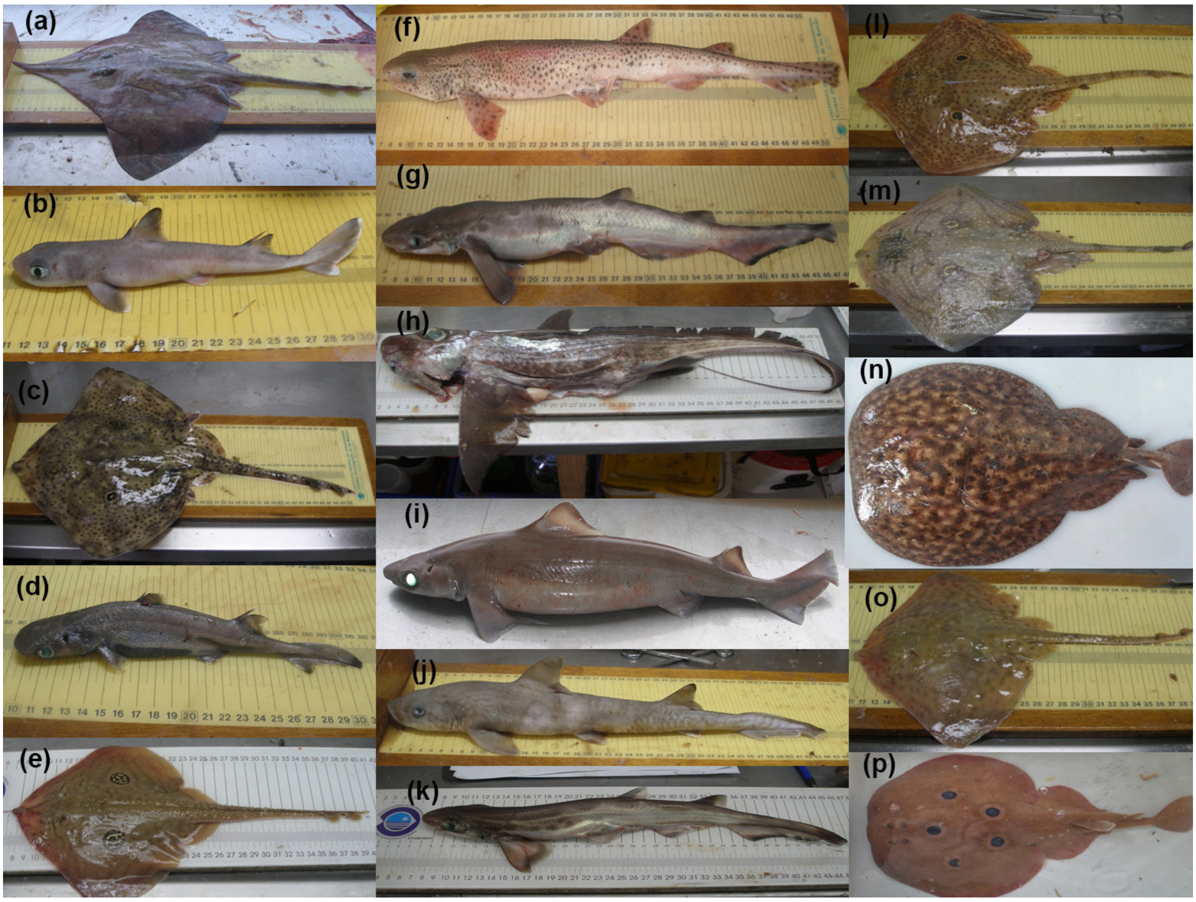


Figure 1.8. Examples of chondrichthyan species from the Mediterranean Sea: (a) *Dipturus oxyrinchus*; (b) *Squalus blainvillei*; (c) *Raja polystigma*; (d) *Etmopterus spinax*; (e) *Leucoraja naevus*; (f) *Scyliorhinus canicula*; (g) *Galeus melastomus*; (h) *Chimaera monstrosa*; (i) *Centrophorus granulosus*; (j) *Mustelus mustelus*; (k) *Galeus atlanticus*; (l) *Raja miraletus*; (m) *Raja radula*; (n) *Torpedo marmorata*; (o) *Raja montagui*; (p) *Torpedo torpedo*.

The spatial pattern of chondrichthyan richness within the Mediterranean is not homogeneous (Serena, 2005; Coll *et al*., 2010). This pattern is often related to historical causes (e.g. extinction followed by a recolonization process,) and ecological and environmental factors such as sea surface temperature, latitude and salinity in depth and typology of the seabed (Serena, 2005;

Meléndez *et al*., 2017). The diversity of cartilaginous fishes was greatest in the western Mediterranean, mainly off Morocco, Algeria and Tunisia, followed by the northwest Mediterranean, including the Iberian Peninsula, Balearic Islands, France and Italy (Figure 1.9). Intermediate and lower levels of diversity have been recorded in the central Mediterranean, including Malta, Sicily, and the Adriatic and Aegean Seas (Coll *et al*., 2010; Dulvy *et al*., 2016; Meléndez *et al*., 2017).

Climate change can have a potential effect on chondrichthyans, particularly their species composition, population dynamics, and, therefore, the functioning of the ecosystem (Harley, 2011; Pistevos *et al*., 2015). The mechanisms by which chondrichthyan populations are vulnerable to global change are a relatively new research area (Pistevos *et al*., 2015). Concerning environmental changes, some models have suggested decreases in body size and the collapse of chondrichthyan populations. In this sense, few empirical studies have investigated the underlying mechanism and have tested how such vulnerable species may respond to multiple global stressors (Sheridan and Bickford, 2011; Pistevos *et al*., 2015). In the Mediterranean, the effect of environmental variability on chondrichthyan populations has been documented; suggesting that heavily exploited populations can alter their resilience, and even, increase their competition with other species (e.g. Puerta *et al*., 2016). Mediterranean chondrichthyan populations are particularly sensitive to changes in sea surface temperature (STT; Quetglas *et al*., 2016), the North Atlantic Oscillation (NAO; Barausse *et al*., 2014), the mesoscale index from western Mediterranean (IDEA; Quetglas *et al*., 2013) and the Western Mediterranean Oscillation index (WeMO; Barausse *et al*., 2014). Unfavourable environmental conditions could accelerate the collapse of chondrichthyan populations, acting synergistically with fishing, or impede the recovery of their population in the

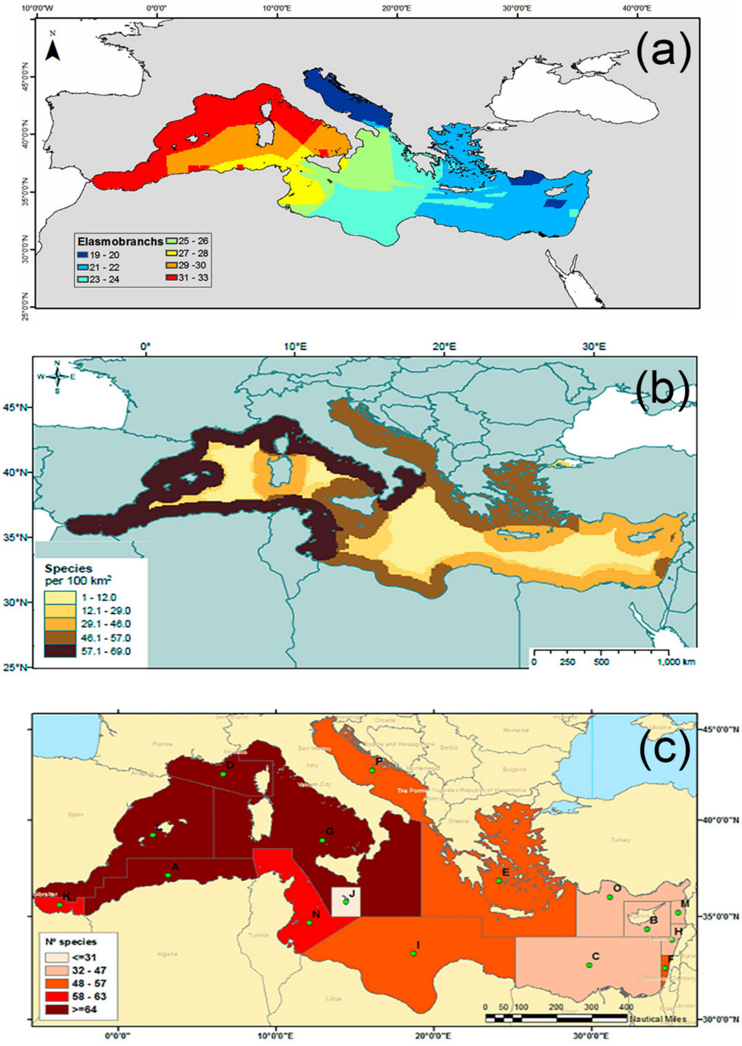


Figure 1.9. Spatial patterns of chondrichthyan richness in the Mediterranean Sea. From (a) Coll *et al*. (2010); (b) Dulvy *et al*. (2016); (c) Meléndez *et al*. (2017).

future (Barausse *et al*., 2014). So, environmental factors must be taken into account for assessment of chondrichthyan populations.

Mediterranean chondrichthyans inhabit demersal ecosystems on continental shelves and slopes, which makes them especially vulnerable to bottom trawl fishing (Compagno, 1990; Serena, 2005; Shepherd and Myers, 2005). Chondrichthyans have a low commercial value compared to that of teleost fishes and shellfishes (Cavanagh and Gibson, 2007). In fact, they represent only 1.1% of the total landings in this area (Serena, 2005). Catches of cartilaginous fishes primarily derive from two different fisheries: pelagic fishery with longlines and gillnets, and demersal trawl fishery. In both fisheries, they represent only a by-catch (Carbonell *et al*., 2003; Serena, 2005; Bradai *et al*., 2012). Bottom trawl fishery is commonly performed throughout the Mediterranean area and its activity has increased on the shelf and slope over the past 50 years (Aldebert, 1997; Serena, 2005; Cavanagh and Gibson, 2007). This increase in trawling activity has resulted in a decline in many chondrichthyan populations such as documented in different Mediterranean areas. In the Gulf of Lions, a study based on historical data from both bottom trawl surveys and commercial landings, revealed a clear decline (around of 50%) of demersal elasmobranch species population since the 1960s, where only 13 of the 25 species recorded in the years 1957-1960 were still caught in the period 1994-1995 (Aldebert, 1997). In the Adriatic Sea, a decrease in elasmobranch diversity and occurrence was observed between 1948 and 1998; larger sharks and some rays disappeared or were rarely found mainly during the last years (Jukic-Peladic *et al*., 2001). In the northern Adriatic Sea a decrease of several elasmobranch species was also documented, 11 species of which have almost completely disappeared (Ferretti *et al*., 2013). Similar results were obtained by Barausse *et al*. (2014), who observed a dramatic decline in elasmobranch landings (around of 80%) over the past 68 years in the same study area. In the Aegean Sea, a study based on bottom trawl fishery data, showed a significant decrease in chondrichthyan species richness, accompanied by an analogous decline in their abundance (catch composition and catch rate) from 1995 to 2006 (Damalas and Vassilopoulou, 2011). Demersal elasmobranch communities in the Balearic Islands showed a clear decreasing trend from 1965 to 2009 (Guijarro *et al*., 2012). Lastly, a general decreasing trend in the catch rates of sharks and skates in the Tyrrhenian Sea was observed from 1961 to mid-1990s (Ligas *et al*., 2013).

The marine ecosystems along the continental shelf and slope off the Mediterranean Iberian Peninsula and Balearic Islands (western Mediterranean) have been subjected to regular trawl fishing since the middle of the 20th century (Gil de Sola, 1993; Quetglas *et al*., 2013). Bottom trawl fishery is performed mainly along the continental shelf and upper slope down to a depth of 800 m (Moranta *et al*., 2008). In this area, chondrichthyan species are mainly caught as a by-catch of the bottom trawling fleet (Figure 1.10), where the main target species are red mullets (*Mullus barbatus* and *M. surmuletus*), European hake (*Merluccius merluccius*), Norway lobster (*Nephrops norvergicus*) and red shrimp (*Aristeus antennatus*) (Carbonell *et al*., 2003; Guijarro *et al*., 2012). The three most frequently caught chondrichthyan species are *Galeus* *melastomus*, *Scyliorhinus canicula* and *Etmopterus spinax*, which comprise around 80% of the total demersal chondrichthyan abundance, followed by *Raja clavata*, *Raja asterias*, *Raja miraletus* and *Leucoraja naevus* (Massutí and Moranta, 2003; Ordines *et al*., 2011; Pennino *et al*., 2013; Mendoza *et al*., 2014). The main predictors of chondrichthyan distribution patterns in the western Mediterranean are depth, slope of seabed and type of substrate (Pennino *et al*., 2013; Mendoza *et al*., 2014).

### *1.2.3 Management and conservation of Mediterranean chondrichthyans*

The substantial change in Mediterranean chondrichthyan species richness and abundance patterns over the past half-century has increased international concern, calling for the assessment of fishery activity and application of effective management and conservation strategies to ensure their populations in a sustainable way. In this sense, at international, national and regional levels, management and conservation tools have been developed and adopted to protect chondrichthyan species. The Mediterranean Red List of the International Union for the Conservation of the Nature (IUCN) assessed 73 Mediterranean chondrichthyans, finding that more than half of these assessed species are regionally threatened, 20 are in critically endangered, status, and 11 in endangered status (Dulvy *et al*., 2016; see Table 1.1). At least half of the sharks (22 out 41 species) face an elevated risk of extinction, as well as 50% of batoids (16 of 32 species), whereas the only chimaera species (*Chimaera monstrosa*) is considered to be of the least concern (Dulvy *et al*., 2016; see Table 1.1).

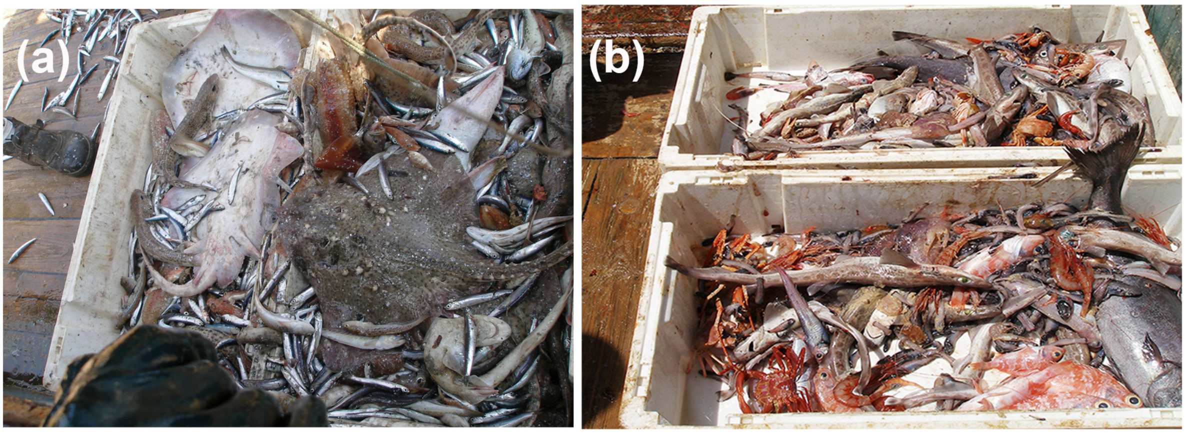


Figure 1.10. Examples of skate species (a) *Raja clavata* and (b) *Galeus melastomus* caught as a by-catch by of the trawl fishery off the Balearic Islands (western Mediterranean).

In 1976, 16 Mediterranean countries and the European Union adopted the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention), related to specially protected areas and biological diversity. The Barcelona Convention identified a list of endangered species with priority protection (UNEP, 2005). In 2003, the framework of the United Nations Environment Programme (UNEP) was developed in collaboration with the IUCN Centre for the Mediterranean. The Cooperation Action Plan for the Conservation of Cartilaginous Fishes (Chondrichthyans) in the Mediterranean, (UNEP-MAP-

RAC/SPA, 2003) promoted the conservation of chondrichthyan populations, the protection of selected chondrichthyan species whose populations are considered endangered, the protection and restoration of critical habitats for chondrichthyans, the improvement of scientific knowledge by research and scientific monitoring of chondrichthyan populations, and the recovery of depleted chondrichthyan stocks. Annex II of the Barcelona Convention lists the species of threatened chondrichthyan in the Mediterranean (Table 1.2), with the objective to ensure the maximum protection possible and recovery of these species. On the other hand, Annex III lists the species whose exploitation is regulated (Table 1.2).

Table 1.1. Summary of the regional IUCN Red List status of all Mediterranean of chondrichthyan species (Dulvy *et al*., 2016).

| **IUCN Red List Category** | **Number of species** | |
|---|---|---|
| | **Sharks** | **Batoids** |
| Critical Endangered (CR) | 12 | 8 |
| Endangered (EN) | 6 | 5 |
| Vulnerable (VU) | 5 | 3 |
| Near Threatened (NT) | 3 | 6 |
| Least Concern (LC) | 5 | 7 |
| Dada Deficient (DD) | 10 | 3 |
| **Total species** | **41** | **32** |

In 2010 and 2012, the GFCM, adopted ad hoc measures to reduce the by-catch of pelagic sharks such as thresher (*Alopias* spp.), mako (*Isurus oxyrhinchus*), and hammerhead (*Sphyrna* spp.) sharks. In 2012, the GFCM also banned finning practices and according to recommendation GFCM/36/2012/3, the chondrichthyan species listed in Annex II of the SPA/BD protocol of the Barcelona Convention (UNEP, 2011) must be released unharmed and alive whenever possible, cannot be retained on board, transshipped, landed, transferred, stored, sold or offered for sale.

Another intergovernmental treaty involved in chondrichthyan conservation and management is the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Its objective is to ensure that international trade of wild animals and plants does not threaten their survival, through a permit system that regulates international trade in species included in the following Appendices: *Appendix* I, includes species threatened with extinction which are or may be affected by trade; *Appendix* II, includes species which, although not necessarily now threatened with extinction, may become so unless trade in specimens of such species is subject to strict regulation in order to avoid utilization incompatible with their survival; and *Appendix* III includes species which any Party has identified as being subjected to regulation within its jurisdiction for the purpose of preventing or restricting exploitation, and that needs the cooperation of other countries to prevent unsustainable or illegal exploitation (García-Núñez, 2008). Chondrichthyans were first included in *Appendix* II in February 2003. According to the last CITES meeting in 2013, there are two and six Mediterranean chondrichthyan species included in *Appendix* I and *Appendix* II, respectively (UNEP/CITES, 2016; see Table 1.2).

At the regional level, Spain has implemented a number of protection measures of chondrichthyan species. On January 21st 2012, the Spanish Official State Gazette published Order AAA/75/2012 announcing the inclusion of eleven shark and ray species in the List of Wild Species under Special Protection (Table 1.2). Inclusion in the list delivers on commitments made by Spain as a party to the Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean. Inclusion in the list prohibits the capture, injury trade, import and export of these species, and requires periodic evaluations of their conservation status. Then, on September 3rd, the Spanish Official State Gazette published Order AAA/1771/2015 notifying the inclusion of seven new shark and ray species (Table 1.2).

In addition, local protection measures have also been adopted, for example, on July 8th 2005, the Balearic Islands Official Gazette published Decree 75/2005 proclaiming the creation of the Balearic Catalogue of Threatened Species. The current version of this catalogue in 2015 is composed of a list of marine protected species including 17 chondrichthyans (Morey, 2015; Table 1.2). This Archipelago also has its own published Red Book with the IUCN categories adapted to its fish fauna (Grau *et al*., 2015). In this Red Book are included 57 chondrichthyans, 30 of which are threatened to different degrees; 15 of them are critically endangered, 11 endangered and 4 vulnerable (Grau *et al*., 2015). The number of locally extinct species in the Balearic Islands ranges from two to four (Grau *et al*., 2015; Morey, 2015).

Table 1.2. Summary of management and conservation tools of threatened chondrichthyans in the Mediterranean according to the Mediterranean Red List of the International Union for the Conservation of the Nature (IUCN) Red List drawn up in 2016 (Dulvy *et al*., 2016). IUCN threatened categories: VU, Vulnerable; EN, Endangered; CR, Critically Endangered. Chondrichthyan species marked with a cross are included in the Barcelona Convention (BCN), the International Trade of Endangered Species of Wild Fauna and Flora (CITES), the Spanish Official State Gazette Orders AAA/75/2012 and AAA/1771/2015 and/or the Balearic Catalogue of Threatened Species (Balearic CTS). Extinct chondrichthyan species in the Balearic Islands are indicated with an asterisk.

| **Family** | **Species** | **IUCN 2016** | **BCN Annex II** | **BCN Annex III** | **CITES App I** | **CITES App II** | **Order AAA 75/2012** | **Order AAA 1771/2015** | **Balearic CTS** |
|---|---|---|---|---|---|---|---|---|---|
| Carcharhinidae | *Carcharhinus plumbeus* | EN | | X | | | | | |
| | *Prionace glauca* | CR | | X | | | | | |
| Triakidae | *Galeorhinus galeus* | VU | X | | | | | X | X |
| | *Mustelus mustelus* | VU | | | | | | | |
| | *Mustelus asterias* | VU | | X | | | | | |
| | *Mustelus punctulatus* | VU | | X | | | | | |
| Sphyrnidae | *Sphyrna zygaena* | CR | X | | | X | | | X |
| Alopiidae | *Alopias superciliosus* | EN | | | | X | | | X |
| | *Alopias vulpinus* | EN | | X | | X | | | X |
| Cetorhinidae | *Cetorhinus maximus* | EN | | | | X | | | X |
| Lamnidae | *Carcharodon carcharias* | CR | X | | | X | | | X |
| | *Isurus oxyrhincus* | CR | X | | | | | X | X |
| | *Lamna nasus* | CR | X | | | X | | X | |
| Odontaspidae | *Carcharias taurus* | CR | X | | | | X | | |
| | *Odontaspis ferox* | CR | X | | | | X | | |
| Centrophoridae | *Centrophorus granulosus* | CR | | X | | | | | |
| Dalatiidae | *Dalatias licha* | VU | | | | | | | |
| Echinorhinidae | *Echinorhinus brucus* | EN | | | | | | | |
| Oxynotidae | *Oxynotus centrina* | CR | X | | | | X | | X |
| Squalidae | *Squalus acanthias* | EN | | X | | | | | |
| Squatinidae | *Squatina aculeate** | CR | X | | | | X | | X |
| | *Squatina oculata** | CR | X | | | | X | | X |
| | *Squatina squatina** | CR | X | | | | X | | X |
| Dasyatidae | *Dasyatis centraura* | VU | | | | | | | |
| | *Dasyatis pastinaca* | VU | | | | | | | |
| Gymnuridae | *Gymnura altavela* | CR | X | | | | X | | X |
| Mobulidae | *Mobula mobular* | EN | X | | | | | | X |
| Myliobatidae | *Mylibatis aquila* | VU | | | | | | | |
| | *Pteromylaeus bovinus* | CR | | | | | | | |
| Pristidae | *Pristis pectinata* | CR | X | | X | | X | | |
| | *Pristis pristis** | CR | X | | X | | X | | X |
| Rajidae | *Dipturus batis* | CR | X | | | | X | | |
| | *Leucoraja circularis* | CR | X | | | | | X | X |
| | *Leucoraja fullonica* | CR | | | | | | | |
| | *Leucoraja melitensis* | CR | X | | | | | X | |
| | *Raja radula* | EN | | | | | | | |
| | *Rostroraja alba* | EN | X | | | | X | | X |
| Rhinobatidae | *Glaucostegus cemiculus* | EN | X | | | | | X | X |
| | *Rhinobatos rhinobatos* | EN | X | | | | | X | |

## 1.3 Population dynamics of exploited fish species: from classical methods to molecular approaches

Stock assessment approaches are often based on the characteristics of individual fish in a population (Pope *et al.*, 2010). Classical data include their length and weight (Anderson and Neumann, 1996), as well as, sex, maturity, gonad weight, and liver weight (Strange, 1996). Likewise, hard structures (e.g. bone, otolith or scales) can be used to estimate the age of individual fishes (DeVries and Frie, 1996), and the amount and type of food in the stomach can be determined (Bowen, 1996). These data from individual fish are summarized with statistics to estimate the parameters of the population from which the sample was taken. In chondrichthyans, these statistics can be combined to provide useful estimates for assessing shark populations such as, growth rates (e.g. Hisano *et al.*, 2011), the condition or "plumpness" of fish in a population (e.g. Reyier *et al.*, 2008), size and age structures (e.g. Sánchez-de Ita *et al.*, 2011), feeding habits (e.g. Valls *et al.*, 2011), size at first maturity (e.g. Romero-Caicedo *et al.*, 2016) and mortality rates (e.g. Poisson *et al.*, 2014).

Population dynamics refer to the processes responsible for changes in abundance or biomass of a population over time and are monitored by a subset of possible population parameters (Pope *et al.*, 2010). Estimates of population dynamics can indicate how a population arrived at its current state and how it might change in the future (Pope *et al.*, 2010). A population assessment might focus on determining whether the size of a population is relatively constant, increasing, or decreasing, which requires recorded data such as abundance, biomass, and individual size. Assessing population dynamics of fish is best achieved using long-term data collected with standardized methods (Kilduff *et al.*, 2009). Fisheries stock assessment involves describing and understanding the basic dynamics of a particular fish population or stock (Kilduff *et al.*, 2009).

Molecular genetics are of universal application in living organisms and make use of the information stored in the genome (Avise, 2000). A great advantage of genetic approaches is that a small tissue sample collected from an animal contains its complete nuclear (bi-parentally inherited) and mitochondrial (matrilineal) genomic information (Dudgeon *et al.*, 2012). By comparing neutral or conserved variation within selected regions of the genome it is possible to assess the genetic relationship at the individual level (e.g. genotyping for parenthood), population (e.g. population structure, connectivity, philopatry), species, (e.g. identify cryptic species), and higher order so as to discern evolutionary relationships (Dudgeon *et al.*, 2012).

The past six decades have seen on increasing application of genetics to fisheries management and ecology (Waples *et al.*, 2008), resulting in significant insights into the individual and population ecology of marine exploited species, with consequent paradigm shifts that have changed the perception of marine environment and the species therein (Hauser and Carvalho, 2008). Fisheries genetics have been dominated by four molecular markers: allozymes, mitochondrial DNA (mtDNA) sequencing, microsatellites, and more recently single nucleotide polymorphims

(SNPs) (Hauser and Seeb, 2008). At present, there have been significant theoretical advances in the analysis of genetic data. These changes are reflected by the recent rapid growth in the number of scientific publications on fisheries genetics (Ovenden *et al*., 2015). Genetic analysis can contribute to fisheries management in at least eleven ways: (i) species identification, (ii) fisheries stock structure, (iii) resolving mixed-stock fisheries, (iv) DNA as a biomarker for age, (v) ecosystem monitoring, (vi) estimating harvest rates and abundance, (vii) genetic diversity, population abundance and resilience, (viii) evolutionary responses to fishing, (ix) genetic effect of stock enhancement, (x) detection of pathogens and invasive species, (xi) product provenance and fisheries surveillance (Ovenden *et al*., 2015).

Genetic studies of chondrichthyans have applications in fisheries management and conservation issues, such as the accurate identification of species and definition of management units based on population structure approaches (Dudgeon *et al*., 2012). Many aspects of fisheries management rely on the accurate identification of both targeted and non-targeted resources. DNA analysis is particularly useful if the specimen lacks the diagnostic characters for routine taxonomic identification, if morphological characters are poorly defined, if no diagnostic morphological characters are known or if the state of preservation precludes morphological analysis (Ovenden *et al*., 2015). The advantages of DNA as a data source for species identification have been incorporated into the "DNA barcoding" approach (Hebert *et al*., 2003). DNA barcoding is the use of a specific mtDNA gene region (cytochrome oxidase subunit 1: COI) to recognize animal species by comparison with validated reference sequences. The application of this tool has been successfully used to accurately identify problematic skate species (e.g. Coulson *et al*., 2011; Serra-Pereira *et al*., 2011) and even to identify unknown species samples with shark, ray and skate fin samples (e.g. Salini *et al*., 2007; Holmes *et al*., 2009).

Chondrichthyan population studies aim to infer historical and contemporary processes responsible for the observed patterns of spatial genetic differentiation, and to identify baseline information regarding stock structure, and unit for fisheries assessment (Beheregaray, 2008; Ovenden *et al*., 2011; Dudgeon *et al*., 2009). Genetic structure is readily measured with genetic markers and, broadly, the detection of genetic differences between spatially or temporally separated samples implies the existence of some level of demographic independence and the presence of separate stocks (Waples *et al*., 2008). An important benefit of the genetic approach is that it measures long-term (i.e. multi-generational) average levels of population connectivity. Detailed regional-scale genetic populations have been studied for many chondrichthyan species, revealing a complex population structure with direct implication for stock identification (e.g. Chevolot *et al*., 2006; Corrigan *et al*., 2008; Ramírez-Amaro *et al*., 2017b).

On the other hand, the determination of the hierarchical relationship between species and divergence time estimation among them based on biological sequences such as proteins or DNA is performed by phylogenetic studies (Liò and Goldman, 1998). DNA phylogenies are being used as the basis for mapping the evolution of morphological, behavioural and ecological characters (Hewitt, 2001). Molecular clocks are commonly used in phylogenetic analysis to estimate the timing of evolutionary events (Ho *et al*., 2015). A number of statistical methods are available (e.g. BEAST, IMa2) for testing the molecular-clock hypothesis for a given set of DNA or protein sequences. When estimating evolutionary timescales in a phylogenetic analysis, the molecular clock needs to be calibrated; this can be done by assigning an absolute age to one or more nodes in the phylogeny, which can then act as a reference point for estimating the ages of the remaining nodes (Bromhan *et al*., 1999). Calibration points in chondrichthyans are often based on fossil records (e.g. Aschliman *et al*., 2012), paleogeographic events (e.g. Richards *et al*., 2009), and dated events from other molecular studies (e.g. Valsecchi *et al*., 2005).

Over the past two decades, phylogenetic approaches have become increasingly prominent in biodiversity conservation (Forest *et al* 2015; Rolland *et al*., 2012). Phylogenetic diversity has been proposed as a measure of biodiversity that attempts to capture the historical dimension of evolutionary processes (e.g. speciation, radiation) that are responsible for present-day patterns of biodiversity, not only based on the topology of phylogenetic trees but also on the length of their branches (Erwin, 1991; Forest *et al*., 2015). Detailed phylogenies also provide a window into speciation and extinction rates, the ecological and biogeographical causes of speciation and extinction, and the timing of these events, all of which were hitherto inaccessible (Vázquez and Gittleman, 1998). Despite that, in chondrichthyans, few studies have focused on the applicability of aspects of phylogenetic diversity in conservation (e.g. Corrigan and Beheregaray, 2009; Stelbrink *et al*., 2010).

# 2. OBJECTIVES

Taking into account the fact that the Mediterranean is one of the most impacted seas in the World (e.g. crowding of coastal zones, habitat degradation, biodiversity loss, overfishing, and pollution) and that chondrichthyans are species highly vulnerable to fishing exploitation, the general aim of the present Thesis is to improve scientific knowledge on demersal chondrichthyans dwelling on circalittoral and bathyal soft bottoms, exploited by the bottom trawl fishery along the continental shelf and slope in the western Mediterranean. This includes knowing their communities and population dynamics, connectivity, phylogenetic relationships, and the spatio-temporal trends of the most abundant species throughout the western Mediterranean. The specific objectives of this Thesis are the following:

1. To describe the compositions and structure of demersal chondrichthyans assemblages, as well as determine the main biological parameters of their most abundant species and to compare them between the four geographical subareas (GSAs) established by the General Fisheries Commission for the Mediterranean in the western Mediterranean: Northern Alboran (GSA01), Alboran Island (GSA02), Balearic Islands (GSA05) and Northern Spain (GSA06).

2. To characterize the genetic structure, connectivity and demographic history of the most abundant species, the demersal sharks *Scyliorhinus canicula* and *Galeus melastomus*, from a fine scale sampling design throughout the western Mediterranean and to compare the genetic approach between the different GSAs and sub-basins in the area.

3. To establish the phylogenetic relationship and divergence time in relation to historical evolutionary events of batoid species in the Mediterranean, to know the possible drivers for their speciation events.

4. To improve the taxonomic identification of some batoids combining morphological traits and molecular markers to identify cryptic species.

5. To assess, at community and population levels, the spatio-temporal evolution of demersal chondrichthyans along the western Mediterranean using fishery-independent data over the last two decades.

To achieve these objectives, classical community and population parameters, as well as genetic approaches, were implemented in the four following chapters (I-IV). These chapters containing original research are presented as a collection of five papers, two already published, two others currently submitted, and the last one in preparation for its submission, preceded by a General Introduction, Objectives, and Material and Methods, and followed by Conclusions and References. For this reason, each chapter of results can be read separately, because it has its own Summary, Keywords, Introduction, Material and Methods, Results, and Discussion. The following scientific papers are partially or totally reflected in the Thesis.

Chapter I: COMMUNITIES (*Objective 1*)

Ramírez-Amaro S., Ordines F., Terrasa B., Esteban A., García C., Guijarro B., Massutí E. (2015). Demersal chondrichthyans in the western Mediterranean: assemblages and biological parameters of their main species. *Marine and Freshwater Research* 67: 636–652.

Chapter II: CONNECTIVITY (*Objective 2*)

Ramírez-Amaro S., Picornell A., Arenas M., Castro J.A., Massutí E., Ramon C., Terrasa B. (Submitted). Contrasting evolutionary patterns in populations of demersal sharks sampled at a fine scale throughout the western Mediterranean.

Chapter III: PHYLOGENY AND TAXONOMY (*Objectives 3 and 4*)

Ramírez-Amaro S., Ordines F., Picornell A., Castro J.A., Ramon C., Massutí E., Terrasa B. (Submitted). The evolutionary history of Mediterranean Batoidea (Chondrichthyes: Neoselachii): a molecular phylogenetic approach.

Ramírez-Amaro S., Ordines F., Puerto M.A., García C., Ramon C., Terrasa B., Massutí E. (2017). New morphological and molecular evidence confirm the presence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea and extend its distribution to the western basin. *Mediterranean Marine Science* 18/2: 253–261 (Chapter III).

Chapter IV: TRENDS (*Objective 5*)

Ramírez-Amaro S., Ordines F., Esteban A., García C., Guijarro B., Salmerón F., Terrasa B., Massutí E. (In preparation). Spatio-temporal trends in demersal Chondrichthyans exploited by bottom trawling in the western Mediterranean: some resist, but others do not.

# 3. MATERIAL AND METHODS

## 3.1 Study area: the western Mediterranean

The study area is located in the western Mediterranean and it is mainly influenced by the inflow of Atlantic Water (AW) through the Strait of Gibraltar and the exchange of these waters with Mediterranean water through the Balearic channels. The inflow of AW, is first directed north-eastwards due to the orientation of strait, and then generally describes a clockwise gyre in the west of the Alboran, between Spain and Morocco. The inflow commonly describes a second clockwise gyre in the east of the Alboran, between Spain and Algeria. The boundary of the eastern Alboran gyre that extends between Almeria and Oran, named the Almeria-Oran Front (Figure 3.1). In the vicinity of Oran, the current splits into a westerly component, which completes the gyre, and into an easterly component, which form a strong easterly current along the Algerian coast (Algerian Current) (Arnone *et al*., 1990; Millot, 1999). The eastward flowing Algerian Current boundary appears in the imagery as a continuous undulating ocean feature lying within 30 km of the Algerian Coast (Arnone *et al*., 1990).

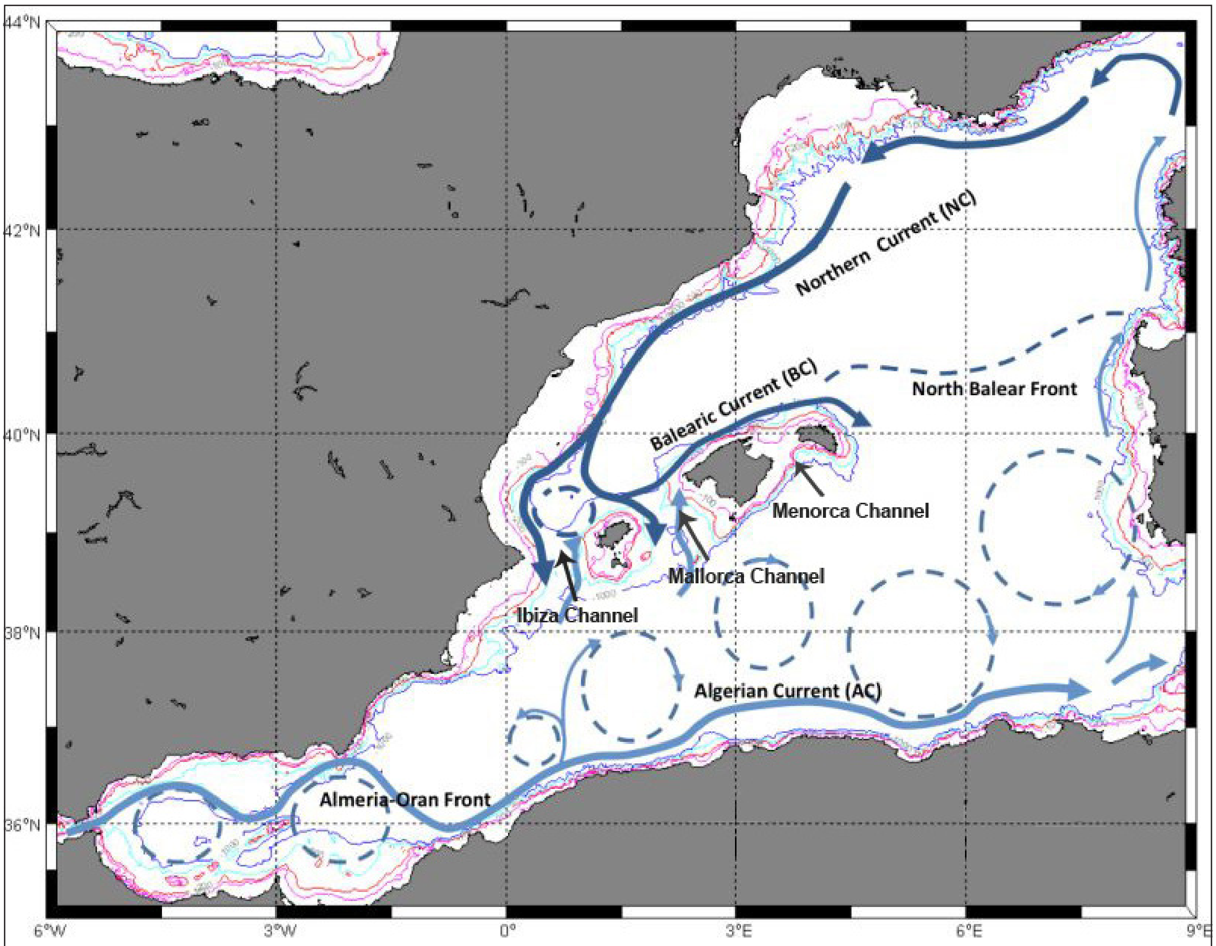


Figure 3.1. Main currents along the western Mediterranean. Lines denote isobaths for 100, 500, 1000 and 2000 m. Modified from Balbín *et al*. (2014).

The Balearic Islands are the natural limit between the Algerian and Balearic sub-basins in the western Mediterranean. In the southern part, the Algerian sub-basin receives fresh surface water from the AW. To the north, the Balearic sub-basin contains colder and more saline surface

AW that has remained longer in the Mediterranean, and its dynamics is affected by atmospheric forcing (Hopkins, 1978; López-Jurado *et al*., 2008). The Mallorca and Ibiza channels play a key role in the regional circulation of this area and their topography condition the exchanges between these two sub-basins (Pinot *et al*., 2002). Because of this, there are significant differences between the hydrodynamic condition that affect the north and the south of the Balearic Islands. The surface waters at the Balearic channels have all an Atlantic origin but with different residence time in the Mediterranean, where the presence of fresher and older AW in the channel produces ocean fronts that affect the sea dynamics (López-Jurado *et al*., 2008).

Four of thirty GSA established by GFCM are located in the Spanish western Mediterranean and were included for the present studies: Northern Alboran Sea (GSA01), Alboran Island (GSA02), Balearic Islands (GSA05) and Northern Spain (GSA06) (Figure 3.2).

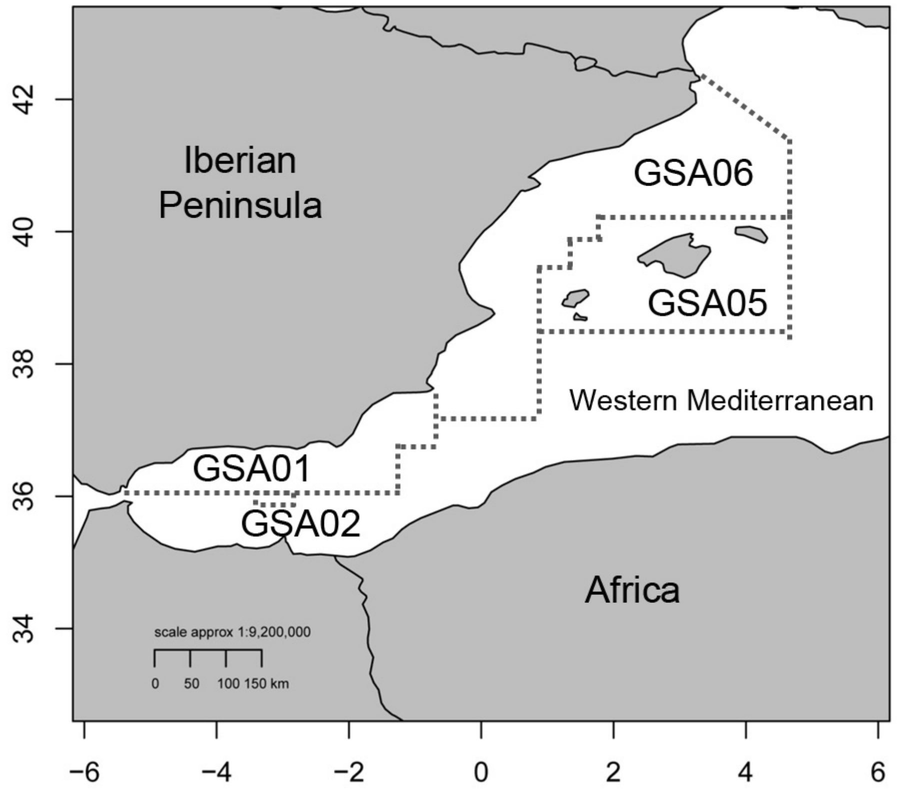


Figure 3.2. Map of the study area (western Mediterranean) showing the geographic sub-areas established by the General Fisheries Commission for the Mediterranean: GSA01, Northern Alboran; GSA02, Alboran Island; GSA05, Balearic Islands; GSA06, Northern Spain.

## 3.2 MEDITS Programme: data source

The biological data and genetic samples used in the present work were obtained from the Mediterranean International Bottom Trawl Survey (MEDITS). The MEDITS programme (http://www.sibm.it/MEDITS%202011/principalemedits.htm) started in 1994 with four partners (Spain, France, Italy and Greece), and is at present performed by ten riparian EU countries.

This survey programme focus to produce basic information on demersal species in terms of population abundance and biomass distribution as well as demographic structure. The surveys intend to include as much as possible all the trawlable areas over the selves and the upper slopes from 10 to 800 m depth off the coast of the partner countries (Figure 3.3). The stations have been distributed applying a stratified scheme with random drawing inside each stratum. This stratification adopted was the depth, with the following limits: 30, 50, 100, 200, 500 and 800 m (Figure 3.3), and the number of stations per stratum are proportional to its area. The sampling scheme, gear and sampling protocol are standardized among all countries (Bertrand *et al*., 2002). Surveys are conducted every year during the spring and the beginning of summer. The Spanish surveys were carried out on board the R/V *Cornide de Saavedra* and *Miguel Oliver* (Figure 3.4). The duration of the hauls is fixed to 30 minutes on depth less than 200 m and 60 minutes for deeper stations.

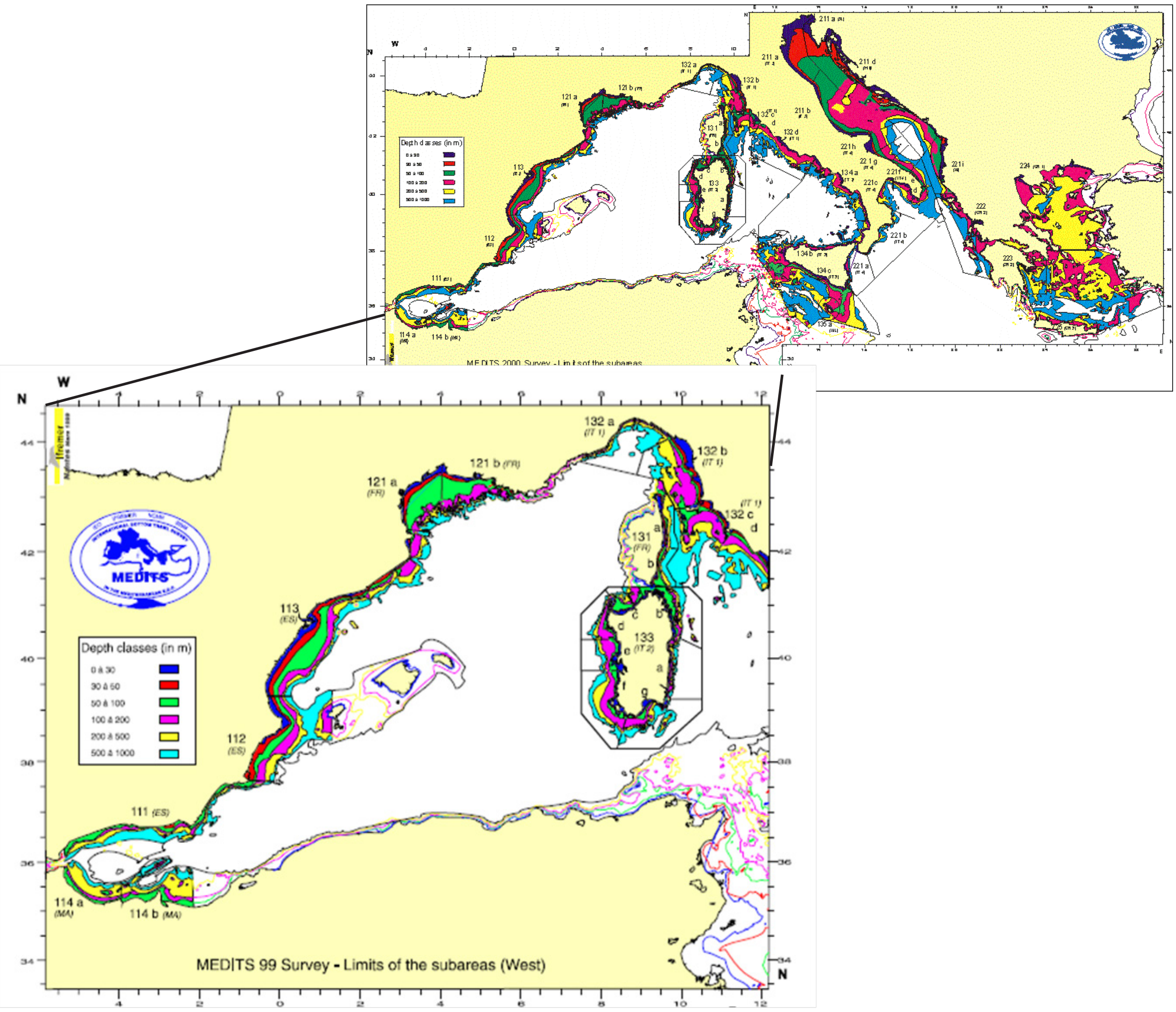


Figure 3.3. Limits of the sampling bathymetric strata of the MEDITS scientific surveys along the western Mediterranean. Modified from Bertrand *et al*. (2002). The bathymetric limits off Balearic Islands are not included, because this area was incorporated to MEDITS programme in 2007.

Figure 3.4. Research vessels used during the Spanish MEDITS surveys: (a) *Cornide de Saavedra* and (b) *Miguel Oliver*.

## 3.3 Biological and ecological parameters

### *3.3.1 Applied statistics*

The biological data used in this Thesis was obtained from 1994 to 2015 for this study. Several statistical analyses were performed using different statistical softwares. These statistical analyses and softwares are described in Table 3.1. Additionally, the methods performed and application purposes are indicated in each chapter.

### *3.3.2 Trends in abundance and biomass*

To identify underlying common trends in abundance and biomass among time series (Chapter IV), a Dynamic Factor Analysis (DFA, Zuur *et al*., 2003a) was used. DFA is a multivariate dimension reduction technique designed for relatively short and non-stationary time-series data. This approach involves fitting a specialized, multivariate, autoregressive state-space model to detect a set of underlying trends that explain temporal variation in a collection of time series (Zuur *et al*., 2003b; Holmes *et al*., 2013). The process component of DFA fits autocorrelated common trends to accommodate the time series of the indices and resulting trends (Stachura *et al*., 2014). Factor loadings indicate the strength of the influence of each survey index on the resulting common trends. Values higher in magnitude denote a stronger effect of the given GSA or species on the corresponding common trend (Zuur *et al*., 2003a; Zuur *et al*., 2003b).

Table 3.1. Statistical methods and indices used in this Thesis including the chapters and they corresponding softwares and references.

| **Methods and Indices** | **Description** | **Chapters** | **Softwares and references** |
|---|---|---|---|
| Length–weight relationship (LWp) | It is the relation between the weight of shark (g) and total length (cm) which is estimated by linear regressions analysis. | I/IV | SigmaPlot v.11.0<br>Systat software |
| Length–frequency distribution (LFd) | The chondrichthyan specimens are grouped in different size classes or categories, and then, the percentage contribution of each category is estimated. | I/IV | |
| Somatic condition (SC) | Is calculated from the residuals of the linear regression between length and weight. | IV | |
| Linear regressions | It is a measure of the relationship between two continuous (quantitative) variables. | I/IV | |
| Length at first maturity ($L_{50}$) | Logistic regressions model were fitted to maturation stages (as binary data) to determine the length at with 50% of specimens had become mature. | I/IV | R 3.3.2<br>R Core Team |
| Bathymetric distribution models (Bm) | The generalised linear model and linear regression are used to model the species response curves (standardized abundance) along the depth gradient. | I | CANOCO v4.5<br>Ter Braak and Smilauer, 2002 |
| Redundancy analysis (RDA) | This method seeks the combinations of explanatory variables that best explain the variation of the dependent matrix. In RDA, the ordination process is directly influenced by a set of explanatory variables: the ordination seeks the axes that are best explained by a linear combination of explanatory variables. | III | |
| Species richness (*S*) | It is a measure of the number of species per unit area. | I/IV | PRIMER v6.1.6<br>Clarke and Warwick, 2001 |
| Shannon diversity index (*H'*) | It is a quantitative measure that reflects how many different species there are in a community and takes into account how evenly the basic entities (such as individuals) are distributed among those communities. | I | |
| Evenness (*J*) | It is a measure of biodiversity which quantifies how equal the community is numerically and is based on Shannon diversity index. | I | |
| Analysis of similitude (ANOSIM) | Analysis of similarities hypothesis for differences between groups of community samples, using permutation methods on resemblance matrix. | I | |
| Similarity percentage analysis (SIMPER) | Examines the contribution of each variable (e.g. abundance, biomass) to average resemblance between sample groups. | I | |
| Multidimensional scaling (MDS) | It is a means of visualizing the levels of similarity of individual cases of a dataset. | I/II | |
| CLUSTER | Implements hierarchical agglomerative clustering, using standardized abundance. | I | |

The correlation of the observation errors was modeled using different covariance matrices: *i*) same variance and no covariance (diagonal-equal); *ii*) different variances and no covariance (diagonal-unequal); *iii*) same variance and covariance (equalvarcov); and *iv*) different variances and covariances (unconstrained). The corrected Akaike's information criterion (AICc) was used as a measure for goodness of fit and for finding the best model having the lowest AIC (Zuur *et al*., 2003a; Zuur and Pierce, 2004). Model implementation was performed using the Multivariate Autoregressive State-Space "MARSS" package (Holmes *et al*., 2013) in R 3.3.2. This approach was used in chapter IV.

## 3.4 Genetic analysis

### *3.4.1 Laboratory procedures*

Figure 3.5. Genetic sampling example of a shark species during the MEDITS surveys.

Genetic samples were collected during the MEDTIS surveys carried out between 2013 and 2016 (Figure 3.5). A small piece of tissue (around 2 cm2) was taken from each individual and stored at 4°C in absolute ethanol and then transported to the laboratory. Genomic DNA was isolated from samples using a standard phenol-chloroform method proposed by Terrasa *et al*. (2009) with minor modifications: *i*) the tissue sample was homogenised using a lysis buffer (Tris-HCl 10mM, KCl 50mM, MgCl2 25 mM, 0.45% Igepal CA-630, 0.45% Tween®20, pH 8); *ii*) sodium dodecyl sulfate (SDS; 20%) and proteinase K (20 mg/ml) were added to samples and then the preparation incubated at 60°C for at four hours in order to induced cell lysis; *iii*) purification of DNA was performed by three consecutive extractions added phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1); *iv*) for DNA precipitation we used ethanol absolute and sodium acetate (5M at –20°C), then the pellet was dried and resuspended in the appropriate volume of water Milli-Q; *v*) later was used the NanoDrop (ND-1000; Thermo Scientific) to quantify the genomic DNA, and *vi*) lastly, a set of aliquots (volume: 20 µl) for PCR reactions were prepared.

### *3.4.2 Mitochondrial fragments*

In total, four mitochondrial fragments were amplified and sequenced for the genetic studies performed in this thesis (chapters II and III): Cytochrome b (*Cyt b*); Dehydrogenase subunit 2

(*ND2*); Cytochrome oxidase subunit I (*COI*) and Control region (*CR*). The mitochondrial fragment was amplified using the Polymerase chain reaction (PCR). PCR reactions were performed in a GeneAmp 2700 (Applied Biosystems) thermocycler. Primers and PCR conditions for each fragment are described in Table 3.2. Amplification products were validated with 1% agarose gel electrophoresis, then PCR product were purified using the commercial kit QIAquick® PCR Purification Kit (QIAGEN). Lastly, both heavy and light strands were sequenced on an automated ABI 3130 sequencer using ABI Prism Terminator BigDye TM Cycle Sequencing Reaction Kit (Applied Biosystems). The resulting DNA sequences were imported into BioEdit 7.0.5.2 (Hall, 1999) and checked for quality and accuracy with nucleotide base assignment. The multiple sequences alignments were obtained with ClustalW (Thompson *et al*., 1994).

Table 3.2. Primers and PCR conditions for each mitochondrial fragment.

| Regions | Primers | PCR condition | | |
|---|---|---|---|---|
| Cytochrome b<br>(Palumbi, 1996) | H15175: 5’-CCCTCAGAATGATATTTGTCCTCA-3’<br>L14724: 5’-TGACTTGAARAACAYCGTTG-3’ | 96.0ºC | 5:00 | |
| | | 94.0ºC | 1:00 | 35c |
| | | 56.0ºC | 1:00 | |
| | | 72.0ºC | 1:00 | |
| | | 72.0ºC | 10:00 | |
| Dehydrogenase subunit 2<br>(Sandoval-Castillo and Rocha-Olivares, 2011) | ND2Met47: 5’-TTTTGGGCCCATACC-3’<br>ND2Trp18: 5’-GCTTTGAAGGCTTTTGGT-3’ | 95.0ºC | 2:00 | |
| | | 94.0ºC | 0:14 | 35c |
| | | 54.0ºC | 1:00 | |
| | | 72.0ºC | 2:00 | |
| | | 72.0ºC | 6:00 | |
| Cytochrome oxidase subunit I<br>(Ward *et al*. 2005) | FishF1: 5’-TCAACCAACCACAAAGACATTGGCAC-3’<br>FishF2: 5’-TCGACTAATCATAAAGATATCGGCAC-3’<br>FishR1: 5’-TAGACTTCTGGGTGGCCAAAGAATCA-3’<br>FishR2: 5’-ACTTCAGGGTGACCGAAGAATCAGAA-3’ | 95.0ºC | 2:00 | |
| | | 94.0ºC | 0:30 | 35c |
| | | 54.0ºC | 0:30 | |
| | | 72.0ºC | 1:00 | |
| | | 72.0ºC | 10:00 | |
| Control region<br>For SC<br>(Gubili *et al*. 2014)<br>For GM<br>(designed in the present thesis study) | ScyD1pF: 5’-ATGACATGGCCCACATATCC-3’<br>Scan2R: 5’-TTCTCTTCTCAAGACCGGGTA-3’<br>GmF: 5’- GTGATATAGCCCACATACCTTA-3’<br>GmR: 5’- TTTCTCTAATTCTCGGAGGG-3’ | 95.0ºC | 2:00 | |
| | | 94.0ºC | 0:14 | 35c |
| | | 54.0ºC | 1:00 | |
| | | 72.0ºC | 2:00 | |
| | | 72.0ºC | 6:00 | |

### *3.4.3 Microsatellites*

Microsatellites markers, also called short tandem repeat loci (STR), were used in *Scyliorhinus canicula* (Chapter II). These markers are typically highly polymorphic regions in the nuclear genome. They are short nucleotide sequences (commonly 2 to 4 base pairs in length) that are tandemly repeated several times (Sun *et al*., 1984). Microsatellites were used to infer the patterns of population structure and dispersal of *S. canicula*. A total of twelve microsatellites described by Griffiths *et al*. (2011) were tested: Scan02, Scan03, Scan04, Scan06, Scan07, Scan09, Scan10, Scan12, Scan13, Scan14, Scan15 and Scan16. The PCR conditions were: 1 µl 1/50 diluted genomic DNA, 0.2 mM of each dNTP (Bioline), 0.05 pM of each primer, 2.5 mM

MgCl2, PCR buffer and 0.25 U of Taq DNA polymerase (Bioline). PCR reactions were performed in a GeneAmp 2700 (Applied Biosustems) thermocycler with a denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C for 0.5 min, annealing at 60°C for 0.5 min, and extension at 72°C for 0.5 min, before a final extension step of 72°C for 10 min. All sets of primers showing consistent amplifications with 1% agarose gel electrophoresis. Then these primers were fluorescently labelled (6FAM, VIC, NED or PET), their fluorescent combination are show in Table 3.3. These sets of primers were amplified again with QIAquick® PCR Purification Kit (QIAGEN) with the same conditions above described.

Table 3.3. Fluorescent primers for microsatellite multiplex PCR.

| Primer name | Sequence (5' to 3') | Fluorescent 5' label |
|---|---|---|
| Scan02F | TGC AGC TTC GCT ATC TTA TGC | 6FAM<br>VIC |
| Scan03F | TGG ATA ATT GAC AGA ATC GGC | |
| Scan15F | TCA TCA TCA TCA CCA CCA GAA | |
| Scan09F | GAG AAT TGT TTC CCA GTG GC | |
| Scan07F | CCT GCA CAC AGC AGG TAG AA | NED |
| Scan12F | GCC AGT GGC TAT AAC GGA AC | |
| Scan13F | CGC CCT CCC CTA AAA TAG AC | |
| Scan04F | ACC AAA GAT CAA GCC AGG AA | |
| Scan16F | CCG ACT CCT TTG GAT GTG TT | PET |
| Scan06F | GGC AGT GAT TGC ATT CTT GA | |
| Scan10F | CGG TAC GAC CTG GAT CAA CT | |
| Scan14F | AAC CAT CCT CCG CAA ATA AA | |

### *3.4.4 Genetic indices and statistical methods*

Different genetic indices and statistical methods for mtDNA and microsatellites markers were used. The genetic analyses and implemented softwares are described in Table 3.4. The methods performed and application purposes are also indicated in each chapter.

### *3.4.5 Population structure and connectivity*

Inferences and analysis of population structure from genetic data are mainly done by clustering individuals into groups, often referred to as demes or subpopulations. Evaluation of population structure and gene flow levels between populations allows inferences of the migration patterns and their genetic consequences, among other processes (Templeton, 2006). To assess this (chapter II) in both molecular markers (mtDNA and microsatellites), the following analyses were performed:

*Analyses of molecular variance* (*AMOVA*): it estimates genetic structure indices using the number of mutations between molecular haplotypes. It is tested using non-parametric permutation procedures and it was performed with ARLEQUIN v3.11 (Excoffier *et al.*, 2005) (applied in both molecular markers).

*Genetic distances*: it is a measure of population differentiation due to genetic structure. Fst (Wright's, 1965) can provide the basis for a measure of genetic distance when divergence is caused by drift (Reynolds *et al*., 1983) (applied in both molecular markers). ΦST is an analogous estimator to Fst that takes into account the distances between alleles thereby correcting for mutation rate (Excoffier *et al*., 2005) (applied in mtDNA fragments). The significances of the genetic distances are tested by permuting the haplotypes or loci between populations and were performed with ARLEQUIN v3.11 (Excoffier *et al*., 2005).

Table 3.4. Basic genetic indices and statistical methods used in this Thesis. Their description, molecular marker in which they were applied (mitochondrial DNA: mtDNA; microsatellites: STRs), softwares and references are indicated.

| **Genetic indices** | **Description** | **Molecular markers** | **Chapters** | **Softwares and references** |
|---|---|---|---|---|
| Nucleotide diversity ($\pi$) | Average number of nucleotide differences per site between two sequences. | mtDNA | II/III | DnaSP v5 Librado and Rozas, 2009 |
| Haplotype diversity ($h$) | A measure of the uniqueness of a particular haplotype in a given population. | mtDNA | II/III | |
| Haplotype differences ($k$) | Average number of differences between haplotypes | mtDNA | II | |
| Tajima (D) | Measure based on the differences between the number of segregating sites and the average number of nucleotide differences. | mtDNA | II | ARLEQUIN v3.11 Excoffier *et al*., 2005 |
| Fu ($F_s$) | Measured based on the haplotype frequency distribution conditional the value of $\theta$ (where, $\theta=4N\mu$; for diploid-autosomal; N and $\mu$ are the effective population size, and the mutation rate per DNA sequence per generation, respectively. | mtDNA | II | |
| Linkage disequilibrium (LD) | It is a measured of deviation from a random association between alleles at different loci. | STRs | II | |
| Garza-Williamson index (GW) | The mean ratio of the number of alleles at given locus with respect to the range of allele size, to detect population bottlenecks. | STRs | II | |
| Allelic frequencies (Na) | Mean number of alleles over loci. | STRs | II | FSTAT 2.9.3.2 Goudet *et al*., 2002 |
| Hardy-Weinberg Equilibrium (HWE) | Describes the expected frequencies of genotypes in a population under random mating. Deviations from HWE are assessed using a chi-square goodness of fit test, which compares observed genotype frequencies with expected genotype frequencies that are calculated from allele frequencies assuming equilibrium. | STRs | II | |
| Allelic richness (Rs) | The effective number of alleles with equal frequencies. | STRs | II | GenAlex 6.502 Peakall and Smouse, 2012 |

*Likelihood analysis with metropolis algorithm using random coalescence* (*LAMARC*): it is a package of programs for computing population parameters such as population size and migration events by using likelihood over all possible gene genealogies that could explain the observed sample. It was estimated with LAMARC v.2.1.10 (Kuhner, 2006) (applied in both molecular markers).

*Haplotype network*: it is used in the analysis of population genetic data to visualise genealogical relationship at the intraspecific level, as well as to make inference about history of population and biogeography. It was estimated with TCS v1.21 using the probability of parsimony (Clement *et al*., 2000) (applied in mtDNA fragments).

*Bayesian phylogeographic and ecological clustering* (*BPEC*): it is used to combine sequence data with geographical and ecological data in order to draw conclusions about the geographically clustered structure consistent with the evolutionary history of the organisms of interest. BPEC combines an evolutionary model for DNA sequences through a haplotype tree together with a geographical model representing migrations forming cluster into a fully model-based framework. Model implementation was performed using the "BPEC" package (Manolopoulou and Emerson, 2012) in R 3.3.2 (applied in mtDNA fragments).

*Mismatch distribution*: it computes the distribution of the observed number of differences between pairs of haplotypes in the sample and also estimates parameters of a sudden demographic expansion using a generalized least-square approach. It was performed with ARLEQUIN v3.11 (Excoffier *et al*., 2005) (applied in mtDNA fragments).

*Extended Bayesian skyline plot* (*EBSP*): it is a model used to infer population history from multilocus genetic data. EBSP estimates the number of population size changes directly from the data using Bayesian stochastic variable selection. The MCMC algorithm is used by BEAST v1.8.2 to explore the state space defined by the set of priors (including parameters) to be inferred by applying stochastic functions (Drummond and Rambaut, 2007) (applied in mtDNA fragments).

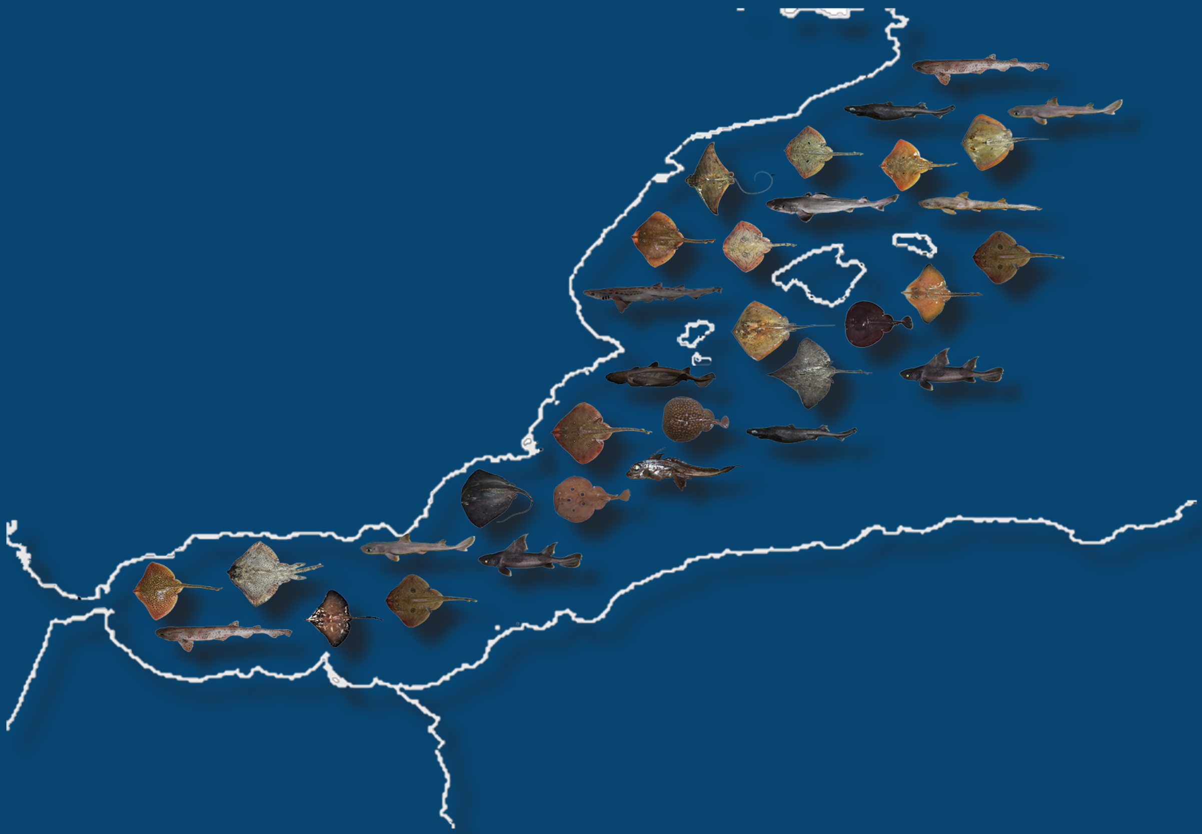
*STRUCTURE software*: it implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. This assumes a model in which there are K populations (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned to populations, or jointly to two or more populations if genotypes indicate that they are mixed (Pritchard *et al*., 2000) (applied in microsatellites).

*BOTTLENECK program*: it is a population genetics computer program that conducts different test for indentifying populations that have recently experienced a severe reduction in effective population size. It requires allele frequency data obtained from one sample of individuals and at least four polymorphic loci. It computes for each population sample and for each locus the distribution of heterozygosity expected from the observed number of alleles, given the sample size under the assumption of mutation-drift equilibrium (applied in microsatellites).

### *3.4.6 Phylogeny*

Evolutionary models (models of nucleotide substitution) are sets of assumptions about the process of nucleotide or amino acid substitution. They describe the different probabilities of change from one nucleotide to another, with the aim of correcting for unseen changes along the phylogeny. The statistical selection of the best-fit model of nucleotide substitution was identified with jModelTest v2 software (Darriba *et al*., 2012). Once a substitution model has been selected, the Bayesian Inference of Phylogeny (MrBayes software; Ronquist *et al*., 2011) and the Bayesian Evolutionary Analysis Sampling Trees (BEAST software; Drummond *et al*., 2012) were used as methods of reconstructing phylogenies. Both methods use Markov Chain Monte Carlo (MCMC) methods to estimate the posterior distribution of model parameters. MCMC is a stochastic algorithm that produces sample-based estimates of a target distribution of choice. The most distinguishing feature of BEAST is its firm focus on calibrated phylogenies and genealogies, that is, rooted trees incorporating a time-scale. This is obtained by explicitly modeling the rate of molecular evolution on each branch in the tree. On the simplest level this can be a uniform rate over the entire tree (e.g. the molecular clock model; Zuckerkandl and Pauling, 1965) with this rate known in advance or estimated from calibration information (Drummond and Rambaut, 2007). BEAST includes a Bayesian framework for species tree estimation named Species Tree Ancestral Reconstruction (*BEAST; Helen and Drummond, 2010). The model assumes no recombination within each locus and free recombination between loci. *BEAST does not require that each gene alignment is input with the same number of sequences. All that is needed is that each sequence in each gene alignment is mapped to the appropriate species (Drummond and Bouckaert, 2015).

# 4. RESULTS



# CHAPTER I

## 4.1 COMMUNITIES

Demersal chondrichthyans in the western Mediterranean: assemblages and biological parameters of their main species

Ramírez-Amaro S., Ordines F., Terrasa B., Esteban A., García C., Guijarro B., Massutí E. (2015)

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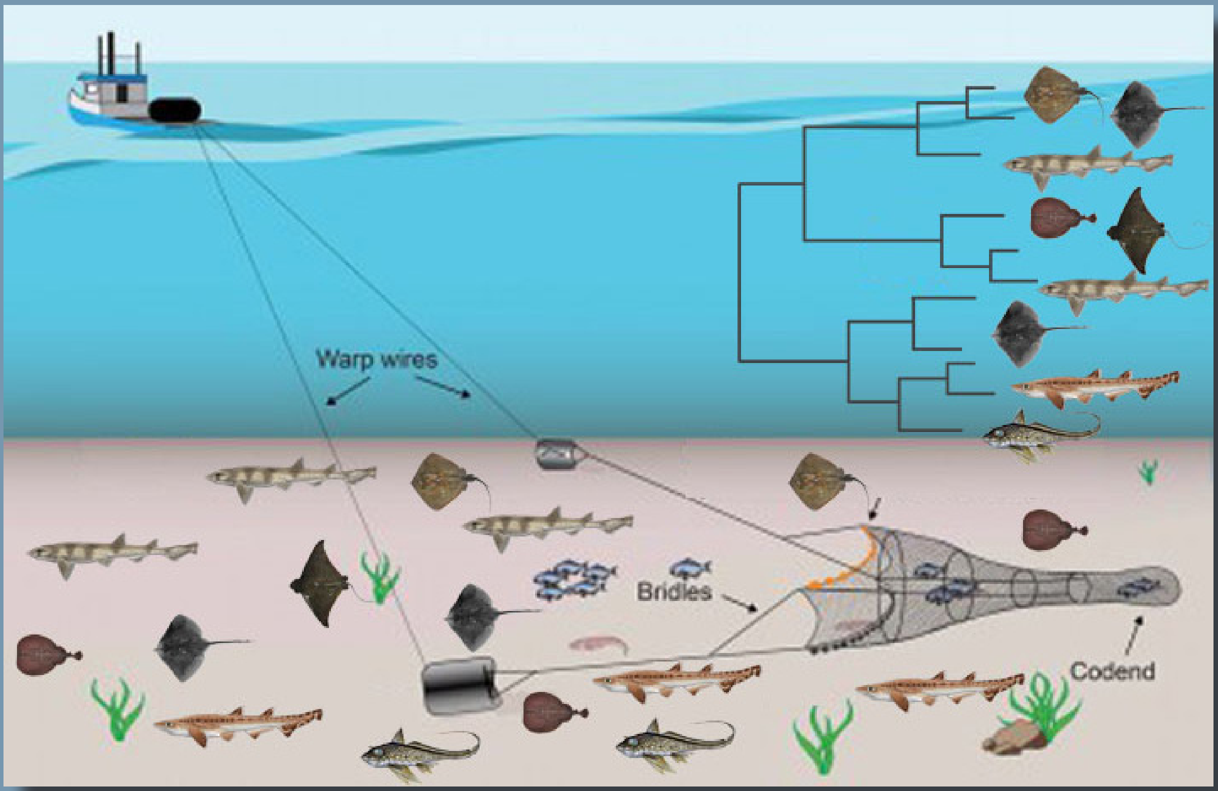


## Summary

The composition and structure of demersal chondrichthyan assemblages and the biological parameters of their main species were compared in four geographical subareas (GSAs) established by the General Fisheries Commission for the Mediterranean in the western Mediterranean: northern Alboran Sea (GSA01) and Alboran Island (GSA02), Balearic Islands (GSA05) and northern Spain (GSA06), with the first two being considered jointly. Data were obtained from 199 hauls undertaken from May to June 2013 during the Spanish International Bottom Trawl Survey in the Mediterranean (MEDITS). Twenty-five different chondrichthyan species were caught in all GSAs: seven were common to all areas, five appeared only in GSA01, nine appeared only in GSA05 and one appeared only in GSA06. Analysis of community structure (clusters, multidimensional scaling, analysis of similitude) revealed two assemblages related to depth, with very similar bathymetric ranges in all GSAs, namely continental shelf (from 41 to 252 m) and slope (from 284 to 813 m). The highest diversity, biomass and abundance values on the shelf and slope assemblages were recorded in GSA05 and GSA01, respectively. These results highlight the effects of the distinct fishing and oceanographic parameters related to the GSAs. Generally, the biological parameters of the most important species did not show differences between GSAs, which could suggest the existence of a single population in the western Mediterranean.

**Keywords:** abundance, bottom trawl fishery, depth-related trends, diversity, length at first maturity, vulnerable species.

## 4.1.1 Introduction

In the Mediterranean Sea, international concern related to changes in the abundance and diversity of chondrichthyans has been increasing. When based on taxa found in a wide variety of ecosystems that are sensitive to human impact (García *et al*., 2008) and have particular life histories (Stevens *et al*., 2000), as well as important ecological functions (Stevens *et al*., 2000; Ferretti *et al*., 2010), spatial biodiversity patterns could be particularly useful to identify areas of high conservation value, not only for a particular group, but also for the entire ecosystem (Lucifora *et al*., 2012).

In the Mediterranean, the chondrichthyan community (82 recorded species) has shown a decline in species diversity and abundance in recent decades (Serena, 2005; Walker *et al*., 2005; Ferretti *et al*., 2008). According to Bradai *et al*. (2012), cartilaginous species have become the most endangered group of marine fish in the Mediterranean, with 31 species assessed as critically endangered, endangered or vulnerable (Cavanagh and Gibson, 2007). In recent decades, a decrease in the presence and abundance of chondrichthyans in fishing landings has been documented in the Gulf of Lions (Aldebert, 1997; Bertrand *et al*., 1998), Balearic Islands (Guijarro *et al*., 2012), Tyrrhenian Sea (Ligas *et al*., 2013) and Adriatic Sea (Jukic-Peladic *et al*., 2001). The effect of fisheries has multiplied during the past 30 years, leading to the overexploitation of the main commercial stocks in most areas (Colloca *et al*., 2013), along with a variety of well-described direct and indirect negative effects on the ecosystem.

In multispecies fisheries, such as Mediterranean bottom trawling performed along the continental shelf and upper slope down to a depth of 800 m, chondrichthyans make up a significant proportion of the bycatch, although they represent a minor part of the landings (Guijarro *et al*., 2012). Depending on the area and the operational depth of the gear, the most frequently caught species are *Galeus melastomus*, *Scyliorhinus canicula*, *Etmopterus spinax*, *Raja clavata*, *Raja asterias* and *Squalus acanthias* (Bertrand *et al*., 2000; Carbonell *et al*., 2003; Damalas and Vassilopoulou, 2011).

The General Fisheries Commission for the Mediterranean (GFCM) has established 30 areas for fisheries assessment and management purposes. Four of these geographical subareas (GSAs) are located in the western Mediterranean: northern Alboran Sea (GSA01), Alboran Island (GSA02), Balearic Islands (GSA05) and northern Spain (GSA06). Recently, the GFCM recognised a lack of knowledge concerning the biology and fishery of elasmobranchs in many parts of the Mediterranean, and strongly encouraged more studies related to conservation issues of these species (Bradai *et al*., 2012). In the present study, we describe the demersal chondrichthyan assemblages on the bottom trawl fishing grounds of these GSAs, and the biological parameters of their most abundant species. The aim of the study was to improve scientific knowledge of the populations of these vulnerable species in the western Mediterranean, thus contributing to a more efficient assessment and management of the associated fisheries, as well as to the conservation of marine ecosystems and their living resources.

## 4.1.2 Materials and methods

*Data source*

Information was obtained from 199 hauls made between depths of 38 and 813 m during the International Bottom Trawl Survey in the Mediterranean (MEDITS) performed from 7 May to 28 June 2013 in the western Mediterranean (Figure 4.1.1). The number of hauls per GSA is given in Table 4.1.1. The sampling scheme and gear, as well as the sampling protocol of catches, were those applied throughout the Mediterranean within the framework of the MEDITS program (Bertrand *et al.*, 2002). The effective trawling time ranged from 30 to 60 min depending on the operating depth, with an average towing speed of 5.5 km/h (3 knots). The raising and lowering of the net from and to the sea bottom, as well as the horizontal and vertical openings of the net (16.4 and 2.8 m respectively), were estimated using the SCANMAR system (Catch Control Systems, Scanmar AS, P.O. Box 44, 3167).

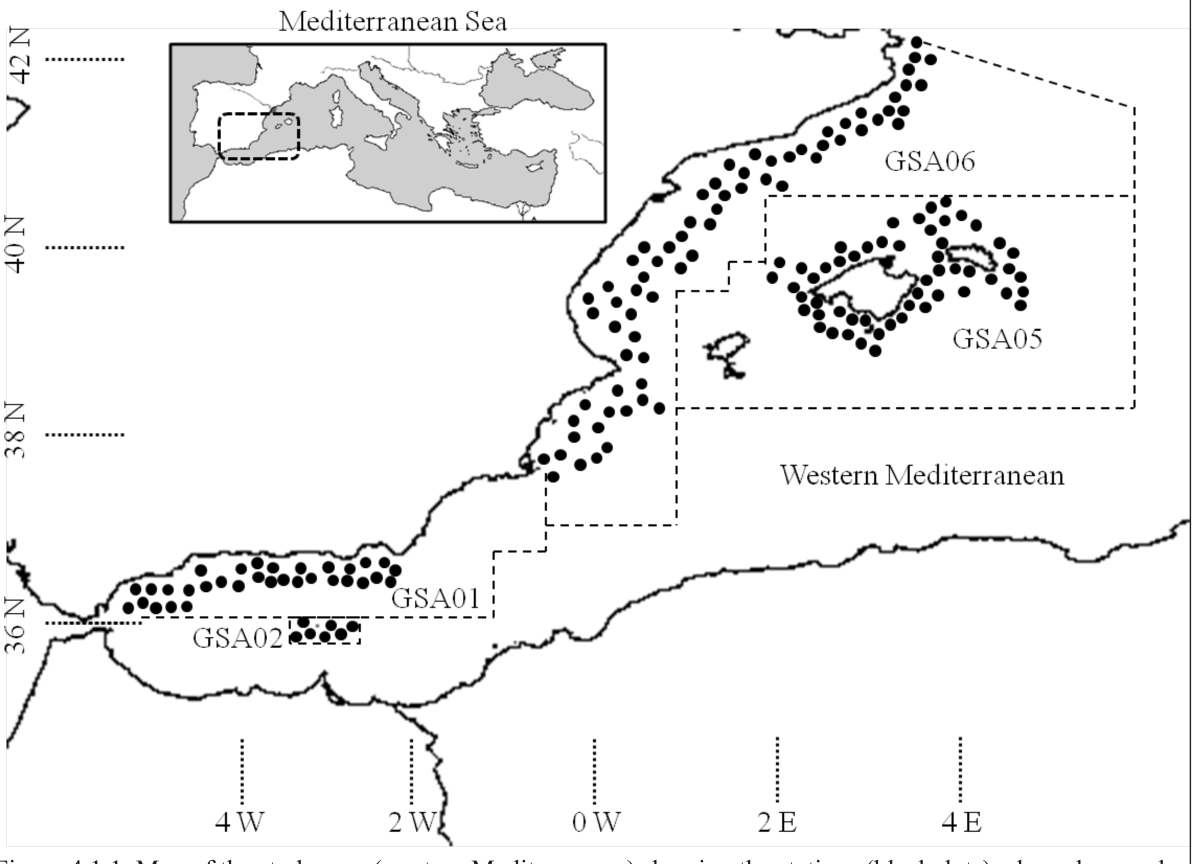


Figure 4.1.1. Map of the study area (western Mediterranean) showing the stations (black dots) where demersal chondrichthyans were captured during the International Bottom Trawl Survey in the Mediterranean (MEDITS) performed in 2013. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain.

In each haul, the chondrichthyans were classified and individuals of each species were counted, weighed, sexed and measured (total length (TL); for chimaeras, TL was measured from the distal tip of the snout to the cloacal opening). For the most abundant species, sampling of the maturity stage and individual weight of either the total catch or a random sample of the catch was also made in each haul. Collected data were standardised to within 1 $km^2$.

Table 4.1.1. Number of experimental bottom trawl hauls analysed by each geographical subarea (GSA) and their depth intervals. Data show the number of hauls in which chondrichthyans were caught (+ hauls) and their depth interval (positive hauls in brackets). GSA01, northern Alboran; GSA02, Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain.

| **Depth strata (m)** | **GSA01** | **GSA02** | **GSA05** | **GSA06** |
|---|---|---|---|---|
| 40–100 | 14 (10) | 0 | 23 | 48 (22) |
| 100–200 | 5 (5) | 1 | 14 | 24 (22) |
| 200–500 | 10 (10) | 2 | 9 | 17 (16) |
| 500–820 | 10 (10) | 4 | 10 (8) | 8 (8) |
| **Total** | **39 (35)** | **7 (7)** | **56 (54)** | **97 (68)** |

*Biological parameters*

For the most abundant species, length–weight parameters (LWp) were estimated for each sex. The model describing this relationship was typically of the following form:

$$W = a \times L^{b}$$

where W is bodyweight (g), L is TL (cm) and a and b are fitting constants. LWp were fitted for males and females in each GSA. The coefficient of determination (R2) was also calculated. Analysis of covariance (ANCOVA) was applied to log-transformed data to compare LWp between sexes.

The maturity parameters (Mp) of species were determined by macroscopic examination of the gonads following the four-stage scale for both sexes established by the Workshop on Sexual Maturity Staging of Elasmobranchs (ICES, 2010). Size at maturity was determined using maturity ogives. The length at first maturity ($L_{50}$; i.e. the length at which 50% of fish had become mature) was estimated by fitting binomial maturity to length data. A logistic equation was fitted using least-squares non-linear regression in the following equation:

$$Y = \frac{1}{(1+e^{-(a+bx)})}$$

where Y is the maturity status and x is TL (cm). The $L_{50}$ was estimated as –a/b. The significance of differences between maturity curves was evaluated using maximum-likelihood tests (Kimura, 1980).

The sex ratio for the whole sample was estimated by GSA and evaluated using the $\chi 2$ test in order to determine deviations from 1 : 1.

The length–frequency distribution (LFd) by sex was estimated for the most abundant species in each GSA and depth strata. Because LFd violated the assumption of normal distribution and homoscedasticity, the non-parametric Mann–Whitney U-test and Kruskal–Wallis test, followed by Dunn's post hoc test, were used to compare LFd between sexes.

*Bathymetric distribution of species*

Bathymetric distribution models were developed for the species appearing in at least five hauls in each GSA. The data used for this analysis were the standardised abundance of each species and the mean depth per haul. Because it has been observed that species typically show unimodal (bell-shaped) response curves with respect to environmental variables, the generalized linear model (GLM) and linear regression were used to model the species response curves along the depth gradient. The response type (unimodal or linear) of a particular species was chosen on the basis of the lowest value of the Akaike information criterion (AIC) as a measure of the parsimony of each regression model (Ter Braak and Smilauer, 2002). The GLM used was a log-linear model, in which the linear predictor is a quadratic polynomial of the environmental variable. This model is precisely the 'Gaussian' response curve that has been put forward as ideal for species responses along an environmental gradient (Ter Braak, 1986).

*Assemblages and community descriptors*

Clusters and multidimensional scaling (MDS) were applied to the standardised abundance data matrix for each GSA, using PRIMER V.6 (Clarke and Warwick, 2001). GSA01 and GSA02 were considered jointly for this and the rest of the analysis because of the low number of hauls in GSA02. Consequently, both GSAs were referred to as GSA01.

Because of the low frequency of appearance of most species, samples were pooled for each 15-m depth interval. In the resulting data matrix of species per depth interval, the value of each cell corresponded to the average abundance value of a particular species in the hauls for the particular depth interval. To reduce the effect of abundant species, data were fourth root transformed (Clarke and Warwick, 2001). The Bray–Curtis index was used as a between-interval similarity measure. Similarity percentage analysis (SIMPER) was used to assess the species contribution (abundance and biomass) to the detected assemblages. Analysis of similitude (ANOSIM) was used to test differences between areas in each assemblage. The community descriptors, total abundance (individuals km$^{-2}$), total biomass (kg km$^{-2}$), species richness (d), Shannon diversity index ($H'$) and evenness ($J$) were also calculated by assemblage using the following equations:

$$d = \frac{\mathrm{S} - 1}{\log\ (\mathrm{N})}$$

$$H' = -\sum_{\mathrm{i}=1}^{S} \mathrm{p}_{\mathrm{i}} \log_{2}$$

$$\mathrm{Y} = \frac{H(s)}{H(\mathrm{max})}$$

where $S$ is the number of species, N is the number of individuals, ρi is the proportion that the ith species contributes to the total abundance of the sample, H(s) is the Shannon–Wiener index and H(max) is the theoretical maximum value for H(s) if all species are equally abundant.

## 4.1.3 Results

*Species caught*

In all, 25 chondrichthyan species belonging to 10 families were caught in all GSAs (Table 4.1.2). Fourteen species (five exclusive species) were caught in GSA01, compared with 18 (nine exclusive) in GSA05 and 10 (one exclusive) in GSA06. Seven species appeared in all areas, with the sharks *S. canicula* and *G. melastomus* being the most abundant species, accounting for the largest proportion of total biomass (Table 4.1.2). Other species commonly caught were *Galeus atlanticus*, *E. spinax* and *Chimaera monstrosa* in GSA01, *R. clavata*, *Raja miraletus* and *Raja polystigma* in GSA05, and only *Raja asterias* in GSA06.

*Biological parameters*

*Scyliorhinus canicula*

Differences in LWp were observed between sexes for all GSAs (F = 40.0; $P < 0.05$; Table 4.1.3), and geographical differences were detected within each sex (Table 4.1.4). The sex ratio differed from 1: 1 only in GSA05 ($\chi^2 = 35.07$; $P < 0.05$; Table 4.1.3). Similarly, there were no differences among GSAs in the Mp and $L_{50}$ estimated by sex. Hence, these curves were calculated combined for all GSAs (Table 4.1.4); $L_{50}$ values for females and males were 41.1 cm ($R^2 = 0.99$) and 39.4 cm ($R^2 = 0.98$), respectively, with no significant differences between sexes ($\chi^2 = -31.02$; $P > 0.05$). The bathymetric distribution was similar in GSA01 and GSA06 (250 and 242 m respectively), but in GSA05 it was located at a shallower depth (163 m; Table 4.1.5; Figure 4.1.2).

Table 4.1.2 Chondrichthyan species caught by geographical subarea (GSA) during the International Bottom Trawl Survey in the Mediterranean (MEDITS) performed in 2013 in the western Mediterranean. Unless indicated otherwise, data show the mean ±s.e.m. D, depth range of appearance; TL, total length. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain.

| GSA | Family | Species | D (m) | % appearance | Average abundance (ind/km$^2$) | Biomass (kg/km$^2$) | Mean (±s.d.) size (TL; cm) | | Number of samples | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Females | Males | Females | Males |
| GSA01 | Dalatiidae | *Dalatias licha* | 541–777 | 22 | 1.5 ±1.2 | 1.9 ±1.8 | 39.6 | 117.8 | 1 | 1 |
| | Etmopteridae | *Etmopterus spinax* | 375–813 | 88 | 89.0 ±30.0 | 10.6 ±4.2 | 29.0 ±0.5 | 26.1 ±0.4 | 159 | 148 |
| | Scyliorhinidae | *Galeus melastomus* | 320–813 | 100 | 1815.0 ±422.0 | 327.1 ±84.0 | 35.7 ±0.6 | 38.1 ±0.5 | 446 | 481 |
| | | *Galeus atlanticus* | 328–648 | 53 | 188.0 ±87.0 | 14.5 ±5.8 | 32.6 ±1.1 | 30.8 ±1.0 | 42 | 44 |
| | | *Scyliorhinus canicula* | 42–637 | 86 | 232.0 ±94.0 | 30.3 ±9.2 | 35.4 ±0.8 | 37.0 ±0.8 | 149 | 138 |
| | Triakidae | *Galeorhinus galeus* | 359–419 | 75 | 2.0 ±2.0 | 26.0 ±19.0 | 133.0 ±10.1 | 141.7 ±6.4 | 2 | 4 |
| | Centrophoridae | *Centrophorus granulosus* | 559 | – | 0.2 ±0.2 | 0.9 ±0.9 | | 82.0 | | 1 |
| | Rajidae | *Leucoraja circularis* | 359–559 | 20 | 0.5 ±0.3 | 0.3 ±0.2 | 43.5 | 42.0 | 1 | 1 |
| | | *Leucoraja naevus* | 246–343 | 50 | 7.8 ±5.2 | 2.8 ±1.9 | 42.0 ±4.9 | 42.4 ±5.4 | 14 | 9 |
| | | *Raja clavata* | 343 | – | 0.3 ±0.3 | 0.04 ±0.01 | | 30.5 | | 1 |
| | Torpedinidae | *Torpedo marmorata* | 65–241 | 43 | 4.7 ±2.2 | 1.5 ±0.7 | 21.6 ±1.8 | 27.4 | 6 | 1 |
| | | *Torpedo nobiliana* | 241–777 | 22 | 1.4 ±0.6 | 5.5 ±3.9 | | 22.6 ±2.4 | | 3 |
| | | *Torpedo torpedo* | 45 | – | 1.1 ±1.1 | 1.1 ±1.1 | 23.5 | 23.3 ±1.7 | 1 | 2 |
| | Chimaeridae | *Chimaera monstrosa* | 359–813 | 83 | 34.0 ±13.0 | 21.2 ±8.5 | 23.5 ±1.1 | 23.5 ±8.8 | 10 | 2 |
| GSA05 | Etmopteridae | *Etmopterus spinax* | 497–737 | 88 | 7.8 ±3.8 | 0.5 ±0.2 | 21.5 ±2.3 | 18.6 ±2.1 | 18 | 13 |
| | Scyliorhinidae | *Galeus melastomus* | 145–754 | 70 | 370.0 ±153.0 | 171.0 ±8.1 | 25.6 ±1.0 | 25.7 ±0.9 | 136 | 143 |
| | | *Scyliorhinus canicula* | 52–385 | 100 | 904.2 ±167.0 | 106.1 ±22.2 | 31.3 ±0.5 | 34.9 ±0.5 | 282 | 388 |
| | Squalidae | *Squalus blainvillei* | 173 | – | 0.4 ±0.4 | 0.02 ±0.01 | 22.1 | | 1 | |
| | Triakidae | *Mustelus mustelus* | 56–138 | 11 | 3.1 ±2.1 | 7.0 ±2.1 | | 46.0 | | 1 |
| | Dasyatidae | *Dasyatis pastinaca* | 58–70 | 25 | 5.1 ±4.0 | 2.9 ±2.5 | 44.1 ±3.5 | 41.1 ±1.8 | 2 | 5 |
| | Rajidae | *Dipturus oxyrinchus* | 134–497 | 32 | 2.7 ±1.5 | 9.3 ±4.5 | 85.0 ±13.1 | 80.3 ±6.4 | 5 | 6 |
| | | *Leucoraja circularis* | 134–497 | 16 | 2.2 ±1.6 | 1.5 ±1.2 | 61.5 ±8.6 | 40.6 ±2.6 | 3 | 4 |
| | | *Leucoraja naevus* | 52–249 | 26 | 8.7 ±3.5 | 3.4 ±1.3 | 41.6 ±3.6 | 36.6 ±2.2 | 7 | 13 |
| | | *Raja brachyura* | 56–63 | 56 | 6.0 ±3.1 | 19.0 ±12.0 | 65.0 ±10.0 | 72.0 ±11.0 | 6 | 3 |
| | | *Raja clavata* | 58–497 | 57 | 50.0 ±14.3 | 55.1 ±14.2 | 49.6 ±1.9 | 49.6 ±1.7 | 71 | 79 |
| | | *Raja miraletus* | 62–150 | 40 | 28.0 ±18.0 | 3.4 ±1.7 | 28.4 ±1.2 | 27.8 ±1.2 | 27 | 22 |
| | | *Raja montagui* | 57 | – | 0.6 ±0.6 | 0.5 ±0.5 | 49.5 | | 1 | |
| | | *Raja polystigma* | 56–414 | 44 | 19.6 ±5.4 | 3.2 ±1.4 | 29.7 ±1.1 | 28.3 ±1.5 | 18 | 25 |
| | | *Raja radula* | 58–80 | 29 | 4.6 ±2.8 | 2.0 ±1.0 | 37.8 ±1.3 | 40.6 ±2.5 | 2 | 5 |
| | | *Rostroraja alba* | 56 | – | 0.7 ±0.7 | 25.0 ±25.0 | 168.0 | | 1 | |
| | Torpedinidae | *Torpedo marmorata* | 134–252 | 25 | 0.8 ±0.5 | 0.3 ±0.2 | | 20.1 ±1.4 | | 3 |
| | Chimaeridae | *Chimaera monstrosa* | 497 | – | 0.2 ±0.2 | 0.003 ±0.001 | 6.8 | | 1 | |
| | Dalatiidae | *Dalatias licha* | 369–729 | 17 | 0.3 ±0.2 | 0.07 ±0.01 | 43.0 | 43.5 | 4 | 2 |
| | Etmopteridae | *Etmopterus spinax* | 369–729 | 54 | 2.7 ±1.4 | 0.2 ±0.1 | 21.6 ±2.6 | 16.5 ±2.1 | 10 | 7 |
| | Scyliorhinidae | *Galeus melastomus* | 291–729 | 94 | 163.1 ±54.0 | 20.3 ±7.0 | 32.6 ±1.0 | 30.9 ±0.9 | 210 | 213 |
| | | *Scyliorhinus canicula* | 47–729 | 88 | 509.0 ±108.2 | 81.0 ±15.0 | 33.9 ±0.5 | 37.0 ±0.5 | 393 | 433 |
| | Rajidae | *Leucoraja naevus* | 48 | – | 1.2 ±1.2 | 1.3 ±1.3 | 50.5 ±5.0 | 47.5 | 2 | 1 |
| | | *Raja asterias* | 41–247 | 13 | 4.6 ±2.2 | 2.4 ±1.3 | 35.6 ±7.4 | 56.9 ±0.2 | 5 | 3 |
| | | *Raja clavata* | 127–155 | 44 | 1.2 ±0.6 | 2.3 ±1.4 | 57.1 ±11.0 | | 3 | |
| | | *Raja montagui* | 180 | – | 0.4 ±0.4 | 0.2 ±0.2 | | 63.0 | | 1 |
| | Torpedinidae | *Torpedo marmorata* | 41–135 | 26 | 4.3 ±1.6 | 1.5 ±0.6 | 23.2 ±1.9 | 22.6 ±2.2 | 8 | 7 |
| | Chimaeridae | *Chimaera monstrosa* | 404–729 | 20 | 0.6 ±0.4 | 0.6 ±0.4 | 6.3 ±2.5 | 9.2 | 2 | 1 |

Table 4.1.3. Length–weight relationship, sex ratio, smallest mature individual (based on total length), length at which 50% of fish had become mature ($L_{50}$) and maturity parameters for *Scyliorhinus canicula* (Sc), *Galeus melastomus* (Gm), *Etmopterus spinax* (Es) and *Raja clavata* (Rc) according to geographical subarea (GSA). The number of specimens analysed (n) and the results of the tests used for comparisons (χ2 and F) are also shown. $^*P < 0.05$. See Materials and Methods for more details regarding maturity and length–weight equations. NS, not significant; GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain; F, female; M, male; a and b, fitting constants for both parameters.

| **Species** | **GSA** | *n* | | **Length–weight** | | | | **R2** | | *F* | **Sex ratio** | | **SM (cm)** | | **$L_{50}$ (cm)** | | **Maturity** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *a* | | *b* | | | | | | | | | | | *a* | | *b* | | *R2* | | *χ2* |
| | | F | M | F | M | F | M | F | M | *P* | F : M | P | F | M | F | M | F | M | F | M | F | M | *P* |
| Sc | GSA01 | 149 | 138 | 0.0004 | 0.0017 | 3.58 | 3.13 | 0.98 | 0.98 | * | 1 : 1 | NS | 40 | 37 | 42.2 | 39.3 | –22.5 | –25.1 | 0.53 | 0.63 | 0.98 | 0.98 | NS |
| | GSA05 | 282 | 388 | 0.0007 | 0.0019 | 3.44 | 3.14 | 0.98 | 0.98 | * | 0.7 : 1 | * | 37 | 36 | 40.6 | 39.2 | –23.4 | –24.1 | 0.57 | 0.61 | 0.99 | 0.98 | NS |
| | GSA06 | 393 | 433 | 0.0013 | 0.0030 | 3.25 | 3.00 | 0.98 | 0.98 | * | 1 : 1 | NS | 36 | 34 | 40.9 | 39.6 | –26.0 | –17.9 | 0.64 | 0.45 | 0.98 | 0.99 | NS |
| | All | 824 | 959 | 0.0009 | 0.0025 | 3.35 | 3.05 | 0.97 | 0.96 | * | 0.8 : 1 | * | 36 | 34 | 41.1 | 39.4 | –23.5 | –20.8 | 0.57 | 0.53 | 0.99 | 0.98 | NS |
| Gm | GSA01 | 446 | 481 | 0.0024 | 0.0050 | 3.02 | 2.83 | 0.99 | 0.99 | * | 1 : 1 | NS | 51 | 46 | 54.1 | 47.2 | –25.9 | –35.8 | 0.48 | 0.76 | 0.98 | 0.99 | * |
| | GSA05 | 136 | 143 | 0.0049 | 0.0044 | 2.86 | 2.89 | 0.99 | 0.99 | NS | 1 : 1 | NS | 51 | 48 | 52.1 | 49.9 | –50.9 | –31.3 | 0.98 | 0.62 | 0.83 | 0.86 | NS |
| | GSA06 | 210 | 214 | 0.0035 | 0.0064 | 2.95 | 2.79 | 0.99 | 0.99 | * | 1 : 1 | NS | 47 | 46 | 52.8 | 49.3 | –34.5 | –39.7 | 0.65 | 0.80 | 0.98 | 0.96 | * |
| | All | 792 | 838 | 0.0026 | 0.0050 | 3.02 | 2.84 | 0.98 | 0.98 | NS | 1 : 1 | NS | 47 | 46 | 53.3 | 47.5 | –29.0 | –32.6 | 0.54 | 0.68 | 0.99 | 0.99 | * |
| Es | GSA01 | 159 | 148 | 0.0101 | 0.0295 | 2.76 | 2.42 | 0.98 | 0.97 | NS | 1 : 1 | NS | 28 | 27 | 36.4 | 29.1 | –18.7 | –33.4 | 0.05 | 0.11 | 0.90 | 0.97 | * |
| Rc | GSA05 | 71 | 79 | 0.0027 | 0.0051 | 3.21 | 3.05 | 0.99 | 0.98 | * | 1 : 1 | NS | 75 | 49 | 78.1 | 68.6 | –21.3 | –13.1 | 0.03 | 0.02 | 0.98 | 0.88 | * |

### *Galeus melastomus*

No differences were recorded for LWp between sexes for all GSAs (F = 0.4; $P > 0.05$; Table 4.1.3) but differences were observed between geographical areas (Table 4.1.4). The sex ratio was not significantly different from 1 : 1 in all GSAs (Table 4.1.3). The Mp by sex did not show any differences between GSAs, so Mp values were estimated combined for all GSAs (Table 4.1.4). Differences were detected between sexes ($\chi^2 = 341.52$; $P < 0.05$), with $L_{50}$ values of 53.2 cm for females ($R^2 = 0.99$) and 47.5 cm for males ($R^2 = 0.99$). The optimum depth was greater in GSA01 (686 m), followed by GS06 (549 m) and GSA05 (483 m; Table 4.1.5; Figure 4.1.2).

Table 4.1.4. Comparison of length–weight relationships and length at first maturity ($L_{50}$) parameters for *Scyliorhinus canicula* (Sc) and *Galeus melastomus* (Gm) by each geographical subarea (GSA). The results of tests used for comparisons ($\chi^2$ and F) are also shown. *$P < 0.05$. See Materials and Methods for more details concerning maturity and length-weight equations. NS, not significant; GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain; F, female; M, male.

| Comp | Length–weight | | | | | | | | $L_{50}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | | | | P | | | | χ2 | | | | P | | | |
| | Gm | | Sc | | Gm | | Sc | | Gm | | Sc | | Gm | | Sc | |
| | F | M | F | M | F | M | F | M | F | *M* | F | M | F | M | F | M |
| GSA01 vs GSA05 | 4.5 | 7.5 | 2.6 | 6.5 | NS | * | NS | * | –41.6 | –66.0 | –114.8 | –28.4 | NS | NS | NS | NS |
| GSA01 vs GSA06 | 3.0 | 0.9 | 7.1 | 3.3 | NS | NS | * | NS | –39.4 | –116.5 | –41.7 | –21.3 | NS | NS | NS | NS |
| GSA05 vs GSA06 | 12.8 | 8.3 | 1.6 | 41.6 | * | * | NS | * | –39.4 | –2.9 | –10.9 | –30.2 | NS | NS | NS | NS |

### *Etmopterus spinax*

For specimens distributed in GSA01, no differences were observed between sexes for LWp (F = 14.76; $P > 0.05$; Table 4.1.3). However, differences were observed for Mp ($\chi^2 = 98.97$; $P < 0.05$), with $L_{50}$ values of 36.4 cm for females ($R^2 = 0.90$) and 29.1 cm for males ($R^2 = 0.97$). The sex ratio was not different from 1 : 1 ($\chi^2 = 15.05$; $P > 0.05$). The bathymetric distribution was the same as *G. melastomus*, with greater depths in GSA01 (832 m), followed by GSA06 (611 m) and GSA05 (558 m; Table 4.1.5; Figure 4.1.2).

### *Raja clavata*

For specimens captured in GSA05, differences between females and males were detected in WLp (F = 0.03; $P < 0.05$; Table 4.1.3) and Mp ($\chi^2 = 8.81$; $P < 0.05$), with $L_{50}$ values of 78.1 cm for females ($R^2 = 0.98$) and 68.6 cm for males ($R^2 = 0.88$). The sex ratio did not differ from 1 : 1 ($\chi^2 = 6.21$; $P > 0.05$), and the optimum depth was recorded at 224 m (Table 4.1.5; Figure 4.1.2).

### *Length–frequency distribution*

The LFd by depth showed clear differences in all GSAs for *S. canicula* (Figure 4.1.3) and *G. melastomus* (Figure 4.1.3), and for *G. atlanticus* and *E. spinax* in GSA01 (Figure 4.1.5). In contrast, no bathymetric differences were observed in the LFd of *R. clavata* in GSA05 (Figure 4.1.6). For *S. canicula* (Figure 4.1.3), individuals <30 cm were more frequent in two depth strata (<100 and 300–600 m) in GSA01 and GSA06, whereas in GSA05 the larger specimens were more frequently found only in waters shallower than 100 m (Figure 4.1.3). Individuals <30 cm were distributed mainly between 100 and 400 m in all GSAs (Figure 4.1.3). For *G. melastomus* (Figure 4.1.4), the bathymetric distribution was similar for both sexes in all GSAs. Individuals <30 cm were more common between 150 and 500 m for GSA01 and GSA06, whereas in GSA05 these specimens were frequently found down to a depth of 700 m. Individuals ≥30 cm predominated between 500 and 800 m in GSA01 and GSA06, whereas these specimens were common in GSA05 only at 700–800 m. Differences in the LFd were observed between sexes in all GSAs ($P < 0.001$). In GSA01, similar bathymetric patterns between juveniles and adult individuals were obtained for *E. spinax* and *G. atlanticus* (Figure 4.1.5). In GSA01, *E. spinax* individuals ≤20 cm were distributed almost exclusively from 350 to 600 m, whereas individuals ≥30 cm predominated between 600 and 850 m. A similar depth pattern was obtained for *G. atlanticus*, with specimens ≤20 cm commonly found from 400 to 500 m and individuals ≥30 cm found almost exclusively between 500 and 650 m. In GSA05, the length of *R. clavata* showed a similar bathymetric distribution of individuals ≤40 and ≥50 cm (Figure 4.1.6). The LFd recorded for *R. miraletus* and *R. polystigma* showed a unimodal distribution, with individuals ≤30 cm predominating (Figure 4.1.6).

Table 4.1.5. Results of the bathymetric distribution models for the most frequent chondrichthyan species captured in each geographical subarea (GSA). The type of best fit is indicated and, in the case of a unimodal response, the optimum depth (Opt. $R^{-2}$) is also shown. Statistical significance: *$P < 0.05$; **$P < 0.001$. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain.

| **Species** | **GSA01** | | | **GSA05** | | | **GSA06** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Response** | **Opt. $R^{-2}$ (m)** | ***P*** | **Response** | **Opt. $R^{-2}$ (m)** | ***P*** | **Response** | **Opt. $R^{-2}$ (m)** | ***P*** |
| *Chimaera monstrosa* | Unimodal | 622 | ** | | | | | | |
| *Dipturus oxyrinchus* | | | | Unimodal | 294 | ** | | | |
| *Etmopterus spinax* | Unimodal | 832 | ** | Unimodal | 558 | ** | Unimodal | 611 | ** |
| *Galeus atlanticus* | Unimodal | 424 | ** | | | | | | |
| *Galeus melastomus* | Unimodal | 686 | ** | Unimodal | 483 | ** | Unimodal | 549 | ** |
| *Leucoraja naevus* | | | | Unimodal | 164 | * | | | |
| *Raja clavata* | | | | Unimodal | 224 | ** | | | |
| *Raja miraletis* | | | | Unimodal | 81 | * | | | |
| *Raja polystigma* | | | | Unimodal | 178 | ** | | | |
| *Scyliorhinus canicula* | Unimodal | 250 | * | Unimodal | 163 | ** | Unimodal | 242 | * |
| *Torpedo marmorata* | | | | | | | Unimodal | 70 | ** |

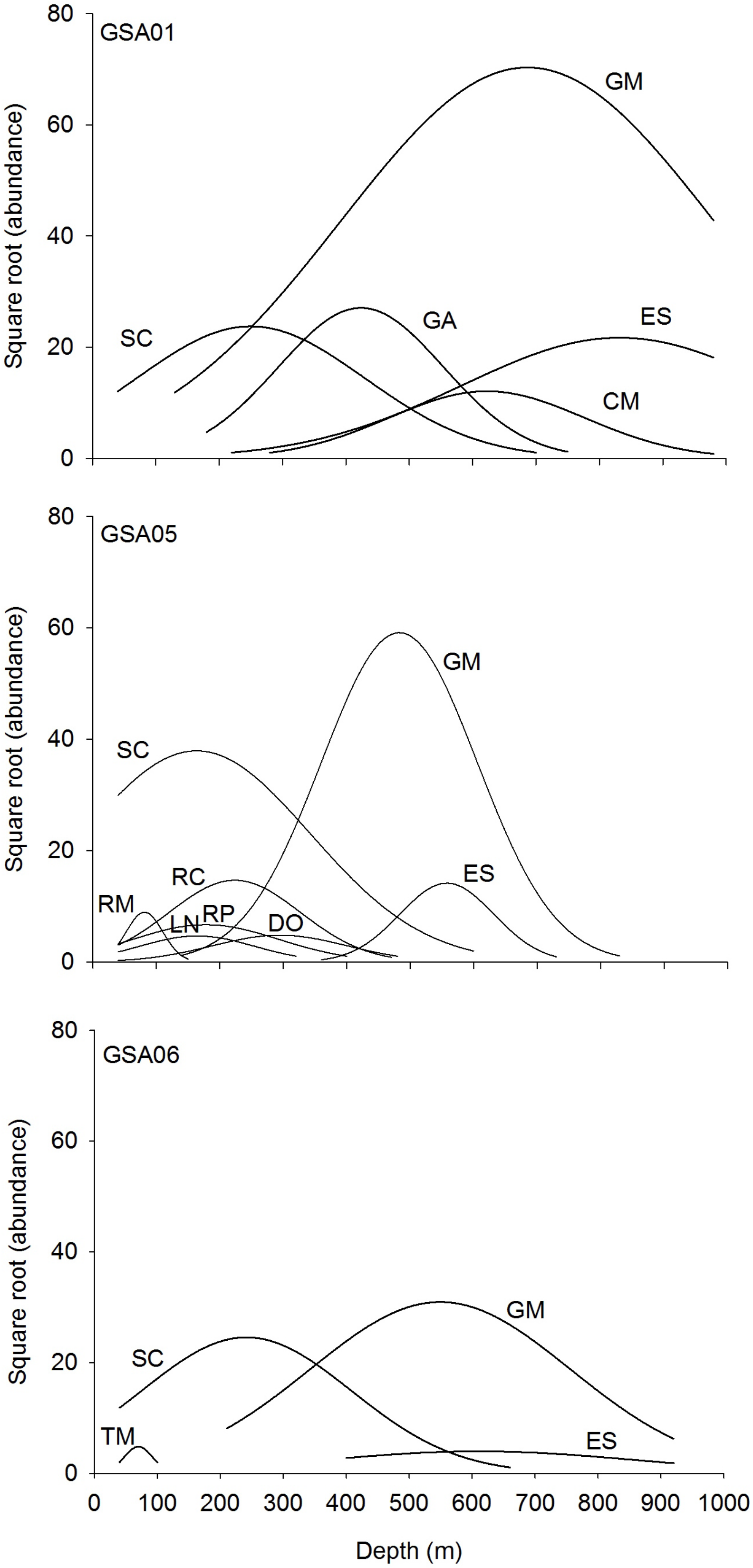


Figure 4.1.2. Bathymetric distribution models for the most abundant chondrichthyan species according to geographical subarea. (GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain. CH, *Chimaera monstrosa*; DO, *Dipturus oxyrinchus*; ES, *Etmopterus spinax*; GA, *Galeus atlanticus*; GM, *Galeus melastomus*; LN, *Leucoraja naevus*; RC, *Raja clavata*; RM; *Raja miraletus*; RP, *Raja polystigma*; SC, *Scyliorhinus canicula*; TM, *Torpedo marmorata*.

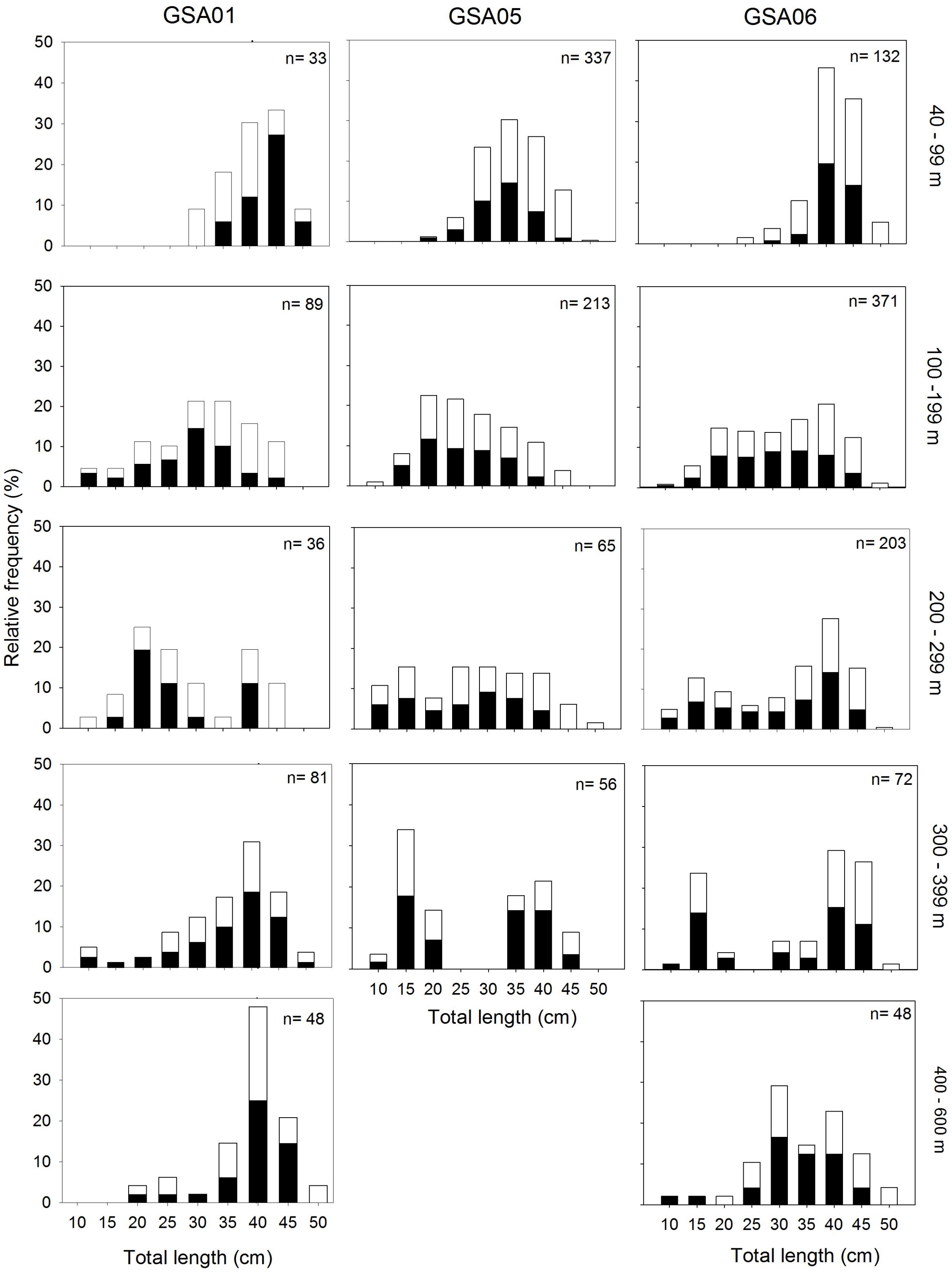


Figure 4.1.3. Length–frequency distributions (percentage by 5 cm size classes) of *Scyliorhinus canicula* captured in each geographical subarea according to sex and depth interval. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain. Closed squares: females; open squares: males. The number of individuals measured is also indicated (n).

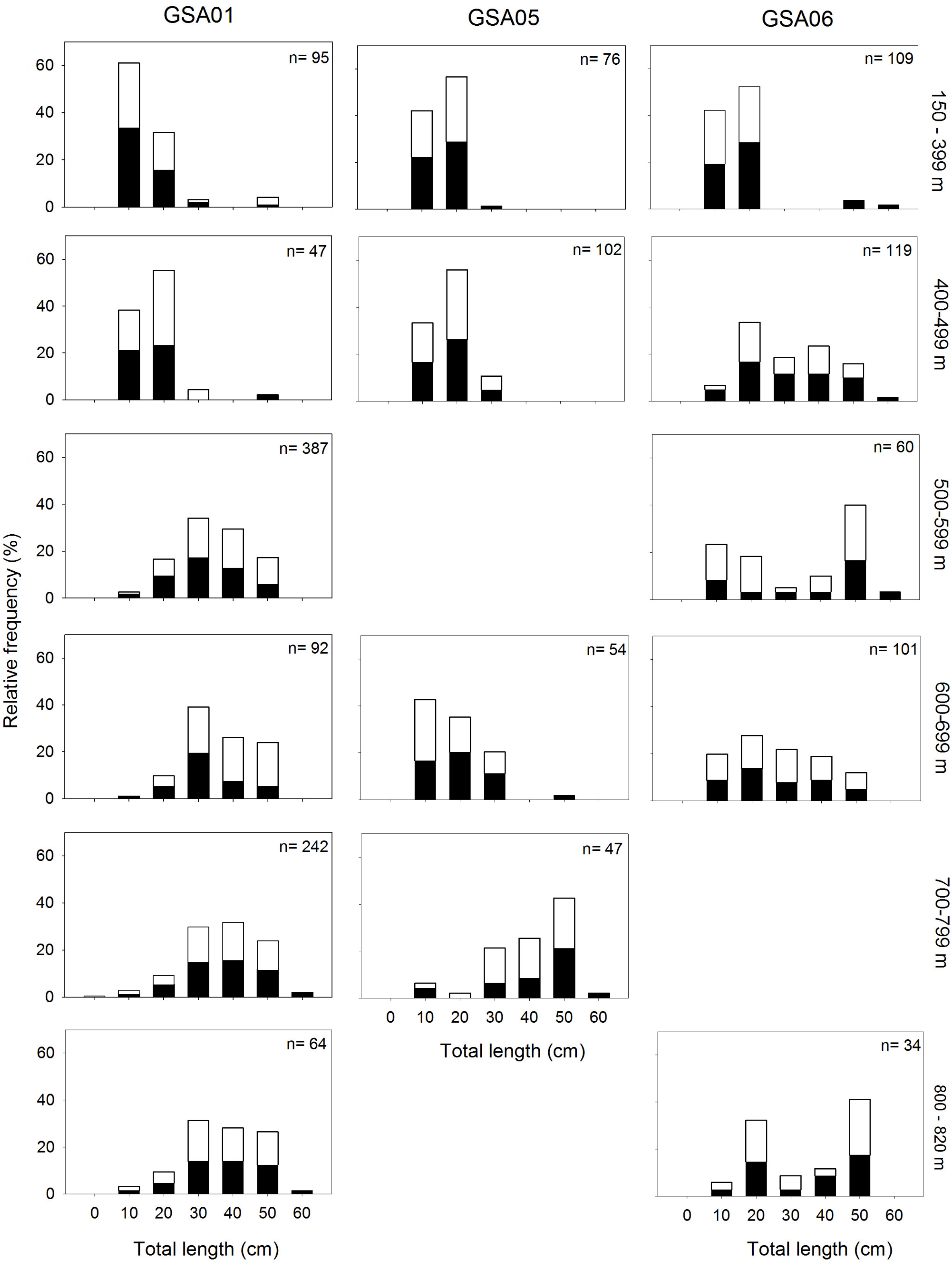


Figure 4.1.4. Length–frequency distributions (percentage by 10 cm size classes) of *Galeus melastomus* captured in each geographical subarea according to sex and depth interval. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain. Closed squares: females; open squares: males. The number of individuals measured is also indicated (n).

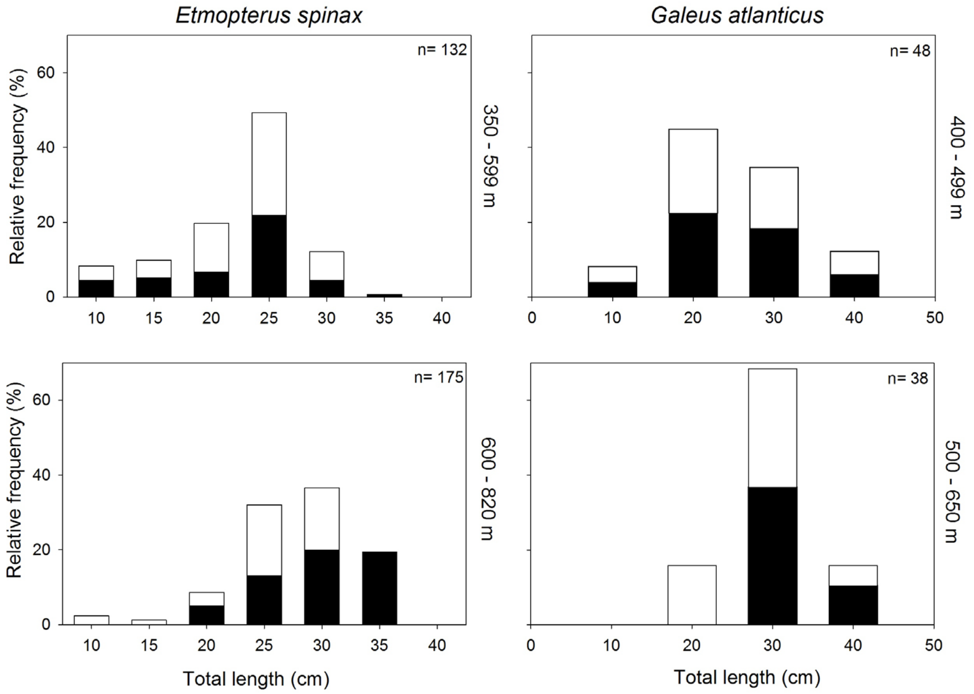


Figure 4.1.5. Length–frequency distribution (percentage by 5 and 10 cm size classes) according to sex and depth interval for *Etmopterus spinax* and *Galeus atlanticus* captured in GSA01 (northern Alboran and Alboran Island). Closed squares: females; open squares: males. The number of individuals measured is also indicated (n).

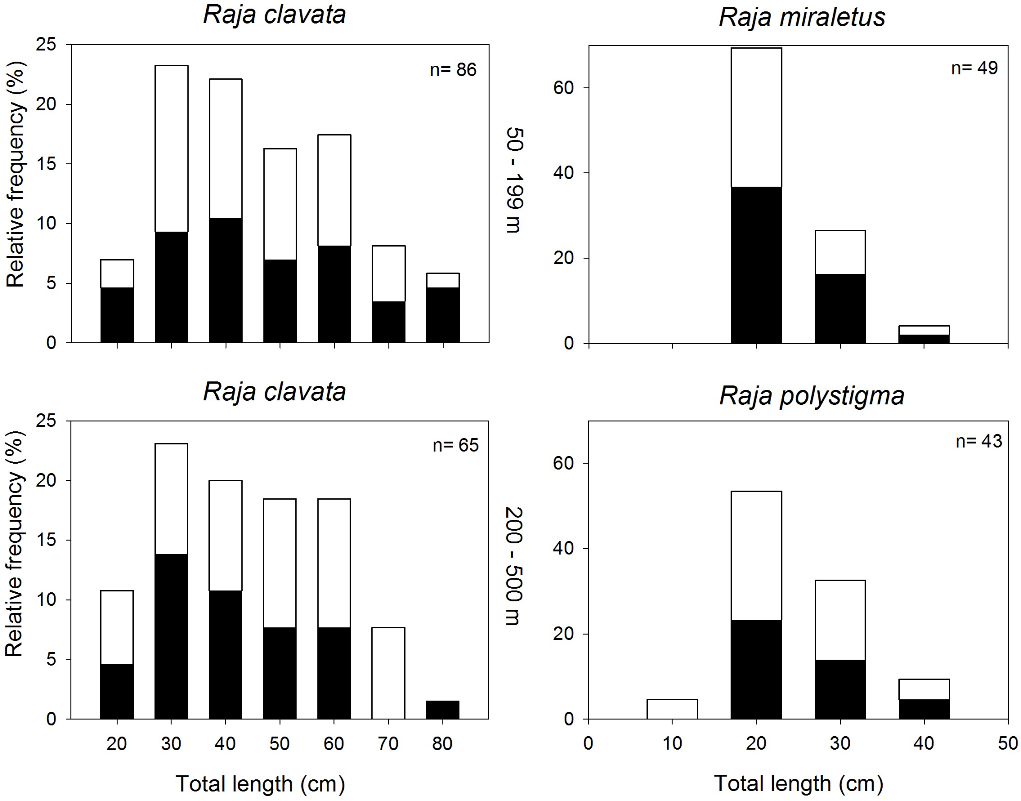


Figure 4.1.6. Length–frequency distribution (percentage by 10 cm size classes) according to sex for *Raja clavata*, *Raja miraletus* and *Raja polystigma* captured in GSA05 (Balearic Islands) and depth interval only for *R. clavata*. Closed squares: females; open squares: males. The number of individuals measured is also indicated (n).

Table 4.1.6. Similarity percentage analysis (SIMPER) results for each chondrichthyan assemblage (CS, continental shelf; S, slope) identified by geographical subarea (GSA) from cluster and multidimensional scaling analyses. Ȳ, mean yields; Ŝi, average similarity; %Ŝi, percentage contribution to similarity; GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain.

| | **Abundance (individuals $\text{km}^{-2}$)** | | | | | | **Biomass (kg $\text{km}^{-2}$)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Ȳ** | **Ŝi** | **Ŝi/s.d.** | **%Ŝi** | **Σ%Ŝi** | | **Ȳ** | **Ŝi** | **Ŝi/s.d.** | **%Ŝi** | **Σ%Ŝi** |
| **GSA01** | | | | | | | | | | | |
| *CS (Ŝi = 38.5)* | | | | | | *CS (Ŝi = 27.0)* | | | | | |
| *S. canicula* | 278 | 37 | 1.7 | 95 | 95 | *S. canicula* | 35 | 24 | 1.3 | 90 | 90 |
| *T. marmorata* | 7.8 | 1.8 | 0.4 | 4.8 | 100 | *T. marmorata* | 2.5 | 2.6 | 0.5 | 9.5 | 100 |
| *S (Ŝi = 47.4)* | | | | | | *S (Ŝi = 31.3)* | | | | | |
| *G. melastomus* | 3166 | 43 | 1.6 | 92 | 92 | *G. melastomus* | 624 | 28 | 0.9 | 88 | 88 |
| *E. spinax* | 185 | 1.5 | 0.7 | 3.1 | 95 | *S. canicula* | 20 | 1.1 | 0.3 | 3.4 | 92 |
| *G. atlanticus* | 216 | 1.5 | 0.4 | 3.1 | 98 | *C. monstrosa* | 34 | 0.8 | 0.5 | 2.5 | 94 |
| *S. canicula* | 170 | 0.6 | 0.4 | 1.2 | 99 | *G. atlanticus* | 18 | 0.6 | 0.3 | 1.9 | 96 |
| | | | | | | *E. spinax* | 23 | 0.6 | 0.5 | 1.8 | 98 |
| **GSA05** | | | | | | | | | | | |
| *CS (Ŝi = 74.2)* | | | | | | *CS (Ŝi = 52.2)* | | | | | |
| *S. canicula* | 1244 | 69 | 3.8 | 94 | 94 | *S. canicula* | 114 | 30 | 3.6 | 57 | 57 |
| *R. clavata* | 99 | 3.1 | 1.2 | 4.1 | 98 | *R. clavata* | 97 | 20 | 1.4 | 38 | 95 |
| *R. polystigma* | 31 | 0.9 | 0.9 | 1.2 | 99 | *D. oxyrinchus* | 18 | 0.8 | 0.3 | 1.5 | 97 |
| | | | | | | *R. polystigma* | 3.6 | 0.7 | 0.9 | 1.4 | 98 |
| *S (Ŝi = 29.1)* | | | | | | *S (Ŝi = 25.6)* | | | | | |
| *G. melastomus* | 1330 | 27 | 1.1 | 94 | 94 | *G. melastomus* | 67 | 22 | 1.1 | 87 | 87 |
| *E. spinax* | 31 | 1.5 | 0.4 | 5.1 | 99 | *E. spinax* | 1.8 | 2.1 | 0.4 | 8.1 | 95 |
| *S. canicula* | 100 | 0.3 | 0.1 | 1.0 | 100 | *R. clavata* | 7.0 | 0.4 | 0.2 | 1.7 | 97 |
| | | | | | | *S. canicula* | 6.9 | 0.4 | 0.1 | 1.4 | 99 |
| **GSA06** | | | | | | | | | | | |
| *CS (Ŝi = 42.2)* | | | | | | *CS (Ŝi = 43.7)* | | | | | |
| *S. canicula* | 1239 | 42 | 1.3 | 99 | 99 | *S. canicula* | 195 | 3.1 | 1.5 | 98 | 98 |
| *R. asterias* | 23 | 0.2 | 0.4 | 0.4 | 100 | *T. marmorata* | 5.7 | 0.3 | 0.2 | 0.7 | 99 |
| | | | | | | *R. asterias* | 11 | 0.3 | 0.3 | 0.6 | 100 |
| *S (Ŝi = 43.6)* | | | | | | *S (Ŝi = 34.4)* | | | | | |
| *G. melastomus* | 534 | 39 | 1.5 | 89 | 89 | *G. melastomus* | 76 | 28 | 1.1 | 80 | 80 |
| *S. canicula* | 179 | 4.3 | 0.6 | 9.8 | 99 | *S. canicula* | 31 | 6.5 | 0.6 | 19 | 99 |
| *E. spinax* | 10 | 0.5 | 0.5 | 1.1 | 100 | *E. spinax* | 0.7 | 0.2 | 0.6 | 0.7 | 100 |

### *Assemblages and community descriptors*

The MDS and cluster analyses (40% similarity) revealed the existence of two bathymetric assemblages in all GSAs (Figure 4.1.7) with similar depth intervals: (1) continental shelf (CS) at 42–246, 52–252 and 41–247 m for GSA01, GSA05 and GSA06 respectively; and (2) slope at 320–813, 356–754 and 284–729 m for GSA01, GSA05 and GSA06 respectively. SIMPER analysis showed that *S. canicula* and *G. melastomus* were the most characteristic species for CS and slope respectively (Table 4.1.6). For all GSAs, *S. canicula* contributed >90% similarity within CS assemblages in terms of abundance. The same results were obtained in terms of biomass, except in GSA05, where this species only represented 50% similarity. In all GSAs, *G.*

*melastomus* contributed >80% similarity within slope assemblages, both in terms of abundance and biomass. For all the assemblages, ANOSIM showed significant differences of dissimilarity between GSAs in terms of both abundance and biomass (Table 4.1.7).

Table 4.1.7. Analysis of similitude (ANOSIM) results for analysing differences between chondrichthyan assemblages (CS, continental shelf; S, slope) identified by geographical subarea (GSA) from cluster and multidimensional scaling analyses. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. A, standardised abundance; B, standardised biomass; GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands.

| **Pair-wise comparison** | **A individuals/km$^2$** | | **B kg/km$^2$** | |
|---|---|---|---|---|
| | **$R^2$** | *P* | **$R^2$** | *P* |
| CSGSA01 v. SGSA01 | 0.62 | *** | 0.71 | *** |
| CSGSA05 v. SGSA05 | 0.86 | *** | 0.74 | *** |
| CSGSA06 v. SGSA06 | 0.70 | *** | 0.25 | ** |
| CSGSA01 v. CSGSA05 | 0.36 | ** | 0.37 | ** |
| CSGSA01 v. CSGSA06 | 0.75 | *** | 0.10 | NS |
| CSGSA05 v. CSGSA06 | 0.99 | *** | 0.20 | ** |
| SGSA01 v. SGSA05 | 0.07 | NS | 0.40 | *** |
| SGSA01 v. SGSA06 | 0.09 | * | 0.61 | *** |
| SGSA05 v. SGSA06 | 0.10 | NS | 0.73 | *** |

The ecological parameters showed differences between assemblages in each GSA (Table 4.1.8). The highest values of mean species richness were obtained in GSA05 for CS (mean ±sd, 6.4 ±0.8) and GSA01 for slope (4.3 ±0.6). For the rest of the assemblages and GSAs, the values of this parameter ranged between 2.3 and 2.7. The diversity of CS assemblages was similar in GSA01 and GSA05 (0.45 and 0.53 respectively), but lower in GSA06 (0.21 ±0.10). For slope assemblages, this index was similar in GSA05 and GSA06 (0.33–0.39), but higher in GSA01 (0.52 ±0.10). The mean abundance of CS assemblages was higher in GSA06 (215.5 ±101.0 individuals km$^{-2}$), followed by GSA01 and GSA05 (129.1 ±50.0 and 89.6 ±28.0 individuals km$^{-2}$ respectively). In slope assemblages, the highest value was estimated in GSA01 (382.9 ±117.0 individuals km$^{-2}$), followed by GSA05 and GSA06 (183.9 ±85.0 and 145.6 ±42.0 individuals km$^{-2}$ respectively). The mean biomass was different between all GSAs and showed high values in the CS assemblage of GSA06 (36.5±17.0 kg km$^{-2}$), whereas in the other GSAs it ranged between 7.7±2.6 and 17.6 kg km$^{-2}$ for GSA01 and GSA05 respectively. In slope assemblage, the highest value was in GSA01 (89.8 ±27.0 kg km$^{-2}$), whereas in the other GSAs it ranged between 11.8 ±4.6 and 22.3 ±8.0 kg km$^{-2}$ in GSA05 and GSA06 respectively.

Table 4.1.8. Mean (±s.e.m.) values of community descriptors by assemblage (CS, continental shelf; S, slope) identified by geographical subarea (GSA) from cluster and multidimensional scaling analyses. The number of hauls and the total number of species by assemblage are also shown. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain.

| **Assemblage** | **GSA01** | | **GSA05** | | **GSA06** | |
|---|---|---|---|---|---|---|
| | ***CS*** | ***S*** | ***CS*** | ***S*** | ***CS*** | ***S*** |
| Hauls | 24 | 18 | 39 | 15 | 47 | 21 |
| Species richness | 2.7 ±0.5 | 4.3 ±0.6 | 6.4 ±0.9 | 2.6 ±0.4 | 2.3 ±0.3 | 2.7 ±0.3 |
| Abundance (individuals km$^{-2}$) | 129 ±49 | 383 ±117 | 90 ±28 | 184 ±85 | 216 ±100 | 146 ±42 |
| Biomass (kg km$^{-2}$) | 7.7 ±2.6 | 90 ±27 | 17.6 ±3.9 | 11.8 ±4.6 | 37 ±17 | 22 ±8 |
| Nº species | 5 | 11 | 16 | 8 | 6 | 5 |
| Diversity (*H*’) | 0.5 ±0.1 | 0.5 ±0.1 | 0.5 ±0.0 | 0.3 ±0.1 | 0.2 ±0.1 | 0.4 ±0.1 |
| Evenness (*J*’) | 0.5 ±0.1 | 0.4 ±0.1 | 0.3 ±0.0 | 0.4 ±0.1 | 0.3 ±0.1 | 0.4 ±0.1 |

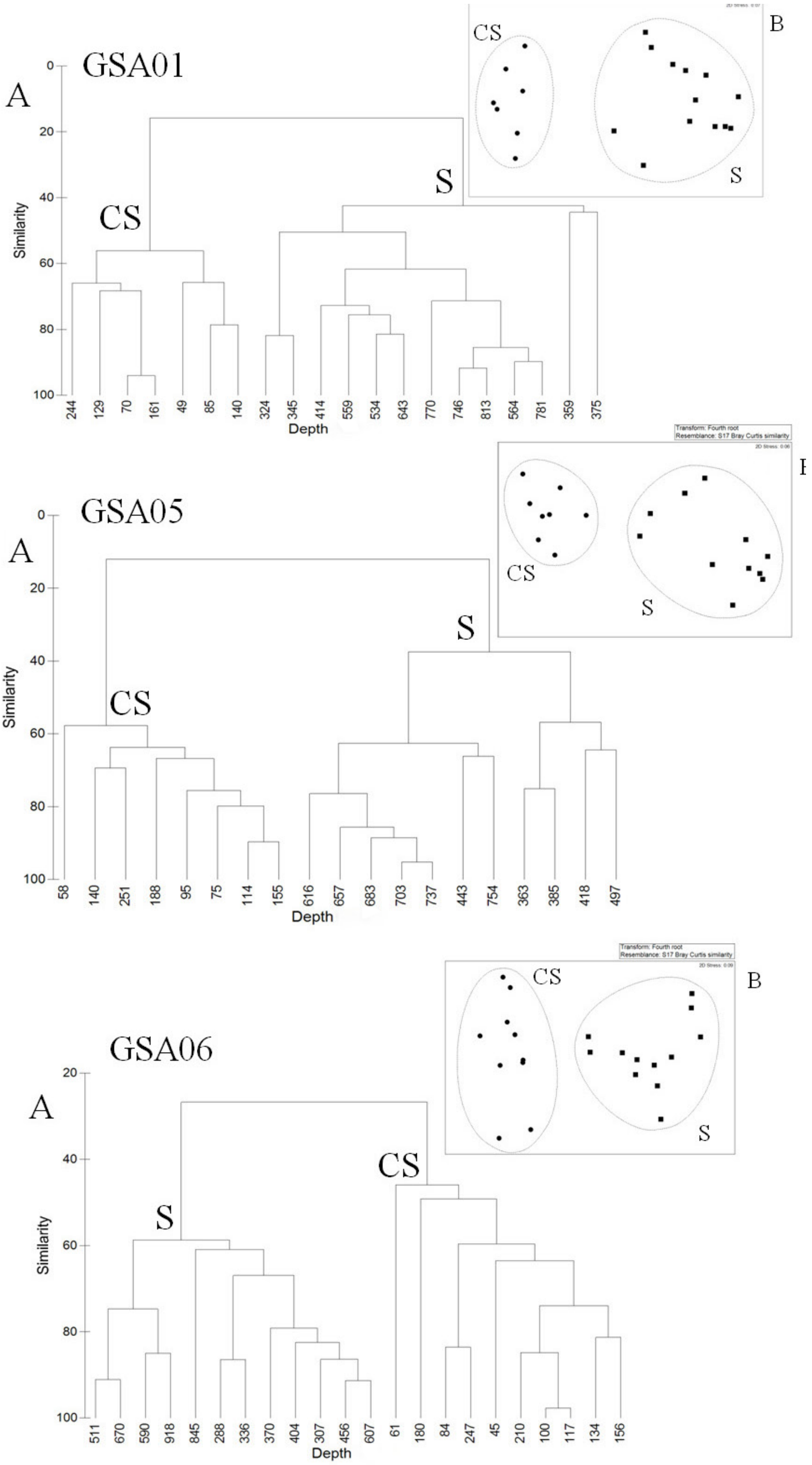


Figure 4.1.7. Dendrograms and multidimensional scaling ordination (insets), performed with PRIMER V.6, showing the chondrichthyan assemblages identified from cluster analysis according to geographical subarea. GSA01, northern Alboran and Alboran Island; GSA05, Balearic Islands; GSA06, northern Spain; CS, continental shelf (closed circles); S, slope (closed squares). Samples are labelled with their mean depths (m).

### 4.1.4 Discussion

Chondrichthyan communities in the circa-littoral zone and on bathyal soft bottoms throughout the western Mediterranean exhibited a high diversity, with a total of 25 species (9 shark, 15 rays and one chimaera). Other areas of the Mediterranean surveyed with the same MEDITS protocol, close to the mainland, showed lower species richness, even considering the higher number of samples analysed, such as for the Gulf of Lions (23 species in 325 hauls; Bertrand *et al*., 2000), Tyrrhenian (21 species in 516 hauls; Biagi *et al*., 2002) and the Adriatic (16 species in 224 hauls; Ungaro *et al*. 1999). In contrast, insular areas have shown similar or higher diversity, such as for Corsica, Sardinia and Sicily (26, 24 and 29 species in 120, 625 and 705 hauls respectively; Bertrand *et al*., 2000) and Malta (26 species in 135 hauls; Dimech *et al*., 2008). This diversity pattern was reflected in our study areas, where the species richness of demersal chondrichthyans was higher in GSA05 (18 species; Balearic Islands), followed by GSA01 (14 species; south-eastern Iberian Peninsula and Alboran Island) and GSA06 (10 species; eastern and north-eastern Iberian Peninsula). This is in agreement with the suggestion by Massutí and Moranta (2003) that demersal elasmobranch diversity in the Balearic Islands is higher than in the adjacent waters off the Iberian Peninsula and similar to other insular areas of the western Mediterranean.

The analysis of species composition detected two assemblages related to depth, with almost similar depth ranges between the three GSAs in the western Mediterranean: (1) the CS between 41 and 252 m; and (2) slope between 284 and 813 m. Depth is generally considered as the most environmental gradient in the sea and has been shown to be the main factor determining the distribution of marine fauna (e.g. Gage and Tyler, 1991). In the Mediterranean, depth has also been reported as the main factor affecting the associations of demersal fish. Similarly, in our study area, the similar bathymetric segregation of demersal assemblages has been reported for the main taxonomic groups of fauna (cephalopods, fish and decapod crustaceans; e.g. Demestre *et al*., 2000; Quetglas *et al*., 2000; Abelló *et al*., 2002; Moranta *et al*., 2008), as well as all the demersal species (García-Rodríguez *et al*., 2011) and even only for the commercial ones (Massutí and Reñones, 2005).

Regional demersal chondrichthyan diversity throughout the western Mediterranean showed clear geographic and depth variations. The highest diversity was detected on the CS of the Balearic Islands, with values double those estimated in GSA01 and GSA06. The high richness reported in GSA05 was because of the presence of many skates (up to 10 species). This could be explained by the heterogeneity of the bottoms, their sedimentation and the widespread presence of benthic habitats. The seabed has been considered as an important factor that enhances the diversity of demersal fish species, both in the Atlantic (Rogers *et al*., 1999) and the Mediterranean (Demestre *et al*., 2000). The sediments in the Balearic Islands area are mainly biogenic sands and gravels, which seem to be more favourable for the presence of rays and skate species than muddy bottoms (Ordines *et al*., 2011). It has been confirmed by Pennino *et al*. (2013), who modelled the importance of the type of substratum for the patterns of elasmobranch spatial distribution in the western Mediterranean, that these species prefer hard and sandy substrates, whereas muddy negative bottoms affect their presence. This preference has also been documented by Skjæraasen and Bergstad (2000) in the north-eastern Atlantic and is consistent with studies on habitat utilisation by some species of the genus in this area, such as *Raja* and *Scyliorhinus* (Hunter *et al*., 2005; Rodríguez-Cabello, 2008; Vaz *et al*., 2008). In contrast with GSA05, GSA01 and GSA06 receive river runoffs and have terrigenous muddy bottoms that predominate in offshore areas of the CS (Demestre *et al*., 2000; García-Rodríguez *et al*., 2011; Baro *et al*., 2012).

Conversely, the diversity of demersal chondrichthyans was lower on the slope assemblages, with the highest values in GSA01, followed by GSA05 and GSA06 (Table 4.1.8). The Alboran Sea is a transition zone between the Atlantic and the western Mediterranean, where some Atlantic species, such as *G. atlanticus* are present (Abad *et al*., 2007). Moreover, the presence of numerous submarine canyons in this area provides an environmental scenario favourable to the enrichment of deep-water ecosystems (Baro *et al*., 2012). The abundance and diversity of marine life is enhanced by canyons through their effect on the circulation of water masses because of the faster currents caused by the funnelling transport of sediment from the CS to the slope bottoms, as well as by providing more varied and complex physical habitats because of their topographic complexity, including the occurrence of rocky outcrops (Marques *et al*., 2005; Würtz, 2012).

Fishing is another factor that can play a central role in the distribution and abundance of vulnerable species such as chondrichthyans. It has been reported that fishing exploitation affects their composition and biodiversity to a greater extent than most other megafaunal species, such as teleosts (Stevens *et al*., 2000). The present study included GSAs with different fishing scenarios. If bottom trawl management rules can be considered very similar throughout the area (e.g. vessels are required to return daily to their home port, with a maximum activity of 12 h per day), the number of bottom trawlers and their gross tonnage, as a proxy of fishing capacity, per potential fishing ground surface covered in the MEDITS surveys can be considered as a simple indicator of the trawl fishing effort exerted (Quetglas *et al*., 2012). From this point of view, GSA06 and GSA05 are the areas with the highest and lowest levels of exploitation respectively, with the density of trawlers around the Balearic Islands being one order of magnitude lower than off the eastern and north-eastern Iberian Peninsula. Historically, the number of trawl fishing vessels has remained very high in GSA06, currently 567 trawlers, followed by GSA01 with 141 trawlers and, finally, GSA05 with only 49 trawlers.

This distinct fishing effort can explain the differences in species richness and diversity of chondrichthyan assemblages detected in the western Mediterranean. On the CS off the Balearic Islands, in addition to a reduced effort compared with the Spanish Peninsula, there are several areas of the shelf that are closed to trawling (e.g. submarine cable zones, marine protected areas, agreements between fishermen to avoid the interaction of the fleet with the small-scale fishery during summer). In addition, the occurrence of benthic habitats with special interest in this area (e.g. maërl and crinoid beds) could enhance the presence and abundance of some elasmobranchs species (e.g. *S. canicula*, *Leucoraja naevus* and *R. clavata* ) (Ordines and Massutí, 2009). Benthic communities are considered highly productive and sensitive habitats and are distributed widely on the trawl fishing grounds of the shallow and deep shelf off the Balearic Islands respectively. Furthermore, displacement of the trawling effort from the shelf to the slope during the past decade, targeting the most vulnerable fishing resource in this area, namely the red shrimp *Aristeus antennatus*, has been argued by Guijarro *et al*. (2012) to explain the slight recovery detected during recent years in the elasmobranch populations on the CS of this archipelago, which has not been observed on the slope.

The highest species richness and diversity detected in the slope assemblage of GSA01 can also be explained by the effect of fishing. The deep-water trawl fishery is not well developed in the Alboran Sea, and its western part has remained almost unexploited between depths of 500 and 800 m (Gil de Sola, 1993). This low level of fishing exploitation is reflected not only in the high diversity, abundance and biomass of chondrichthyans on the GSA01 slope, but also in some deep-water bony fish populations, such as *Trachyrinchus scabrus* and *Helicolenus dactylopterus*, which show a predominance of large individuals in these fishing grounds. However, in GSA05 and GSA06, where the deep-water trawl fishery is well developed down to almost 900 m, large fish were very scarce down to this depth, being distributed on rocky bottoms or at depths greater than 800–1000 m where there is no trawl fishing activity (Massutí *et al*., 2001; Moranta *et al*., 2007). Furthermore, GSA01 incorporate in this study the area of Alboran Island, a marine reserve created in 1998 where regulated fishing exists only beyond a fully protected area around the island. As suggested above, these measures could have contributed to maintaining more stable chondrichthyan populations in GSA01 and GSA05 than in GSA06, where these species have changed drastically as a consequence of the effects of trawl fishing.

According to the International Union for Conservation of Nature (IUCN) Red List status of Mediterranean chondrichthyans (Cavanagh and Gibson, 2007), some threatened species were detected on the CS of GSA05 and slope of GSA01. Two vulnerable species (*Mustelus mustelus*, *Leucoraja circularis*) and one endangered (*Rostroraja alba*) species were recorded on the continental shelf and three vulnerable species were recorded on the slope (*Galeorhinus galeus*, *Centrophorus granulosus*, *L. circularis*). According to recommendation GFCM/36/2012/3 (http://www.gfcmonline.org/decisions/) on fisheries management measures for conservation of sharks and rays in the GFCM area, elasmobranch species listed in Annexe II of the SPA/BD protocol of the Barcelona Convention (*G. galeus*, *L. circularis*, *R. alba*) must be released unharmed and alive whenever possible, cannot be retained on board, transhipped, landed, transferred, stored, sold or offered for sale (UNEP, 2011).

The small-sized sharks *S. canicula* and *G. melastomus* were the most abundant species of the CS and slope assemblages of the western Mediterranean. Both represented >85% of the total abundance and biomass, except on the shelf of GSA05, where the importance of *S. canicula* was considerably lower (50% of total biomass) because of the presence of *R. clavata* (38% of total biomass). Our results, and previous studies in the western Mediterranean (e.g. Massutí and Moranta, 2003; Rey *et al*., 2004; García-Rodríguez *et al*., 2011; Gouraguine *et al*., 2011; Ordines *et al*., 2011) and the north-eastern Atlantic (Rodríguez-Cabello, 2008), showed that *S. canicula* and *G. melastomus* could maintain their populations despite the high fishing efforts exerted in some areas. This could be due to their biological adaptations. According to Camhi *et al*. (1998), Scyliorhinidae have a tendency to mature earlier, have a shorter generation time and higher rates of population increment than other chondrichthyans, and *S. canicula* and *G. melastomus* also have a continuous reproductive cycle in the Mediterranean (Tursi *et al*., 1993; Rey *et al*., 2004; Capapé *et al*., 2007). Moreover, although smaller individuals of these species captured in the bottom trawl fishery of the western Mediterranean are usually discarded and returned to the sea (Carbonell *et al*., 2003), *S. canicula* has shown high survival rates (Rodríguez-Cabello *et al*., 2001) and it has even been suggested that this species can modify its natural diet to benefit from food provided by discards from the bottom trawl fishery (Olaso *et al*., 2005).

The biological parameters of the most abundant species showed subtle differences compared with previous studies in other areas of the Mediterranean (Table 4.1.9). The LWp recorded a positive allometry (Table 4.1.4), where the females in all species showed a higher allometry than males, indicating a faster weight increase in relation to length (Bottari *et al*., 2013). For Mp, the comparison between GSAs for *S. canicula* and *G. melastomus* did not detect significant differences in the $L_{50}$ values of females and males, which could suggest the existence of the same stocks throughout the western Mediterranean, although other studies (e.g. genetics, parasites, tagging) would be required to complement and confirm this. The subtle differences recorded may have been caused by spatial or seasonal differences, or may simply reflect differences in the size of the individuals analysed (Stevens and Wiley, 1986; McCully *et al*., 2012) and could even also be related to the distinct fisheries scenarios discussed previously between GSAs. Fishing efforts can affect fish condition by lowering the stock biomass through the reduction of the intraspecific competition for food and space (Bottari *et al*., 2013).

The LFd of the most abundant chondrichthyan species followed different bathymetric patterns. Juveniles of *S. canicula* were distributed mainly between 150 and 400 m, whereas adults predominated at shallower (<200 m) and deeper (300–600 m) depths. This pattern has been observed in other studies in the Mediterranean (D'Onghia *et al*., 1995; Massutí and Moranta, 2003; Serena and Relini, 2006) and the north-eastern Atlantic (Rodríguez-Cabello *et al*., 2007). In contrast, juveniles of *G. melastomus* and *E. spinax* were mostly distributed in the upper strata, with the adults in deeper strata. A similar pattern has been recorded in the Balearic Islands (Massutí and Moranta, 2003), the Ligurian Sea (Relini-Orsi and Wurtz, 1977) and the Ionian

Sea (Tursi *et al*., 1993). In GSA05, no differences were observed in the bathymetric distribution of juveniles and adults of *R. clavata*, although a high proportion of juveniles was observed, which contrasted with the results obtained a decade previously in the same area by Massutí and Moranta (2003), who recorded a higher abundance of adults. Such changes could be a consequence of fishing impact (Walker and Hislop, 1998), as has been recorded previously in the North Sea (Walker and Heessen, 1996).

Table 4.1.9. Summary of information related to length at first maturity ($L_{50}$; total length) and length–weight relationship parameters (a and b, fitting constants for length-weight relationship) for *Scyliorhinus canicula*, *Galeus melastomus*, *Etmopterus spinax* and *Raja clavata* in the Mediterranean and Black Sea. F: females; M: males; C: both sexes combined.

| **Species** | **Study area** | **Sex** | **$L_{50}$ (cm)** | **a** | **b** | **Source** |
|---|---|---|---|---|---|---|
| *S. canicula* | Mediterranean | C | 37–44 | | | Leloup and Olivereau (1951) |
| | Tunisia | F | 40–45 | | | Capapé (1977a) |
| | | M | 40 | | | |
| | France | F | 41–47 | | | Capapé *et al.* (1991) |
| | | M | 44 | | | |
| | Aegean | F | | 0.0018 | 3.1514 | Savaş-Mater (2002) |
| | | M | | 0.0015 | 3.2112 | |
| | Aegean | F | 39.9 | | | Kousteni *et al.* (2010) |
| | | M | 39.6 | | | |
| | Algeria | F | | 0.00578 | 3.176 | Taleb-Bendiab *et al.* (2012) |
| | | M | | 0.01675 | 3.0215 | |
| | | C | 36 | | | |
| | Western basin | F | | 0.009 | 3.3475 | Present study |
| | | M | | 0.0025 | 3.0467 | |
| | | C | 39.8 | | | |
| *G. melastomus* | Ionian | F | 49 | 0.0257 | 3.05 | Tursi *et al.* (1993) |
| | | M | 45 | 0.0305 | 2.99 | |
| | Alboran | F | 48.8 | | | Rey *et al.* (2004) |
| | | M | 44.3 | | | |
| | Tyrrhenian | F | | 0.084 | 2.960 | Rinelli *et al.* (2005) |
| | | M | | 0.078 | 3.019 | |
| | Western basin | F | 53.25 | | | Present study |
| | | M | 47.50 | | | |
| | | C | | 0.0033 | 2.9459 | |
| *E. spinax* | Tyrrhenian | F | 37.48 | 0.0031 | 3.0814 | Porcu *et al.* (2012, 2014) |
| | | M | 34.39 | 0.0036 | 3.0335 | |
| | Western basin | F | 36.4 | 0.0101 | 2.7558 | Present study |
| | | M | 29.1 | 0.0295 | 2.4158 | |
| *R. clavata* | Adriatic | F | 73 | | | Jardas (1973) |
| | | M | 54 | | | |
| | Aegean | F | | 0.0018 | 2.2532 | Savaş-Mater (2002) |
| | | M | | 0.0006 | 3.5629 | |
| | France | F | 75.6 | | | Capapé *et al.* (2007) |
| | | M | 59.9 | | | |
| | Adriatic | F | 61.2 | 0.0011 | 3.4250 | Krstulović-Šifner *et al.* (2009) |
| | | M | 59.3 | 0.0012 | 3.3969 | |
| | Black Sea | F | 74.6 | | | Saglam and Ak (2012) |
| | | M | 71.8 | | | |
| | Western basin | F | 78.1 | | | Present study |
| | | M | 68.6 | | | |
| | | C | | 0.0029 | 3.1827 | |

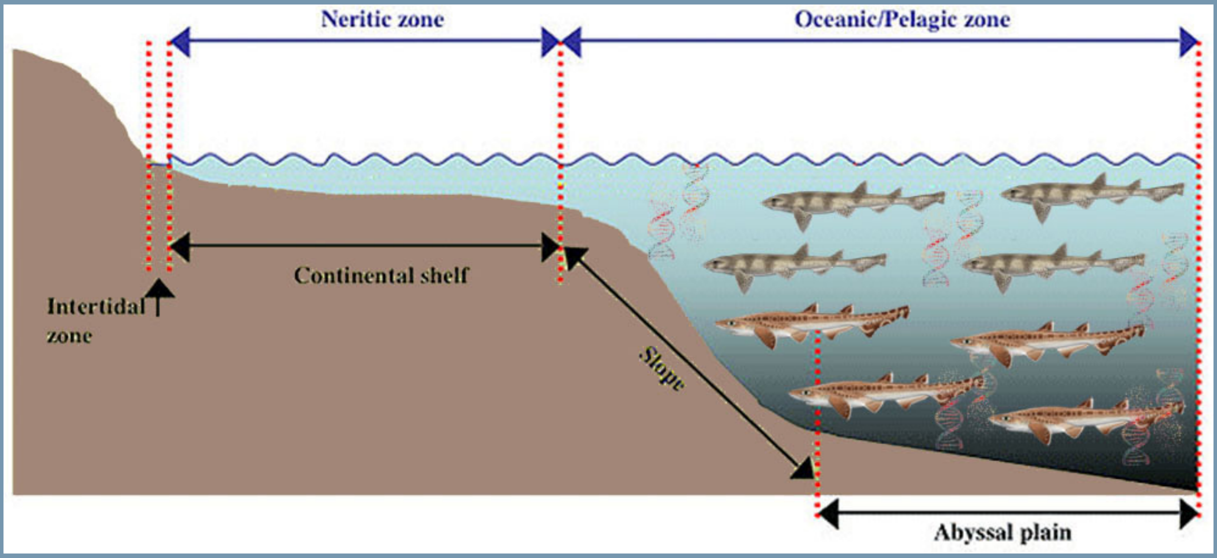
In conclusion, the present study has shown clear differences in the diversity and abundance of chondrichthyans in three different GFCM GSAs of the western Mediterranean. Taking into account the vulnerability of these species, and within the framework of the current ecosystem approach to fisheries assessment and management, which are among the main targets of the new European Common Fisheries Policy, these results can provide useful information for action plans targeting these species (Bradai *et al*., 2012). In the western Mediterranean, the challenge should be to make the sustainability of the bottom trawl fishery compatible with the recovery (GA06) and the protection (GSA01 and GSA05) of the chondrichthyan populations.

# CHAPTER II

## 4.2 CONNECTIVITY

Contrasting evolutionary patterns in populations of demersal sharks sampled at a fine scale throughout the western Mediterranean.

Ramírez-Amaro S., Picornell A., Arenas M., Castro J.A., Massutí E., Ramon C., Terrasa B. (Submitted).



## Summary

The spotted catshark (*Scyliorhinus canicula*) and the blackmouth catshark (*Galeus melastomus*) represent more than 85% of the total abundance of the demersal chondrichthyans in the western Mediterranean. Our study provides a complete analysis of the genetic population structure, connectivity and demographic history of two demersal sharks with a distinct bathymetric distribution in the western Mediterranean. To reach this aim, we performed a fine-scale sampling, in particular throughout the geographical subareas (GSAs) established by the General Fisheries Commission for the Mediterranean. Three mitochondrial fragments were analysed for both species and 12 microsatellite loci for *S. canicula*. We found contrasting patterns of population structure and connectivity for both species. *G. melastomus* showed absence of a population structure and high connectivity between GSAs. In contrast, *S. canicula* displayed significant genetic differences and low connectivity between some GSAs corresponding to different sub-basins (Alboran *vs*. Balearic). These findings would be in accordance with the fact that both species exhibit different dispersal behaviour, which leads to distinct bathymetric distributions. Contrasting demographic histories were also identified between both species. *S. canicula* revealed a stable population in the present, but after suffering bottleneck events. On the other hand, *G. melastomus* showed a recent population expansion. Our results indicate a mismatch between fisheries subareas and population structure for both sharks, which must be considered for fisheries management purposes.

**Keywords**: catshark, *Galeus melastomus*, Mediterranean population, mtDNA, *Scyliorhinus canicula*.

## 4.2.1 Introduction

The study of a shared pattern of genetic differentiation and connectivity is fundamental to unravelling population dynamics (Hanski, 1998), ecological processes (Treml and Halpin, 2012), and for effective management of marine communities (Cowen *et al*., 2000). Indeed, the degree of connectivity between populations has direct consequences on species evolution, development of disease resistance, local adaptation, and the capacity of a metapopulation to adapt to climate change (Mona *et al*., 2014; Padrón and Guizien, 2015). In the particular case of elasmobranchs, they display different patterns of population connectivity in comparison to teleost fishes (Le Port and Lavery, 2012). Molecular studies have shown that the degree connectivity in elasmobranch species appears to be correlated with fidelity to breeding areas (Dudgeon *et al*., 2009), habitat discontinuities (Duncan *et al*., 2006), life history strategies (Verissimo *et al*., 2010), and dispersal capabilities (Chapman *et al*., 2015).

Shark dispersal has been recognized as a key mechanism that influences their intraspecific genetic differentiation (Verissimo *et al*., 2010). While a variety of genetic population studies have been performed on pelagic or coastal sharks [revealing genetic homogeneity or differentiation over a large spatial scale in basking sharks (*Cetorhinus maximus*; Hoelzel *et al*., 2006) and whale sharks (*Rhyncodon typus*; Vignaud *et al*., 2014), respectively], fewer studies have focused on the analysis of less vagile species, such as small sized demersal species, despite these having a complex population structure (Portnoy and Heist, 2012).

Dispersal of juvenile and adult elasmobranch fishes depends on their vagility (dispersal capabilities) (Cunha *et al*., 2012), which complicates the comparative analysis of ecologically similar codistributed species, but comparative studies are rarely applied (e.g., Corrigan *et al*., 2016). Therefore, we compared population structure and connectivity patterns between two codistributed species of small and medium sized demersal sharks belonging to the same family (Scyliorhinidae): the small spotted catshark (*Scyliorhinus canicula* Linnaeus 1758), and the blackmouth catshark (*Galeus melastomus* Rafinesque 1810). Both species are distributed in the Eastern North Atlantic, from Norway to Senegal, including the Azores, and throughout the Mediterranean (Ebert *et al*., 2013). They actually represent more than 85% of the total abundance and biomass of demersal chondrichthyans in the western Mediterranean (Ramírez-Amaro *et al*., 2015). The main difference between the two species is their bathymetric distribution: *S. canicula* is mainly found along the continental shelf and upper slope up to 500 m depth approximately, with an optimum depth of 160 to 250 m (Ramírez-Amaro *et al*., 2015); whilst *G. melastomus* is distributed deeper, along the whole slope between 300 and 1800 m depth approximately (Stefanecu *et al*., 1992; Moranta *et al*., 1998), with an optimum depth of 480 to 685 m (Ramírez-Amaro *et al*., 2015).

Previous dispersal studies on *S. canicula* suggested limited movements, with a distance recorded for the majority of the recaptured specimens of less than 20 km, which pointed towards philopatric behaviour (Sims *et al*., 2001; Rodríquez-Cabello *et al*., 2004). Limited dispersal and philopatry may be the reason for the complex genetic structure of this species even on a small spatial scale throughout the Mediterranean Sea (Barbieri *et al*., 2014; Kousteni *et al*., 2014; Gubili *et al*., 2014). On the other hand, taking into account that deep water sharks (below 200 m depth) include a large diverse group and represent almost half of known shark species (Simpfendorfer and Kyne, 2009), it is necessary to encourage genetic studies of these species in order to disentangle dispersal behaviour, genetic structure, and connectivity. Along these lines, there are no studies on dispersal and genetic structure for *G. melastomus*.

In fact, being elasmobranchs, *S. canicula* and *G. melastomus* should be considered vulnerable species, even though they have been catalogued as of "least concern" by The IUCN Red List of marine fishes in the Mediterranean (Malak *et al*. 2011). It is most remarkable that recent assessments have diagnosed both species as overfished (Cardinale and Osio 2013), like the majority of the demersal resources exploited by Mediterranean bottom trawl fishery. In particular, the western basin is being widely exploited between 50 to 800 m depth, including the entire bathymetric distribution range of *S. canicula*, but only the shallower distribution range of *G. melastomus*. At any rate, both species constitute a significant proportion of the by-catch of fishes in this fishery (Carbonell *et al*. 2003).

Despite the substantial increase in the use of genetic markers for evaluating population structure (reviewed in Dudgeon *et al*., 2012), to date there is not a shark study focused on comparing fisheries management units by genetic analyses. Note that comparison between fisheries management units can provide relevant information for management strategies (Reiss *et al*., 2009). Concerning the Mediterranean Sea, the General Fisheries Commission for the Mediterranean (GFCM) has established 30 areas for fisheries assessment and management purposes. Four of these geographical subareas (GSAs) are located in the western Mediterranean: the northern Alboran Sea (GSA01), Alboran Island (GSA02), Balearic Islands (GSA05) and northern Spain (GSA06).

The main purposes of the present study are: i) in-depth analysis of the population structure, connectivity, and demographic history of *S. canicula* and *G. melastomus*, from a fine scale sampling design throughout the western Mediterranean; and ii) put forward recommendations (concerning the conservation of these elasmobranchs species) for fisheries assessment and management in the Mediterranean.

## 4.2.2 Material and methods

### *Study area*

The western Mediterranean is a small-scale ocean system, mainly influenced by the inflow of Atlantic water through the Strait of Gibraltar and the exchange of these waters with Mediterranean water through the Balearic channels. The Alboran Sea, in the south-western Mediterranean, is characterized by complex bottom topography, with a maximum depth of 2000 m and the presence of the Alboran Island. The Balearic Islands delimit the Balearic sub-basin (to the north) and the Algerian sub-basin (to the south), which are characterized by different oceanographic conditions (reviewed in Massutí *et al*., 2014) and are connected by a series of channels with depths between 100 and 800 m (Pinot *et al*., 2002). These two sub-basins are characterized by the presence of permanent hydrographic fronts. The Almeria-Oran hydrographic front affects the Alboran sub-basin (Millot, 1999). Two additional slope fronts affect the Balearic sub-basin: the Northern Current that flows southwards along the whole continental slope, and the Balearic Current that flows north-eastwardly along the northern slope of the Archipelago (Monserrat *et al*., 2008).

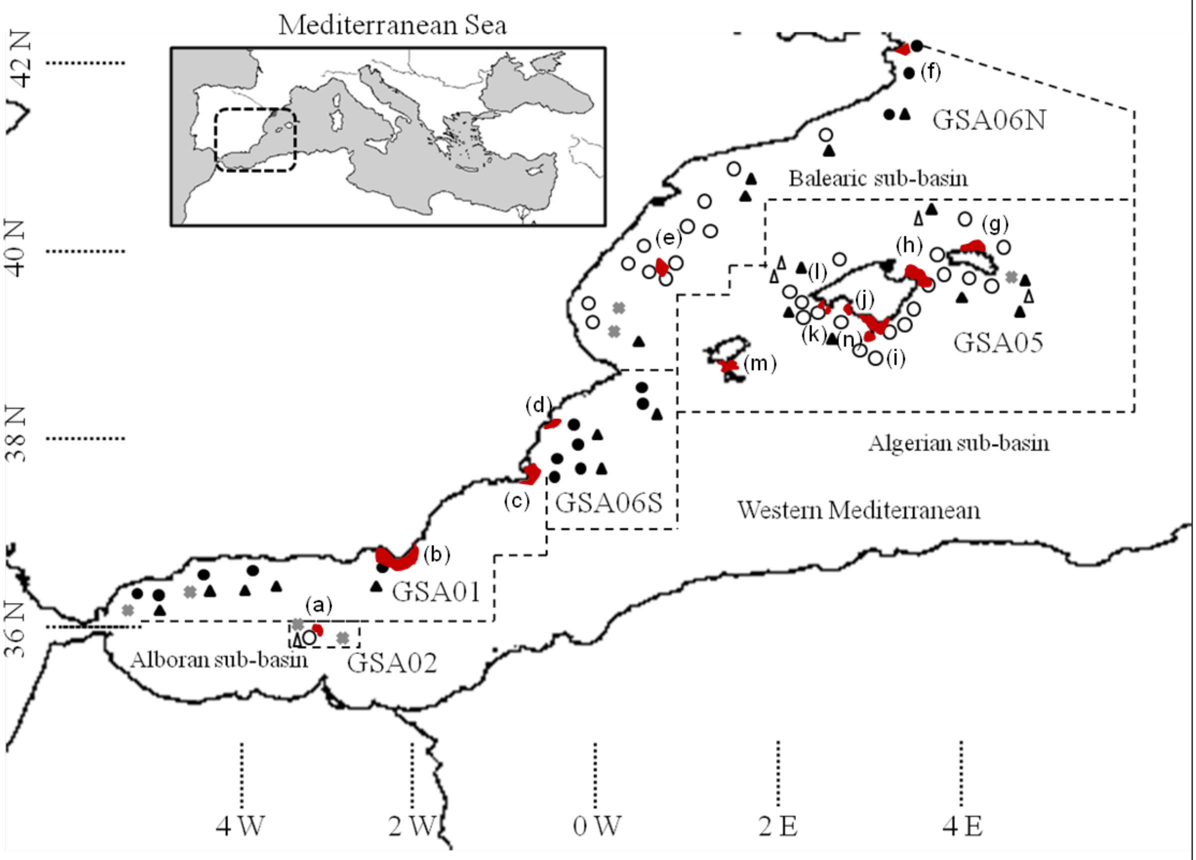


Figure 4.2.1. Locations of the shark samples collected during the MEDITS bottom trawl survey carried out in 2013 throughout the western Mediterranean. Hauls of *Scyliorhinus canicula* are indicated with circles, and hauls of *Galeus melastomus* are indicated with triangles. Filled and unfilled shapes indicate the collection of multiple samples or a single sample, respectively. Cross shapes indicate hauls with collection of samples of both species. Marine protected areas are indicated in red: (a) Alboran Island, (b) Cape Gata, (c) Cape Palos, (d) Tabarca Island, (e) Columbretes Islands, (f) Cape Creus [National Park], (g) North of Menorca, (h) Llevant of Mallorca/Cala Rajada, (i) Palma Bay, (j) Migjorn of Mallorca, (k) Toro Island, (l) Malgrats Islands, (m) Freus of Eivissa and Formentera and (n) Archipelago of Cabrera [National Park].

### *Sample collection and DNA extraction*

Shark samples were obtained during the MEDITS bottom trawl survey (for sampling protocol see Bertrand *et al*. 2002) carried out in 2013 along the western Mediterranean (Figure 4.2.1). A total of 80 individuals from each species were collected at depths between 52 and 559 m for *Scyliorhinus canicula* and between 221 and 813 m for *Galeus melastomus*. Samples were taken at any of the four geographic subareas included in the study area. Conveniently, GSA01 and GSA02 were jointly studied because of the low number of samples in GSA02 (4 samples) and their geographic proximity (Figure 4.2.1) and for simplicity, both GSAs are hereafter referred to as GSA01. Additionally, GSA06 was subdivided into north (GSA06N) and south (GSA06S) due to its large extension. Samples size and location areas are indicated in Table 4.2.1.

A small piece of muscular tissue was excised from each individual and stored at 4°C in absolute ethanol. Genomic DNA was extracted using a standard phenol-chloroform method (Terrasa *et al*., 2009).

### *Mitochondrial DNA sequencing*

Three mitochondrial DNA (mtDNA) fragments were amplified (PCR) and sequenced for each specimen: partial cytochrome b (*cytb*), partial dehydrogenase subunit 2 (*ND2*) and partial control region (*CR*). Primers and PCR conditions are described in Supplementary Table S4.2.1.

PCR products were purified using the QIAquick® PCR Purification Kit (QIAGEN, Valencia, CA, USA). Both heavy and light strands were sequenced using the ABI Prism Terminator BigDye® Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City CA, USA) and separated in an ABI PRISM 3130 Genetic Analyzer. The resulting DNA sequences were imported into BioEdit 7.0.5.2. (Hall, 1999) and checked for quality and accuracy of nucleotide base assignment. Multiple sequence alignments (MSA) were obtained with ClustalW (Thompson *et al*., 1994). Additionally, a fragment of cytochrome oxidase I (*COI*) was sequenced from ten specimens of each species, in order to contribute to the Barcode of Life Data System of both species in the study area (BOLD, http://www.boldsystems.org/).

### *Microsatellite genotyping*

*S. canicula* samples were genotyped for the twelve microsatellite loci described by Griffiths *et al*. (2011): Scan02, Scan03, Scan04, Scan06, Scan07, Scan09, Scan10, Scan12, Scan13, Scan14, Scan15 and Scan16. They were amplified with the QIAGEN® PCR kit (QIAGEN) following Griffiths *et al*. (2012) but with modifications (multiplex PCR details are shown in Supplementary Table S4.2.2). Allele sizes were determined with GeneMapper v.3.1 (Applied Biosystems, Foster City, CA). Additionally, these sets of primers were tested in *G. melastomus* without positive amplification.

*Neutrality, genetic diversity and structure*

For mtDNA concatenated sequences, genetic diversity and neutrality indices (Tajima's D and Fu's Fs) were calculated with ARLEQUIN v3.11 (Excoffier *et al*. 2005) and DnaSP v5 (Librado and Rozas, 2009). The parsimony network for the concatenated mtDNA haplotypes was inferred with TCS v.1.21 (Clement *et al*., 2000).

Microsatellite data were analysed with ARLEQUIN v.3.11, FSTAT 2.9.3.2 (Goudet *et al*., 2002) and GenAlex 6.502 (Peakall and Smouse, 2012) to estimate allelic frequencies (Na), allelic richness (RS), Garza-Williamson index (GW) and, observed (HO) and expected heterozygosities (HE). We also tested whether the observed allelic frequencies conform to those expected under the Hardy-Weinberg Equilibrium (HWE). Finally, linkage disequilibrium (LD) was applied to examine potential correlations of allelic frequencies between pairs of loci.

In order to identify genetically distinct geographical population clusters, the Bayesian Phylogeographic and Ecological Clustering (BPEC) method (Manolopoulou *et al*., 2011) was applied to analyse the mtDNA sequences of each species. The purpose of this method is to combine sequence data with geographical data to infer a geographically clustered structure consistent with the underlying evolutionary history. BPEC applies parsimonious approaches within a model-based framework, assigning probabilities to each location based on the haplotypes observed in each one (Manolopoulou and Emerson, 2012). Different scenarios of trees and migration events are explored through MCMC to estimate geographically clustered structures and rates of dispersal. MCMC simultaneously estimates high probability trees, number of migration events, and corresponding clusters. Following the recommendations of the authors, the following settings were specified: number of iterations of the MCMC chain (10,000,000), number of saved iterations (2,000), dimension (2), parsimony relaxation parameter dS (1), and maximum number of migrations (3).

For each species, the genetic distances, based on mtDNA concatenated sequences, between GSAs, and between clusters identified with BPEC were estimated with ARLEQUIN through genetic distance-based $\Phi_{ST}$ and frequency-based $F_{ST}$. Level of genetic differentiation for microsatellite data was estimated with ARLEQUIN by calculating pairwise $F_{ST}$. Statistical pairwise significances were assessed through 10,000 permutations, for both molecular markers. Next, for *S. canicula*, $\Phi_{ST}$ (for mtDNA) and $F_{ST}$ (for microsatellites) were represented in a multidimensional scaling (MDS) plot with PRIMER v.6 (Clarke and Warwick, 2001). Hierarchical structure was assessed for each molecular marker by analysis of molecular variance with ARLEQUIN.

Further, for *S. canicula* the existence of distinct genetic groups was explored in the set of individual multilocus genotypes with the Bayesian clustering approach implemented in STRUCTURE v.2.3 (Pritchard *et al*., 2000). Probabilities of admixture and no admixture were tested for K clusters, ranging from 1 to 8, assuming correlated allele frequencies. A total of 4 independent runs were performed for each K value, where each MCMC chain presented 1,000,000 iterations and a burn-in of 250,000 iterations. To determine the K value that best fitted the data we applied the highest average maximum-likelihood score and Evanno's delta K (ΔK) (Evanno *et al.*, 2005) with the STRUCTURE HARVESTER frameworks (Earl and von Holdt, 2012).

Additionally, for *S. canicula*, data corresponding to the mtDNA control region (mtDNA-CR) of samples collected in the Atlantic (Bristol, Bri; Scotland, Sco; western Channel, Wca; Portugal, Por) and Mediterranean (Mallorca, Mall; Cyprus, Cyp; Crete, Cre) (Gubili *et al.*, 2014), localities were downloaded from GenBank (accession numbers: KM873790-KM874065). Our mtDNA-CR sequences were added to this dataset in order to perform comparisons. Next, mtDNA-CR data were analyzed through pairwise ($\Phi_{ST}$) among localities (including GSAs) with ARLEQUIN under 10,000 permutations and plotted in MDS using PRIMER. The assigned clusters were evaluated again by pairwise analysis based on both $\Phi_{ST}$ and $F_{ST}$ under 10,000 permutations.

*Demographic history and migration events*

For each species, the demographic history of the population groups was studied with the analysis of mtDNA data through three approaches. Firstly, evidence of population expansion was tested by the mismatch distribution (Rogers and Harpending, 1992) analysis implemented in ARLEQUIN. Harpending's raggedness index (r), quantifying the smoothness of the observed mismatch distributions, was computed to determine the goodness of fit to a unimodal distribution (Harpending, 1994). Secondly, evidence for population expansion was evaluated with Tajima's D (1989) and Fu's Fs (1997) indices. Thirdly, in order to reconstruct the past population dynamics of both species, the Extended Bayesian Skyline Plot (EBSP) was implemented in BEAST v1.8.2 software (Drummond and Rambaut, 2007). For this analysis, the HKY +I substitution model identified with jModelTest v.2 (Darriba *et al.*, 2012) was assigned. For each dataset the prior distributions suggested by default were applied, and 2 MCMC chains of 200,000,000 iterations were run, sampling every 10,000 iterations, and a burn-in of 10% was applied. Convergence between MCMC chains and EBSP analysis was performed with TRACERv.1.5 software (Rambaut and Drummond, 2009).

Additionally, concerning the detection of a genetic bottleneck from microsatellite data, we applied the heterozygosity excess method of Luikart *et al.* (1998) that is implemented in the program BOTTLENECK v.1.2.02 (Piry *et al.*, 1999), under the step-wise mutation model (SMM), and 2-phase model (TPM; considering the following proportions of step-wise mutation: 80%, 90%, 95%, 99%), including all microsatellite loci, and for a total of 20,000 replicates. Next, we applied the following statistical tests: were applied: the sign test (Cornuet and Luikart, 1996) and Wilcoxon's signed rank (Luikart and Cornuet, 1998).

Migration events were identified with the coalescent approach implemented in LAMARC v2.1.10 (Kuhner, 2006) using mtDNA (for both species) and microsatellites (only for *S. canicula*) data. Gene regions were analysed with independent Bayesian searches. For the MCMC, 10 runs were performed with 17 chains each: 12 short chains of 1,000 steps, and 5 long chains of 5,000,000 million iterations following the author's recommendations. Next a burn-in of 10,000 iterations was applied. Sampling was performed every 1,000 steps and convergence was identified with TRACER.

## 4.2.3 Results

*Genetic diversity*

A concatenated fragment of 2,091 bp (*cytb*: 417 bp, *ND2*: 948 bp, *CR*: 726 bp) for *Scyliorhinus canicula* and 2,073 bp (*cytb*: 411 bp, *ND2*: 933 bp, *CR*: 729 bp) for *Galeus melastomus* was sequenced for each sample. All the sequences were deposited in the GenBank database (accession numbers: KX278828-KX279307). COI sequences were also deposited in GenBank (KX283359-KX283378; Supplementary Table S4.2.3) and their corresponding BOLD (GDSWM001-GDSWM020).

We found a total of 43 and 49 different haplotypes derived from the mtDNA data of *S. canicula* and *G. melastomus*, respectively (Table 4.2.1). Overall haplotype diversity (*h*) was similar for both species (0.97 for *S. canicula* and 0.94 for *G. melastomus*). However, overall nucleotide diversity (π) for *G. melastomus* (0.0023) was approximately half that of *S. canicula* (0.0044). Diversity indices h and π were similar in the different GSAs for both species (Table 4.2.1).

Table 4.2.1. Genetic diversity statistics estimated from mtDNA data of *Scyliorhinus canicula* (SC) and *Galeus melastomus* (GM) for each GSA, and the Alboran and Balearic sub-basins.

| Areas | Sn | | Ns | | Hn | | h | | π | | k | | D (Tajima 1989) | | F (Fu 1997) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *SC* | *GM* | *SC* | *GM* | *SC* | *GM* | *SC* | *GM* | *SC* | *GM* | *SC* | *GM* | *SC* | *GM* | *SC* | *GM* |
| GSA01 Alboran sub-basin | 20 | 20 | 28 | 25 | 11 | 17 | 0.89 | 0.97 | 0.0044 | 0.0025 | 9.20 | 5.27 | 0.647 | –0.977 | 0.371 | –9.975$^{***}$ |
| GSA05 | 20 | 20 | 29 | 21 | 16 | 15 | 0.97 | 0.94 | 0.0036 | 0.0021 | 7.51 | 4.38 | –0.318 | –0.990 | –5.547 | –7.498$^{*}$ |
| GSA06S | 20 | 20 | 34 | 21 | 13 | 13 | 0.93 | 0.91 | 0.0042 | 0.0022 | 8.83 | 4.62 | –0.310 | –0.990 | –1.362 | –4.184$^{*}$ |
| GSA06N | 20 | 20 | 29 | 21 | 15 | 16 | 0.92 | 0.97 | 0.0034 | 0.0024 | 7.07 | 4.91 | –0.522 | –0.654 | –4.455 | –8.499$^{***}$ |
| Balearic sub-basin | 60 | 60 | 45 | 35 | 35 | 38 | 0.96 | 0.94 | 0.0040 | 0.0022 | 8.38 | 4.62 | –0.441 | –1.265 | –14.328$^{**}$ | –25.673$^{***}$ |
| All | 80 | 80 | 55 | 42 | 43 | 49 | 0.97 | 0.94 | 0.0044 | 0.0023 | 9.19 | 4.75 | –0.564 | –1.415 | –17.965$^{**}$ | –25.617$^{***}$ |

Sn: number of samples; Ns: polymorphic sites; Hn: number of haplotypes; h: haplotype diversity; π: nucleotide diversity; k: pairwise differences.
Statistical significance: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

The average number of differences between haplotypes (k) was greater for *S. canicula* (9.19) than for *G. melastomus* (4.75). This genetic homogeneity between mtDNA haplotypes in the *G. melastomus* species was also identified in the haplotype network (Figure 4.2.2). The network obtained from *S. canicula* showed fewer shared haplotypes among GSAs than that obtained from *G. melastomus*. In addition, this network analysis revealed more mutational steps between haplotypes and a larger number of missing haplotypes in *S. canicula*.

Table 4.2.2. Genetic diversity statistics estimated from 12 microsatellite loci of *Scyliorhinus canicula* samples collected in each GSA and the Alboran and Balearic sub-basins.

| GSA's | *Na* | *Ho* | *HE* | *Rs* | *HWE* | *G-W* |
|---|---|---|---|---|---|---|
| GSA01 (Alboran sub-basin) | 5.83 (2.11) | 0.612 (0.17) | 0.672 (0.16) | 3.191 | 0.348 | 0.498 (0.08) |
| GSA05 | 5.17 (2.27) | 0.527 (0.23) | 0.600 (0.17) | 2.971 | 0.53 | 0.432 (0.11) |
| GSA06S | 6.33 (2.21) | 0.594 (0.21) | 0.624 (0.20) | 3.502 | 0.541 | 0.428 (0.12) |
| GSA06N | 5.17 (1.72) | 0.586 (0.23) | 0.607 (0.21) | 3.38 | 0.375 | 0.464 (0.10) |
| Balearic sub-basin | 7.67 (2.59) | 0.568 (0.21) | 0.628 (0.19) | 3.382 | 0.319 | 0.472 (0.11) |

Na: mean number of alleles over loci; Ho: observed heterozygosity; HE: expected heterozygosity; Rs: allelic richness; HWE: probability to conformance to Hardy-Weinberg equilibrium; G-W: Garza-Williamson index; standard deviation is shown in parenthesis.

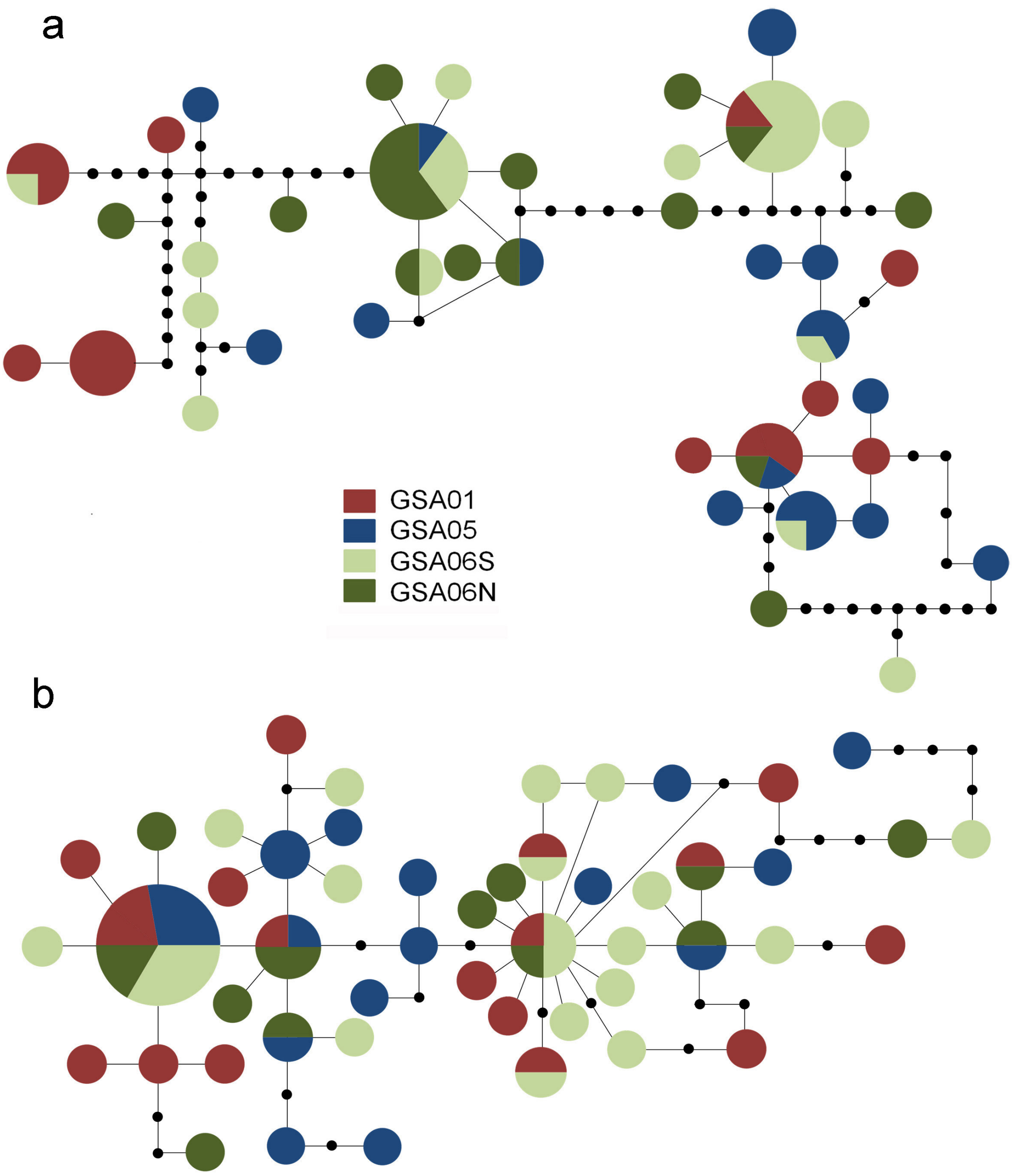


Figure 4.2.2. Haplotype network derived from mtDNA data of *Scyliorhinus canicula* (a) and *Galeus melastomus* (b). Relative area of circles reflects sample size. Connecting black circles represent mutational steps.

Multilocus microsatellite genotypes were obtained for all *S. canicula* samples (Supplementary Table S4.2.4). Analyses of 12 microsatellites showed no evidence for null alleles and short allele dominance. Neither were departures revealed for HWE equilibrium for all locus/GSAs/population combinations (Supplementary Table S4.2.5). Mean observed heterozygosity was similar between GSAs, ranging from 0.527 in GSA05 to 0.612 in GSA01, whereas allelic richness ranged from 2.97 in GSA05 to 3.502 in GSA06S (Table 4.2.2).

*Population differentiation and migration events*

For *S. canicula*, pairwise $\Phi_{ST}$ and $F_{ST}$ values ranged from 0.014 to 0.266 for mtDNA (Table 4.2.3), and $F_{ST}$ values ranged from 0.0007 to 0.026 for microsatellite data (Table 4.2.4). Pairwise estimates were significant for GSAs comparisons (Table 4.2.3 and 4.2.4). In contrast, for *G. melastomus*, pairwise $\Phi_{ST}$ and $F_{ST}$ values ranged from -0.24 to 0.005, suggesting no significant genetic differences between GSAs (Table 4.2.5).

Table 4.2.3. Mitochondrial pairwise differentiation between GSAs for *Scyliorhinus canicula*. Below diagonal: pairwise $F_{ST}$. Above diagonal: pairwise $\Phi_{ST}$.

| Areas | *GSA01* | *GSA05* | *GSA06S* | *GSA06N* |
|---|---|---|---|---|
| GSA01 | ---- | 0.128** | 0.14*** | 0.266*** |
| GSA05 | 0.061** | ---- | 0.049 | 0.176** |
| GSA06S | 0.073*** | 0.03* | ---- | 0.062 |
| GSA06N | 0.085*** | 0.033* | 0.014 | ---- |

Statistical significance: *$p$<0.05; **$p$<0.01; ***$p$<0.001.

Table 4.2.4. Microsatellite pairwise differentiation between GSAs for *Scyliorhinus canicula*. Below diagonal: pairwise $F_{ST}$.

| Areas | *GSA01* | *GSA05* | *GSA06S* | *GSA06N* |
|---|---|---|---|---|
| GSA01 | ---- | | | |
| GSA05 | 0.016* | ---- | | |
| GSA06S | 0.012 | 0.026** | ---- | |
| GSA06N | 0.015* | 0.025** | 0.0007 | ---- |

Statistical significance: *$p$<0.05; **$p$<0.01; ***$p$<0.001.

Table 4.2.5. Mitochondrial pairwise differentiation between GSAs for *Galeus melastomus*. Below diagonal: pairwise $F_{ST}$. Above diagonal: pairwise $\Phi_{ST}$.

| Areas | *GSA01* | *GSA05* | *GSA06S* | *GSA06N* |
|---|---|---|---|---|
| GSA01 | ---- | -0.004 | -0.023 | -0.24 |
| GSA05 | -0.008 | ---- | 0.013 | -0.023 |
| GSA06S | -0.013 | -0.004 | ---- | -0.007 |
| GSA06N | -0.011 | -0.006 | 0.005 | ---- |

Statistical significance: *$p$<0.05; **$p$<0.01; ***$p$<0.001.

BPEC results showed two clusters ($P = 0.7$; Figure 4.2.3a) for *S. canicula*. The spatial location of both clusters corresponds to two geographical areas in the western Mediterranean: the Alboran and Balearic sub-basins. Pairwise values for mtDNA ($\Phi_{ST}$ =0.159, $F_{ST}$ = 0.06, $p < 0.0001$) and microsatellite ($F_{ST}$ = 0.09, $p < 0.05$) were significant between both sub-basins, which verified the two previously identified clusters. Besides, the MDS plot (Figure 4.2.4) supported the presence of two clusters: one cluster including GSA01, which corresponds to the Alboran sub-basin; and the other grouping the GSA05, GSA06S and GSA06N sub-areas, which correspond to the Balearic sub-basin.

By contrast, the BPEC test indicated only one cluster ($p$ = 0.8; Figure 4.2.3b) for *G. melastomus* throughout the study area, which did not present significant pairwise differences between both sub-basins ($\Phi_{ST}$ = -0,009, $F_{ST}$ = -0.013, $p > 0.05$).

Hierarchical AMOVA results for *S. canicula* were generally in agreement between mtDNA and microsatellite data, revealing significant variation between GSAs and also between the Alboran and Balearic sub-basins (Table 4.2.6).

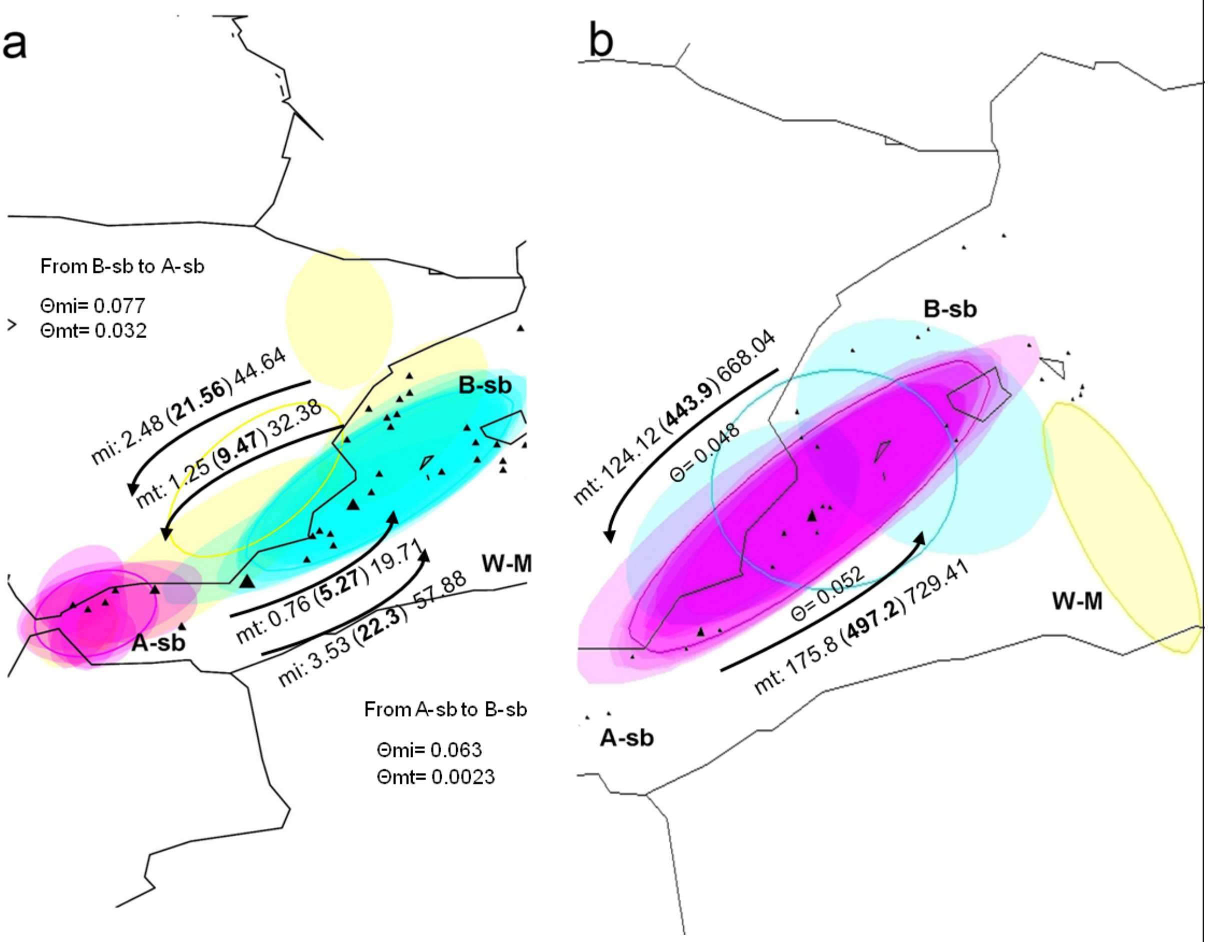


Figure 4.2.3. Bayesian phylogeographic and ecological clustering (BPEC) analyses (coloured areas) and migration rates (arrows) between Alboran (A-sb) and Balearic (B-sb) sub-basin populations for (a) *Scyliorhinus canicula* and (b) *Galeus melastomus* (hypothetical populations). Contour regions are centred for each population cluster and the shaded areas show the radius of 50% concentration contours around them. "mt" means migration rates for mitochondrial data and "mi" means migration rates for microsatellite data. Θ indicates time of mutation rate. Arrows indicate number of migrants per generation and include most probable estimates (in bold) flanked by upper and lower 95% confidence interval (CI, in regular text). Black triangles indicate haplotype locations and W-M indicates western Mediterranean.

Table 4.2.6. Hierarchical AMOVA for *Scyliorhinus canicula* (SC) and *Galeus melastomus* (GM) derived from mitochondrial and microsatellite data.

| Data | Species | Area | Source variation | Variance components | Percentage variance | $F_{ST}$ |
|---|---|---|---|---|---|---|
| Mitochondrial | SC | GSAs | Among | 0.683 | 14.34 | 0.143*** |
| | | | Within | 4.078 | 85.66 | |
| | | Sub-basins | Among | 0.808 | 15.86 | 0.158*** |
| | | | Within | 4.289 | 84.14 | |
| Microsatellite | SC | GSAs | Among | 0.061 | 1.58 | 0.015** |
| | | | Within | 3.785 | 98.42 | |
| | | Sub-basins | Among | 0.0359 | 0.93 | 0.093* |
| | | | Within | 3.817 | 99.07 | |
| Mitochondrial | GM | GSAs | Among | -0.028 | -1.19 | -0.011 ns |
| | | | Within | 2.416 | 101.19 | |
| | | Sub-basins | Among | -0.032 | -1.40 | -0.014 ns |
| | | | Within | 2.387 | 101.40 | |

Statistical significance: **p*<0.05; ***p*<0.01; ****p*<0.001. ns: non-significant.

These differences were also identified in the analysis of specific mitochondrial fragments (Supplementary Table S4.2.7). On the other hand, for *G. melastomus* significant differences were found between GSAs or between both sub-basins (Table 4.2.6).

The genetic subdivision throughout the Mediterranean for *S. canicula* inferred in our above analyses was also consistent with the number of clusters we identified with STRUCTURE. According to the Evanno method, two distinct clusters (K=2; Figure 4.2.5) were identified under either of the two implemented models [average log probability of data for the no admixture model (Figure 4.2.5a) Ln[P(D|K)] = − 2684 .8 ±1.55 (s.d.), and for the admixture model (Figure 4.2.5b) Ln[P(D|K)]= − 2834.4 ±18.87 (s.d.)].

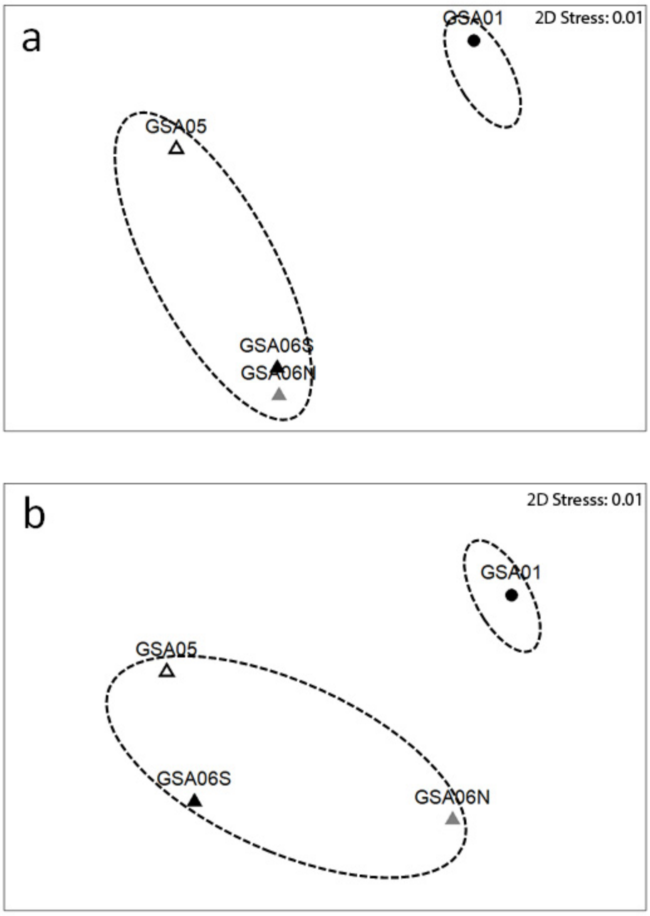


Figure 4.2.4. Clustering derived from multidimensional scaling analysis (MDS) of *Scyliorhinus canicula* for all GSAs using pairwise FST from mtDNA (a) and microsatellite (b) data.

Concerning the global analysis in the Atlantic and Mediterranean Sea, the pairwise $F_{ST}$ and $\Phi_{ST}$ values of the mtDNA-CR dataset for *S. canicula* revealed five separate groups (Table 4.2.7): i) North Atlantic (Bristol, Scotland and western Channel samples), ii) Atlantic Transition (Portugal and GSA01 samples), iii) Balearic Islands (Mallorca and GSA05 samples), iv) North Spain (GSA06S and GSA06N samples), and v) East Mediterranean (Cyprus and Crete samples). These groups were assigned according to the distances observed in the MDS plot (Supplementary Figure S4.2.1).

Migration events were identified by considering the two clusters identified by BPEC in both species (even for *G. melastomus* despite its lack of population structure but to enable a comparison between the studied species). The magnitude of the number of migrant individuals per generation using mtDNA data between the Alboran and Balearic sub-basins was considerably higher for *G. melastomus* than for *S. canicula* (Figure 4.2.3), suggesting a different degree of connectivity in both species. Peculiarly, for *S. canicula* the number of migrant individuals per generation was greater based on microsatellite data than on mtDNA data (Figure 4.2.3).

*Demographic history*

Historical demographic analyses of both species were performed on mtDNA data and following the clustering obtained in previous analyses. Mismatch distribution analysis showed a bimodal distribution for the two clusters of *S. canicula*, which could have been caused by recent demographic stability (Figure 4.2.6). Yet, at the same time, significant positive values of raggedness indices for the Alboran ($r = 0.095$; $p < 0.001$) and Balearic ($r = 0.02$; $p < 0.05$) sub-basins suggest stationary or bottlenecked populations (Harpending, 1994). In contrast, neutrality tests generated different results for each clusters. They exhibited positive values for the Alboran sub-basin and negative values for the Balearic sub-basin, suggesting demographic stability and a recent sudden population expansion, respectively (Table 4.2.1). The EBSP results revealed a constant size during the last one million years for both sub-basins, followed by a recent sudden population for the Balearic group (Figure 4.2.6). For microsatellite data, demographic analyses showed significant values for Wilcoxon and Sing tests (Supplementary Table S4.2.6), suggesting past population bottleneck events in both sub-basins.

Concerning *G. melastomus*, the unimodal distribution (Figure 4.2.6), negative neutrality values (Table 4.2.1), and non-significant raggedness index ($r = 0.007$; $p > 0.05$) suggest a sudden population expansion. The EBSP analysis also suggests a sudden population growth for this species, which was estimated to have begun 200,000-250,000 years ago (Middle Pleistocene) (Figure 4.2.6).

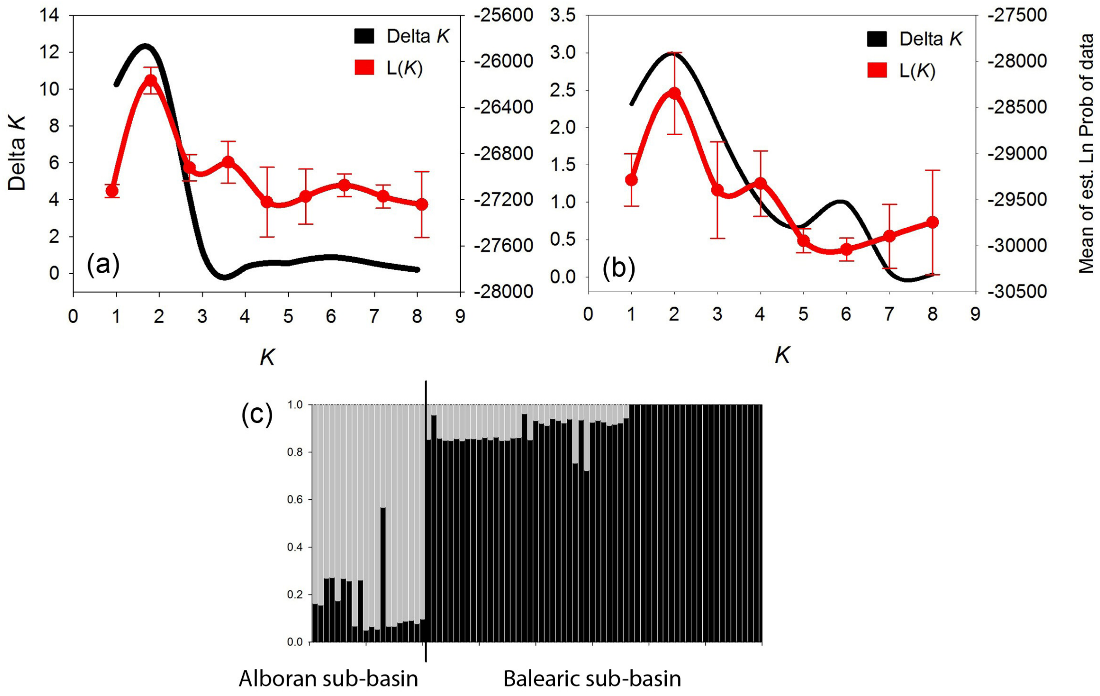


Figure 4.2.5. Clustering of baseline samples of *Scyliorhinus canicula* into regional groups for the no-admixture (a) and admixture (b) models implemented in STRUCTURE. The Evanno method (ΔK) and log probability of the data at a given modeled K value are indicated in black and red lines, respectively. Finally (c) presents the inferred population structure for the best K (K = 2), where each individual is represented by a narrow vertical column broken into 2 colored segments.

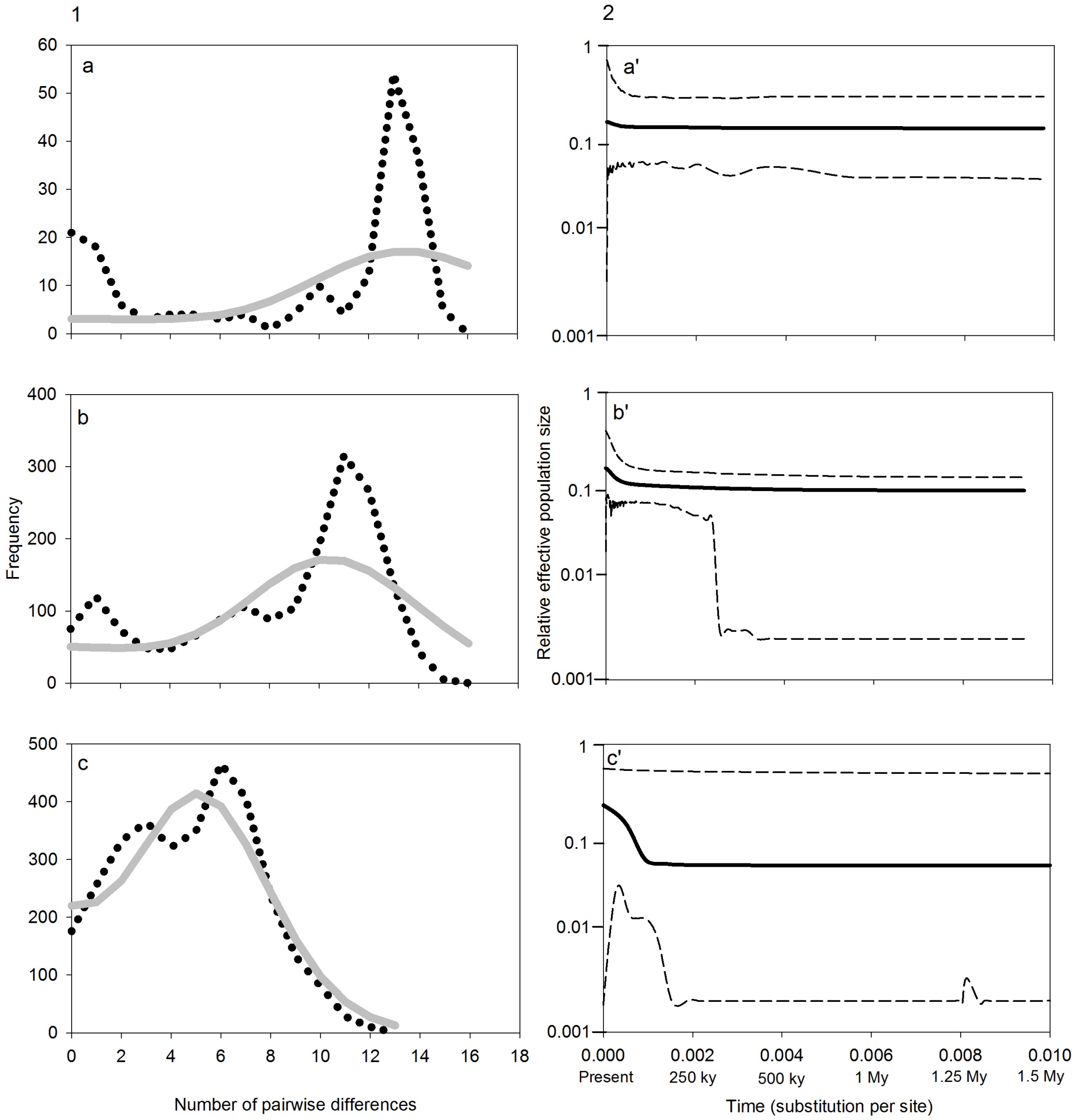


Figure 4.2.6. Variation of the relative effective population size based on mtDNA data through pairwise mismatch distribution (1) and Extended Bayesian Skyline Plot (EBSP) (2). Analysis performed for Alboran (a; a') and Balearic (b; b') populations of *Scyliorhinus canicula*, and for the western Mediterranean (c; c') population of *Galeus melastomus*. For the mismatch distribution, the solid line represents expected frequency and the dotted line represents observed frequency. For EBSP, the dotted line indicates the 95% highest posterior density interval (HPDI).

Table 4.2.7. Genetic distance by pairwise $F_{ST}$ (above diagonal) and $\Phi_{ST}$ (below diagonal) from mtDNA-CR of *Scyliorhinus canicula* including available GenBank sequences.

| | **North Atlantic** | **Atlantic transition** | **Balearic Islands** | **North Spain** | **East Mediterranean** |
|---|---|---|---|---|---|
| North Atlantic (73) | ---- | 0.0343* | 0.050** | 0.167*** | 0.335*** |
| Atlantic transition (49) | 0.011* | ---- | 0.011 | 0.165*** | 0.346*** |
| Balearic Islands (60) | 0.035* | 0.027* | ---- | 0.145*** | 0.348*** |
| North Spain (40) | 0.259*** | 0.221*** | 0.172** | ---- | 0.281*** |
| East Mediterranean (35) | 0.560*** | 0.515*** | 0.525*** | 0.421*** | ---- |

Statistical significance: *$p<0.05$; **$p<0.01$; ***$p<0.001$. Sample size is shown in parenthesis.

## 4.2.4 Discussion

Small sized demersal sharks exhibit variable dispersal behaviour even on a small geographic scale, which has ecological, conservation, and evolutionary implications. Here we present the first comparative analysis of genetic structure, connectivity, and dispersal patterns of the most abundant demersal sharks in the western Mediterranean: the small and medium sized catsharks *Scyliorhinus canicula* and *Galeus melastomus*. These species exhibit a distinct bathymetric distribution and we performed a fine scale sampling approach along the western Mediterranean.

*Contrasting population structure and connectivity*

Based on mitochondrial data, we found contrasting patterns with regard to genetic population structure and connectivity when comparing both species throughout the study area. In particular, *S. canicula* showed genetic differences between GSAs derived from analysis of both mitochondrial and microsatellite data. Next, BPEC was applied to define population boundaries across the study area, which is not fragmented by any obvious barrier to gene flow; this framework indicated two genetic clusters corresponding to the Alboran and Balearic sub-basins, which was supported by additional genetic analyses. In contrast, *G. melastomus* did not exhibit signatures of genetic differences between the studied GSAs.

In elasmobranchs, vagility tends to be lower in coastal demersal species than in deep-sea species (Musick *et al*. 2004), which is in agreement with the genetic differences that were observed between both species. The dispersal differences between these species can also be reflected in the estimated migration events, where the number of effective migrants per generation is clearly higher for *G. melastomus* than for *S. canicula* (Figure 4.2.3). The dispersal capacity of both species could be influenced by their morphological traits, sea topography and oceanic events, and even by their reproductive behaviour.

Musick *et al*. (2004) suggest that vagility increases with body size. Thus, smaller demersal species would be expected to exhibit more genetic heterogeneity between populations than larger pelagic species (Portnoy and Heist 2012). Considering that both species have a similar body size in the study area (Ramírez-Amaro *et al*. 2015), both species would be expected to have a similar degree of genetic heterogeneity, but our results only showed high heterogeneity in *S. canicula*. Due to the intrinsic difficulties in obtaining samples of deep-sea sharks, a number of hypotheses based on prior knowledge with similar species were formulated, although they need testing. For example, a recent genetic study on a small-deep shark *Etmopterus spinax* (smaller than *G. melastomus*) revealed its high dispersal and connectivity in the Mediterranean Sea (Gubili *et al*. 2016). Likewise, genetic studies on similar deep-water sharks such as Centroscymnus coelolepis (Veríssimo *et al*. 2011) and Centroscymnus crepidater (Cunha *et al*. 2012) showed different structure patterns depending on their distribution scale.

In general, catsharks have a limited ability of sustained swimming due to low tail beat frequency, little passive buoyancy, and a limited production of swim power (Scacco *et al*. 2010). The deep-sea *G. melastomus* counteracts a higher drag than shape comparable shallower species such as *S. canicula* due to the higher density of deep-water masses. Assuming this aspect, *G. melastomus* might have a larger caudal area caused by an adaptation to counteract higher drags. Therefore, the more powerful thrusts of deep-water species can lead to greater dispersal than that from species living in shallow waters (Scacco *et al*. 2010), which can be reflected in the migration events that we have estimated for both species.

The most important and widespread canyon systems of the Mediterranean Sea are located along the western Mediterranean (Würtz 2012). These deep waters may influence the low connectivity of *S. canicula* populations between the Alboran and Balearic sub-basins. A physical barrier to dispersal of the ocean deep has been suggested for other demersal elasmobranchs such as Squatina californica (Gaida 1997) and *Raja clavata* (Chevolot *et al*. 2006). However, occasional migrations between areas may occur for *S. canicula*, which is reflected in the haplotype network (Figure 4.2.2), where we identified haplotypes shared between both sub-basins. Previous genetic studies of *S. canicula* also recorded this fact even between the Atlantic and the Mediterranean Sea (Barbieri *et al*. 2014; Kousteni *et al*. 2014; Gubili *et al*. 2014). This pattern may result from the retention of ancestral polymorphism, secondary contacts, mixed stocks, past population declines, or genetic drift caused by habitat fragmentation (Rozenfeld *et al*. 2008; Kousteni *et al*. 2014). In contrast, the genetic homogeneity found in the *G. melastomus* samples suggests gene flow and high connectivity throughout the western Mediterranean (either currently or in a recent past). Because of the probable high dispersal of this species, the study areas do not seem to present major barriers. In fact, taking into account the wide depth range of *G. melastomus* across the western Mediterranean, from 145 to 1400 m (Moranta *et al*. 1998; Ramírez-Amaro *et al*. 2015), we hypothesize that the deep ocean currents located in the study area [e.g., Levantine Intermediate Water (at 150-600 m depth; Millot 1999) and western Mediterranean Deep Water (at 1000-2000 m depth; Millot 1999)] could promote its dispersal.

The population structure of sharks can be influenced by life-history traits (Veríssimo *et al*. 2011). Despite their similar life-history traits [both are oviparous (producing egg cases throughout the year that are anchored to macroalgae or another kind of substrate); tend to reach an early maturity, have a short generation time, evolve with high population growth rates, and have a continuous reproductive cycle (Camhi *et al*. 1998; Rey *et al*. 2005; Capapé *et al*. 2014; Kousteni *et al*. 2010)], both demersal sharks are likely to have different reproductive strategies, such as site fidelity and philopatry. These life-history traits can also affect genetic structure and connectivity, especially for coastal sharks with a low individual potential for dispersal (Chapman *et al*. 2015). Site fidelity and philopatry based on biological, tagging markers and genetic analysis are well documented for *S. canicula* (Sims *et al*. 2001; Rodríguez-Cabello *et al*. 2004; Kousteni *et al*. 2014). Thus here we propose an effect of regional philopatry on population structure and connectivity for *S. canicula*, as recently proposed for three species of demersal shark from eastern Australia (Corrigan *et al*. 2016). This behaviour describes an individual preference in returning to their region of birth, although not necessarily their exact birthplace, within their region to reproduce (Chapman *et al*. 2015). Regional philopatry, inferred from population genetic analysis, can demonstrate the existence of two or more genetically differentiated populations even if the distance between sampling sites is smaller than the individual dispersal distance (Chapman *et al*. 2015). Our results are in agreement with this approach for *S. canicula*. In contrast, the genetic homogeneity and high connectivity that we found for *G. melastomus* suggest the presence of long-distance dispersal (LDD) events within the span of a generation, thus avoiding site fidelity as recorded in other deep water sharks (Veríssimo *et al*. 2011; Cunha *et al*. 2012).

Contrary to Gubili *et al*. (2014), mtDNA-CR analysis supports a genetic discontinuity of *S. canicula* between the Atlantic Ocean and the Mediterranean Sea populations occurring in the Strait of Gibraltar. Here, we hypothesize an Atlantic transition cluster (i.e., the Portugal and Alboran sub-basins) as an important zone of secondary contact between the North Atlantic (i.e. Bristol, Scotland and western Channel) and the Balearic Islands (GSA05), a scenario that has also been observed in teleost species (Bargelloni *et al*. 2003; Magoulas *et al*. 2006). The Strait of Gibraltar and the adjacent Alboran sub-basin are transition zones between the Atlantic Ocean and the Mediterranean Sea that are influenced by the inflow of Atlantic water and its exchange with Mediterranean waters coming from the Balearic channels (Millot 1999; Baro *et al*. 2012). Given that *S. canicula* has a wide depth range across the Alboran sub-basin [42 to 637 m with an optimal depth of 250 m (Ramírez-Amaro *et al*. 2015)], we suggest that the Strait of Gibraltar would not represent a barrier for the exchange of individuals of this species between the Atlantic Ocean and the Mediterranean Sea. The differences with Gubili *et al*. (2014) may be derived from their lack of samples from such a key area as the Alboran sub-basin. This fact highlights the importance of adequately sampling areas to properly analyze biological questions related to geographic genetic structure (Mona *et al*. 2014).

Concerning the comparison of *S. canicula* between Mediterranean sub-basins, we observed differences between the Iberian coast (GSA06S and GSA06N) and the Balearic Islands (GSA05) based on mtDNA-CR (Table 4.2.7). Further, Kousteni *et al*. (2014) found low genetic differentiation (even no significant differences in mtDNA) between the Balearic and Algerian sub-basins. In the light of our results, considering the geographical location, we suggest that the Balearic Islands could operate as a connectivity area or transition zone between the Balearic and Algerian basins. The Balearic channels are important passages for the exchange of surface water between the cooler and more saline waters of the Balearic sub-basin (in the north of the Balearic archipelago) and the warmer and less saline waters from the Atlantic Ocean through the Algerian sub-basin (to the south of the Balearic archipelago) (Pinot *et al*. 2002). These different oceanographic conditions between both sub-basins strongly affect the community structures (Ramón *et al*. 2014) and dynamics of the trophic webs in these deep ecosystems, as well as their demersal assemblages (Massutí *et al*. 2014). Indeed, transition zones are used as feeding areas (Nielsen *et al*. 2004), and it is worth noting that the Balearic Islands are commonly used as feeding ground for a wide range of species such as nekto-benthic fish (Ordines *et al*. 2015). These facts could promote the potential mixing of *S. canicula* between the different recruitment areas.

### *Contrasting demographic histories*

We found different scenarios of demographic history for *S. canicula* and *G. melastomus* species throughout the study area. The bimodal distribution and the missing intermediate haplotypes [identified by mismatch and haplotype network analyses respectively] found in SC, suggest that both sub-basins have undergone a past bottleneck event. Lastly, our bottleneck tests supported and confirmed this population effect in both sub-basins (Supplementary Table S4.2.6). This population event could have been caused by climatic changes during the Pleistocene glacial/interglacial period. It is known that the different conditions and/or habitat availability during this period caused dramatic shifts of the European coastline (Lykousis 2009). The Alboran and Balearic sub-basins were fragmented to islands delimited by deep marine basins and connected to neighbouring areas by narrow strips of continental shelf. Consequently, the habitat available to *S. canicula* was severely restricted, with a negative effect on its populations, which has also been detected for this species in the Aegean Sea (Kousteni *et al*. 2014). Indeed, it should be noted that genetic diversity can also be influenced by population bottlenecks derived from climate changes (Arenas *et al*. 2012; Arenas *et al*. 2014). Our analyses based on neutrality tests support a recent demographic stability for the Alboran sub-basin and a recent increase in population size for the Balearic sub-basin, which suggests the hypothesis of a fast re-colonisation from the North Atlantic during the current interglacial cycle. The recovery of this species could have been caused by its life-history traits (above discussed) and can also be reflected in the recovery of allelic diversity, which was detected in both populations.

In contrast to *S. canicula*, the unimodal distribution, negative neutrality indices, and starlike haplotype network clearly support a population expansion of *G. melastomus* through the western Mediterranean that started during the Middle Pleistocene. This finding is in agreement with studies based on other elasmobranch species such as *Raja clavata* (Chevolot *et al*. 2006). The complex geomorphology caused by multiple submarine canyons across the study area (Würtz 2012) could provide refuge zones.

### *Implications for management and conservation*

The present study followed several key aspects for the integration of genetic data into fisheries assessment and management of marine species. These include a fine scale sampling design, selection of large, variable markers, and multiple analytical methods (Reiss *et al*. 2009; Ovenden *et al*. 2015). Our results indicate a mismatch between the fisheries management units currently considered in the Mediterranean Sea (General Fisheries Commission for the Mediterranean Geographical Sub-Areas: GFCM-GSAs) and the biological divisions (at population level) of both demersal sharks. In the Western basin, *S. canicula* was composed of two different stocks or management units, whereas *G. melastomus* showed the existence of a unique stock, in contrast to the four GSAs considered in the study area.

Although the GFCM-GSAs are based on political and statistical considerations rather than biological factors (Lleonart and Maynou 2003), they probably follow the concept of the precautionary principle: a spatial separation of assessment and management units based on a smaller scale rather than the “real” spatial population structure would be less detrimental than the opposite (Reiss *et al*. 2009). However, the adoption of genetic and evolutionary criteria in the management of natural resources has led to the recognition of "Management Units" (MUs), which represent functionally independent populations, or groups of populations, characterized by low levels of gene flow (Moritz 1994). If fisheries management does not take into consideration the congruence of spatial scales between population structures it can result in reduced productivity and local decline of populations (Worm *et al*. 2006). It could also result in inflated management procedures beyond requirements, and would not only have significant ecological but also economic consequences. Therefore, the different management units detected in this study for the two species throughout the western Mediterranean should be considered for fisheries assessment and management purposes. Following our study we believe that management and conservation of the species targeted by the bottom trawl fishery developed in the area (e.g. European hake, red mullets and red shrimp) could be improved by contemplating further genetic studies.

Identification of barriers to population connectivity is also essential for species with a limited distribution, in order to maximize population resilience and to reduce their vulnerability to extirpation (Botsford *et al*. 2009). Movements of individuals define a spatial neighbourhood that can help to determine management strategies such as the appropriate location of Marine Protected Areas (MPAs; Palumbi 2003; Soria *et al*. 2014). There are numerous MPAs in the western Mediterranean as well as 7 marine reserves and a National Park in the area of the Balearic Islands (Figure 4.2.1). Mainly for *S. canicula*, the results of our study show the potential value of some of these MPAs that are located at the boundary of distinct oceanographic systems in order to ensure connectivity between individuals from the Atlantic and Mediterranean (Alboran Island) and between the Balearic and Algerian sub-basins (with numerous MPAs in the Balearic Islands). The effectiveness of MPAs for protecting exploited species depends, at least partly, on connectivity, due to its effects on population dynamics and genetics (Gaines *et al*. 2010; Soria *et al*. 2014).

The bottlenecks detected in both populations of *S. canicula* are important for conservation biology because they can lead to inbreeding and the appearance of mildly deleterious alleles, thereby increasing the risk of extinction and compromising adaptive evolutionary potential (Frankham *et al*. 1999; Arenas *et al*. 2012). Many natural populations have experienced bottlenecks due to over-exploitation, habitat destruction, or climate changes (e.g. Arenas *et al*. 2014). The present study shows that *S. canicula* is a species sensitive to past climate changes, which should be taken into account for future evolutionary scenarios. The current climate change, which in the western Mediterranean is causing an increase in sea temperatures of both deep and surface waters (Rohling *et al*. 2014), combined with the impact of fishing exploitation of these species, could entail a threat to these populations.

## Supplementary material

### Tables

Table S4.2.1. Primers and PCR conditions for each mitochondrial region.

| Regions | Primers | PCR condition | | |
|---|---|---|---|---|
| Cytochrome b<br>(Palumbi, 1996) | H15175: 5’-CCCTCAGAATGATATTTGTCCTCA-3’<br>L14724: 5’-TGACTTGAARAACAYCGTTG-3’ | 96.0ºC<br>94.0ºC<br>56.0ºC<br>72.0ºC<br>72.0ºC | 5:00<br>1:00<br>1:00<br>1:00<br>10:00 | 35c |
| Dehydrogenase subunit 2<br>(Sandoval-Castillo and Rocha-Olivares, 2011) | ND2Met47: 5’-TTTTGGGCCCATACC-3’<br>ND2Trp18: 5’-GCTTTGAAGGCTTTTGGT-3’ | 95.0ºC<br>94.0ºC<br>54.0ºC<br>72.0ºC<br>72.0ºC | 2:00<br>0:14<br>1:00<br>2:00<br>6:00 | 35c |
| Cytochrome oxidase subunit I<br>(Ward *et al*. 2005) | FishF1: 5’-TCAACCAACCACAAAGACATTGGCAC-3’<br>FishF2: 5’-TCGACTAATCATAAAGATATCGGCAC-3’<br>FishR1: 5’-TAGACTTCTGGGTGGCCAAAGAATCA-3’<br>FishR2: 5’-ACTTCAGGGTGACCGAAGAATCAGAA-3’ | 95.0ºC<br>94.0ºC<br>54.0ºC<br>72.0ºC<br>72.0ºC | 2:00<br>0:30<br>0:30<br>1:00<br>10:00 | 35c |
| Control region<br>For SC<br>(Gubili *et al*. 2014)<br>For GM<br>(designed in the present thesis study) | ScyD1pF: 5’-ATGACATGGCCCACATATCC-3’<br>Scan2R: 5’-TTCTCTTCTCAAGACCGGGTA-3’<br>GmF: 5’- GTGATATAGCCCACATACCTTA-3’<br>GmR: 5’- TTTCTCTAATTCTCGGAGGG-3’ | 95.0ºC<br>94.0ºC<br>54.0ºC<br>72.0ºC<br>72.0ºC | 2:00<br>0:14<br>1:00<br>2:00<br>6:00 | 35c |

Table S4.2.2. Fluorescent primers for microsatellite multiplex PCR.

| Primer name | Sequence (5' to 3') | Fluorescent 5' label |
|---|---|---|
| Scan02F | TGC AGC TTC GCT ATC TTA TGC | 6FAM<br>VIC |
| Scan03F | TGG ATA ATT GAC AGA ATC GGC | |
| Scan15F | TCA TCA TCA TCA CCA CCA GAA | |
| Scan09F | GAG AAT TGT TTC CCA GTG GC | |
| Scan07F | CCT GCA CAC AGC AGG TAG AA | |
| Scan12F | GCC AGT GGC TAT AAC GGA AC | NED |
| Scan13F | CGC CCT CCC CTA AAA TAG AC | |
| Scan04F | ACC AAA GAT CAA GCC AGG AA | PET |
| Scan16F | CCG ACT CCT TTG GAT GTG TT | |
| Scan06F | GGC AGT GAT TGC ATT CTT GA | |
| Scan10F | CGG TAC GAC CTG GAT CAA CT | |
| Scan14F | AAC CAT CCT CCG CAA ATA AA | |

Table S4.2.3. Biological database from the genetic samples of *S. canicula* and *G. melastomus* of COI sequences available at http://www.boldsystems.org/. F: female; M: male; Lat: latitude; Long: longitude.

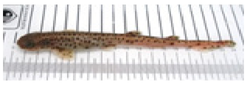
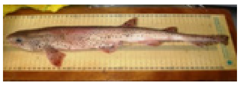
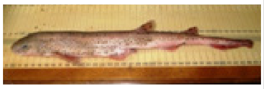
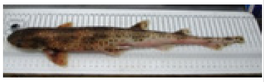
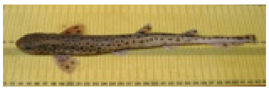
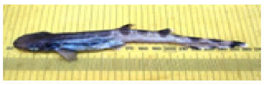
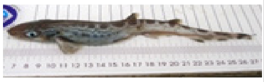
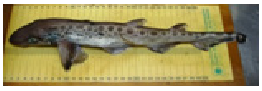
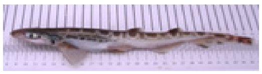
| Species name | ID's | Depth (m) | Length (cm) | Sex | Lat | Long | GenBank ID's | Picture |
|---|---|---|---|---|---|---|---|---|
| *Scyliorhinus canicula* | SC7 | 359 | 17 | F | 36.276 | -5.081 | KX283359 |  |
| *Scyliorhinus canicula* | SC10 | 559 | 43.3 | F | 36.389 | -4.534 | KX283360 |  |
| *Scyliorhinus canicula* | SC21 | 127 | 39.1 | M | 35.930 | -3.101 | KX283361 |  |
| *Scyliorhinus canicula* | SC26 | 328 | 51 | M | 36.008 | -2.790 | KX283362 |  |
| *Scyliorhinus canicula* | SC49 | 107 | 35.5 | F | 37.723 | -0.554 | KX283363 |  |
| *Scyliorhinus canicula* | SC51 | 247 | 37.5 | F | 37.663 | -0.356 | KX283364 |  |
| *Scyliorhinus canicula* | SC123 | 113 | 47.5 | M | 41.073 | 1.476 | KX283365 |  |
| *Scyliorhinus canicula* | SC127 | 87 | 47.5 | F | 42.286 | 3.333 | KX283366 |  |
| *Scyliorhinus canicula* | SC134 | 336 | 47 | M | 41.485 | 3.176 | KX283367 |  |
| *Scyliorhinus canicula* | SC182 | 150 | 25 | M | 40.024 | 3.560 | KX283368 |  |
| *Galeus melastomus* | GM7 | 359 | 17.5 | M | 36.276 | -5.081 | KX283369 |  |
| *Galeus melastomus* | GM8 | 762 | 39 | F | 36.291 | -4.775 | KX283370 |  |
| *Galeus melastomus* | GM23 | 541 | 34 | F | 35.882 | -3.270 | KX283371 |  |
| *Galeus melastomus* | GM26 | 328 | 18.6 | M | 36.008 | -2.790 | KX283372 |  |
| *Galeus melastomus* | GM28 | 530 | 33.3 | F | 36.603 | -3.581 | KX283373 |  |
| *Galeus melastomus* | GM81-2 | 670 | 22.3 | F | 39.045 | 0.433 | KX283374 |  |
| *Galeus melastomus* | GM125 | 314 | 25 | M | 40.967 | 1.466 | KX283375 |  |
| *Galeus melastomus* | GM126 | 511 | 20.3 | M | 40.866 | 1.433 | KX283376 |  |
| *Galeus melastomus* | GM132 | 608 | 32 | M | 41.458 | 3.313 | KX283377 |  |
| *Galeus melastomus* | GM150 | 615 | 20.6 | F | 39.119 | 2.580 | KX283378 |  |

Table S4.2.4. Allelic frequency of twelve microsatellite loci in *Scyliorhinus canicula* for the Alboran (A-sb) and Balearic (B-sb) sub-basins.

| N Allele | Scan02 | | Scan03 | | Scan04 | | Scan06 | | Scan07 | | Scan09 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **A-sb** | **B-sb** | **A-sb** | **B-sb** | **A-sb** | **B-sb** | **A-sb** | **B-sb** | **A-sb** | **B-sb** | **A-sb** | **B-sb** |
| 100 | | | | | | | | | | | 0,425 | 0,4 |
| 102 | | | | | | | | | | | 0,4 | 0,392 |
| 103 | | | | | | | | | | | 0 | 0,008 |
| 104 | 0,025 | 0 | | | | | | | | | 0,05 | 0,117 |
| 106 | 0,025 | 0,017 | | | | | | | | | 0,025 | 0,008 |
| 108 | 0 | 0,008 | | | | | | | | | 0,1 | 0,067 |
| 110 | | | | | | | | | | | 0 | 0,008 |
| 112 | 0,1 | 0,125 | | | | | 0,875 | 0,85 | | | | |
| 114 | 0,3 | 0,267 | | | | | 0,125 | 0,15 | | | | |
| 116 | 0,125 | 0,067 | | | | | | | | | | |
| 118 | 0,1 | 0,225 | | | | | | | | | | |
| 120 | 0,125 | 0,092 | | | | | | | | | | |
| 122 | 0,1 | 0,133 | | | | | | | | | | |
| 124 | 0,075 | 0,042 | | | | | | | | | | |
| 126 | 0,025 | 0,025 | | | | | | | | | | |
| 166 | | | 0,05 | 0,075 | | | | | | | | |
| 168 | | | 0,425 | 0,392 | | | | | | | | |
| 170 | | | 0,025 | 0,125 | | | | | | | | |
| 172 | | | 0,075 | 0,058 | | | | | | | | |
| 174 | | | 0,125 | 0,117 | | | | | | | | |
| 176 | | | 0,1 | 0,117 | | | | | | | | |
| 178 | | | 0,05 | 0,1 | | | | | | | | |
| 180 | | | 0,05 | 0,008 | | | | | | | | |
| 182 | | | 0,1 | 0 | | | | | | | | |
| 184 | | | 0 | 0,008 | | | | | | | | |
| 226 | | | | | 0 | 0,008 | | | | | | |
| 228 | | | | | 0,075 | 0,067 | | | 0 | 0,026 | | |
| 230 | | | | | 0,05 | 0,008 | | | | | | |
| 232 | | | | | 0 | 0,008 | | | 0 | 0,043 | | |
| 234 | | | | | 0,225 | 0,217 | | | 0,45 | 0,509 | | |
| 236 | | | | | 0,45 | 0,425 | | | 0,35 | 0,121 | | |
| 238 | | | | | 0,125 | 0,175 | | | 0,15 | 0,129 | | |
| 240 | | | | | 0,05 | 0,05 | | | 0,025 | 0,086 | | |
| 242 | | | | | 0,025 | 0,042 | | | 0,025 | 0,034 | | |
| 244 | | | | | | | | | 0 | 0,009 | | |
| 248 | | | | | | | | | 0 | 0,017 | | |
| 250 | | | | | | | | | | | | |
| 252 | | | | | | | | | 0 | 0,026 | | |

Table S4.2.4. (continued)

| N Allele | Scan10 | | Scan12 | | Scan13 | | Scan14 | | Scan15 | | Scan16 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A-sb | B-sb | A-sb | B-sb | A-sb | B-sb | A-sb | B-sb | A-sb | B-sb | A-sb | B-sb |
| 108 | | | | | | | | | 0 | 0,009 | | |
| 110 | | | | | | | | | 0 | 0,035 | | |
| 112 | | | | | | | | | 0,325 | 0,184 | | |
| 114 | | | | | | | | | 0,025 | 0,044 | | |
| 116 | | | | | | | | | 0,6 | 0,658 | | |
| 118 | | | | | | | | | 0,05 | 0,07 | | |
| 120 | | | | | | | | | 0 | 0 | | |
| 170 | 0 | 0,025 | | | | | | | | | | |
| 172 | 0 | 0,008 | | | | | | | | | | |
| 174 | 0,175 | 0,025 | | | | | | | | | | |
| 176 | 0,025 | 0 | | | | | | | | | | |
| 182 | 0,525 | 0,85 | | | | | | | | | | |
| 184 | 0,175 | 0,067 | | | | | | | | | | |
| 186 | 0,075 | 0,017 | | | | | | | | | | |
| 188 | 0,025 | 0 | | | | | | | | | | |
| 190 | 0 | 0,008 | | | | | | | | | | |
| 212 | | | | | | | 0,375 | 0,308 | | | | |
| 214 | | | | | | | 0,025 | 0,017 | | | | |
| 216 | | | | | | | 0,15 | 0,108 | | | | |
| 218 | | | | | | | 0,125 | 0,217 | | | | |
| 220 | | | | | | | 0,225 | 0,167 | | | | |
| 222 | | | | | | | 0,075 | 0,117 | | | | |
| 224 | | | | | | | 0 | 0,008 | | | | |
| 226 | | | | | | | 0 | 0,042 | | | | |
| 228 | | | | | | | 0,025 | 0 | | | | |
| 232 | | | | | | | 0 | 0,008 | | | | |
| 234 | | | 0,025 | 0,008 | | | | | | | | |
| 236 | | | 0,475 | 0,617 | | | | | | | | |
| 242 | | | 0,375 | 0,342 | | | | | | | | |
| 244 | | | 0,125 | 0,033 | | | | | | | | |
| 248 | | | | | | | | | 0 | 0,009 | | |
| 250 | | | | | | | 0 | 0,008 | | | | |
| 252 | | | | | 0 | 0,008 | | | 0 | 0,035 | | |
| 254 | | | | | 0,225 | 0,233 | | | 0,325 | 0,184 | | |
| 256 | | | | | 0 | 0,008 | | | 0,025 | 0,044 | | |
| 258 | | | | | 0,075 | 0,083 | | | 0,6 | 0,658 | | |
| 260 | | | | | 0,2 | 0,183 | | | 0,05 | 0,07 | | |
| 262 | | | | | 0,225 | 0,192 | | | | | | |
| 264 | | | | | 0,175 | 0,242 | | | | | | |
| 266 | | | | | 0,1 | 0,017 | | | | | | |
| 268 | | | | | 0 | 0,008 | | | | | | |
| 270 | | | | | 0 | 0,008 | | | | | | |
| 272 | | | | | 0 | 0,017 | | | | | 0 | 0,008 |
| 274 | | | | | | | | | | | 0,025 | 0,017 |
| 276 | | | | | | | | | | | 0,025 | 0,05 |
| 278 | | | | | | | | | | | 0,5 | 0,367 |
| 280 | | | | | | | | | | | 0,325 | 0,45 |
| 282 | | | | | | | | | | | 0,125 | 0,075 |
| 284 | | | | | | | | | | | 0 | 0,033 |

Table S4.2.5. Population genetic parameters summary statistic for each microsatellite locus. Number of alleles (Na), allelic richness (Rs), observed (Ho) and expected (He) heterozygosities and probability of conformance to Hardy-Weinberg (HWE).

| Area | | Sca02 | Sca03 | Sca04 | Sca06 | Sca07 | Sca09 | Sca10 | Sca12 | Sca13 | Sca14 | Sca15 | Sca16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GSA01 (Alboran sub-basin) | Na | 10 | 9 | 9 | 2 | 10 | 7 | 7 | 4 | 11 | 10 | 6 | 7 |
| | Rs | 5.858 | 4.642 | 3.744 | 1.342 | 3.309 | 3.015 | 1.372 | 2.007 | 5.236 | 5.067 | 2.106 | 2.886 |
| | Ho | 0.883 | 0.650 | 0.650 | 0.233 | 0.466 | 0.650 | 0.200 | 0.433 | 0.867 | 0.800 | 0.439 | 0.550 |
| | He | 0.829 | 0.785 | 0.733 | 0.255 | 0.698 | 0.668 | 0.271 | 0.502 | 0.809 | 0.803 | 0.525 | 0.653 |
| | HWE | 0.501 | 0.092 | 0.853 | 0.510 | 0.001 | 0.162 | 0.000 | 0.716 | 0.997 | 0.999 | 0.000 | 0.190 |
| GSA05 | Na | 6 | 8 | 5 | 2 | 7 | 4 | 3 | 3 | 9 | 7 | 2 | 6 |
| | Rs | 3.738 | 3.941 | 2.556 | 1.600 | 3.574 | 2.730 | 1.436 | 2.005 | 4.469 | 5.031 | 1.471 | 3.101 |
| | Ho | 0.900 | 0.650 | 0.550 | 0.400 | 0.421 | 0.650 | 0.150 | 0.300 | 0.800 | 0.800 | 0.200 | 0.500 |
| | He | 0.733 | 0.746 | 0.609 | 0.375 | 0.720 | 0.634 | 0.304 | 0.501 | 0.776 | 0.801 | 0.320 | 0.678 |
| | HWE | 0.770 | 0.617 | 0.684 | 0.766 | 0.038 | 0.038 | 0.000 | 0.197 | 0.971 | 0.977 | 0.094 | 0.114 |
| GSA06S | Na | 10 | 8 | 8 | 2 | 9 | 5 | 6 | 3 | 6 | 7 | 6 | 6 |
| | Rs | 6.452 | 5.714 | 4.444 | 1.220 | 2.188 | 2.878 | 1.455 | 1.942 | 5.031 | 5.333 | 2.531 | 2.837 |
| | Ho | 0.900 | 0.550 | 0.700 | 0.200 | 0.474 | 0.650 | 0.250 | 0.500 | 0.900 | 0.850 | 0.500 | 0.650 |
| | He | 0.845 | 0.825 | 0.775 | 0.180 | 0.543 | 0.653 | 0.313 | 0.485 | 0.801 | 0.813 | 0.605 | 0.648 |
| | HWE | 0.988 | 0.019 | 0.963 | 0.619 | 0.176 | 0.135 | 0.155 | 0.782 | 0.232 | 0.702 | 0.790 | 0.978 |
| GSA06N | Na | 7 | 7 | 5 | 2 | 6 | 6 | 3 | 3 | 7 | 7 | 5 | 4 |
| | Rs | 5.797 | 4.020 | 3.687 | 1.220 | 3.791 | 2.952 | 1.225 | 2.036 | 4.598 | 4.188 | 2.407 | 2.367 |
| | Ho | 0.850 | 0.750 | 0.700 | 0.100 | 0.500 | 0.650 | 0.200 | 0.500 | 0.900 | 0.750 | 0.632 | 0.500 |
| | He | 0.828 | 0.751 | 0.729 | 0.180 | 0.736 | 0.661 | 0.184 | 0.509 | 0.783 | 0.761 | 0.584 | 0.578 |
| | HWE | 0.871 | 0.870 | 0.806 | 0.047 | 0.150 | 0.063 | 0.970 | 0.676 | 0.460 | 0.229 | 0.005 | 0.833 |
| Balearic sub-basin | Na | 10 | 9 | 9 | 2 | 10 | 7 | 7 | 4 | 11 | 10 | 6 | 7 |
| | Rs | 5.858 | 4.642 | 3.744 | 1.342 | 3.309 | 3.015 | 1.372 | 2.007 | 5.236 | 5.067 | 2.106 | 2.886 |
| | Ho | 0.883 | 0.650 | 0.650 | 0.233 | 0.466 | 0.650 | 0.200 | 0.433 | 0.867 | 0.800 | 0.439 | 0.550 |
| | He | 0.829 | 0.785 | 0.733 | 0.255 | 0.698 | 0.668 | 0.271 | 0.502 | 0.809 | 0.803 | 0.525 | 0.653 |
| | HWE | 0.501 | 0.092 | 0.853 | 0.510 | 0.001 | 0.162 | 0.000 | 0.716 | 0.997 | 0.999 | 0.000 | 0.190 |

Table S4.2.6. Wilcoxon and sign test probabilities (*P* values) obtained using the stepwise (SMM) and two-phase (TPM) mutation models implemented in the program BOTTLENECK for the SC Alboran and Balearic sub-basin populations.

| **Mutation model** | **Wilcoxon test (*P* values)** | | **Sign test (*P* values)** | |
|---|---|---|---|---|
| | Alboran sub-basin | Balearic sub-basin | Alboran sub-basin | Balearic sub-basin |
| SMM | <0.01 | <0.001 | <0.01 | <0.001 |
| TPM80 | 0.622 | 0.063 | 0.182 | 0.075 |
| TPM90 | 0.203 | <0.01 | 0.069 | <0.01 |
| TPM95 | 0.077 | <0.001 | <0.05 | <0.01 |
| TPM99 | <0.05 | <0.0001 | <0.01 | <0.001 |

## Figures

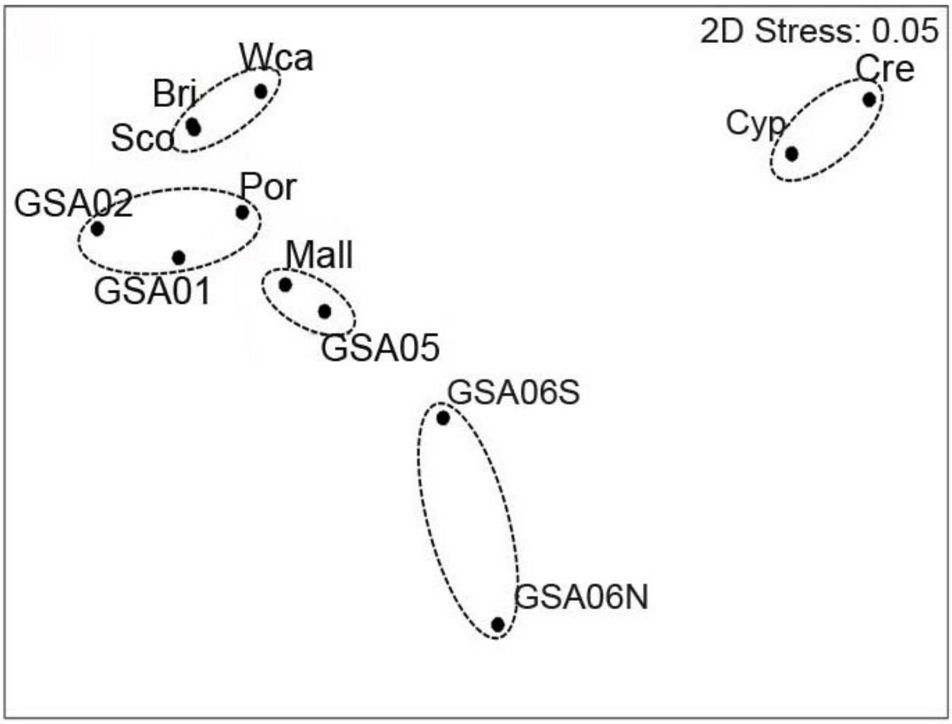


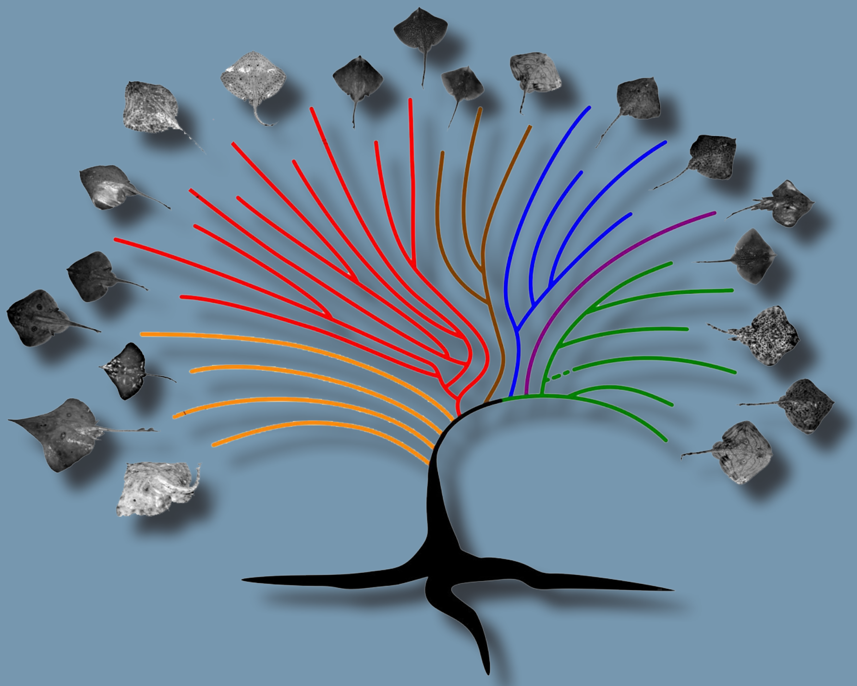
Figure S4.2.1. Multidimensional scaling analysis (MDS) plot for *Scyliorhinus canicula* using pairwise $\Phi_{ST}$ distances with mtDNA-CR data. Localities are named as: Bri: Bristol; Sco: Scotland; Wca: western Channel; Por: Portugal; Mall: Mallorca; Cyp: Cyprus; Cre: Crete.

# CHAPTER III

## 4.3 PHYLOGENY AND TAXONOMY

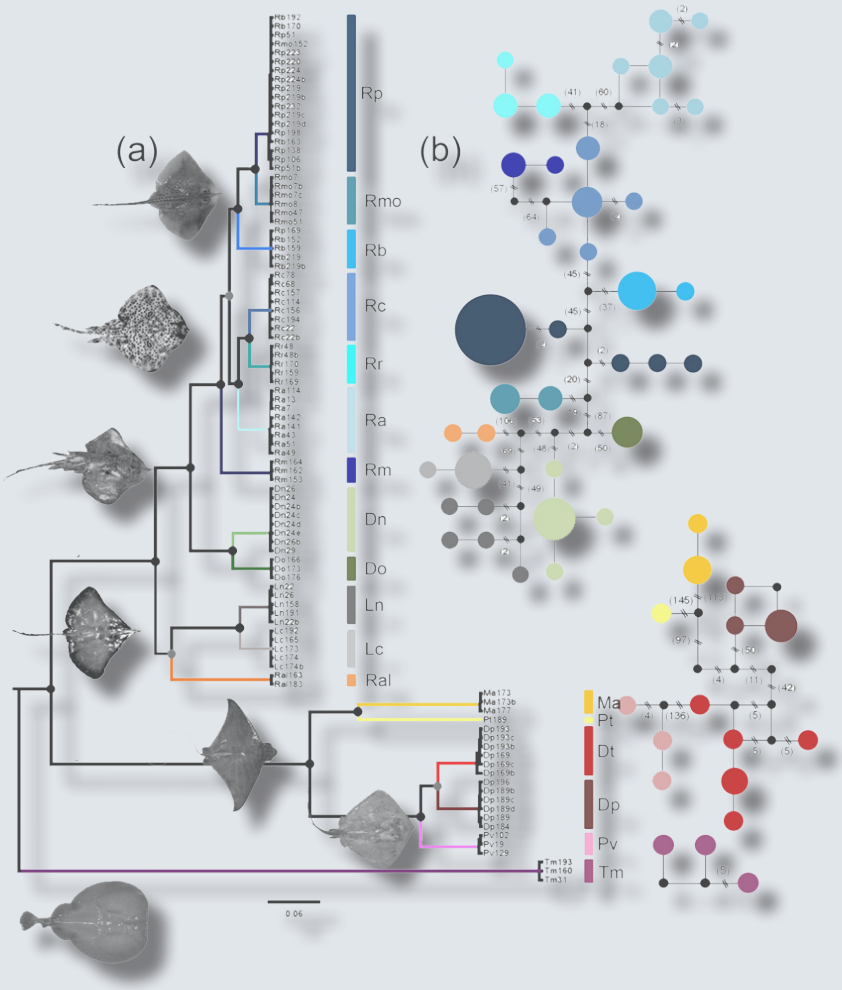
Ramírez-Amaro S., Ordines F., Picornell A., Castro J.A., Ramon C., Massutí E., Terrasa B. (Submitted). The evolutionary history of Mediterranean Batoidea (Chondrichthyes: Neoselachii): a molecular phylogenetic approach.

Ramírez-Amaro S., Ordines F., Puerto M.A., García C., Ramon C., Terrasa B., Massutí E. (2017). New morphological and molecular evidence confirm the presence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea and extend its distribution to the western basin. *Mediterranean Marine Science* 18/2: 253–261.



### 4.3.1 The evolutionary history of Mediterranean Batoidea (Chondrichthyes: Neoselachii): a molecular phylogenetic approach

Ramírez-Amaro S., Ordines F., Picornell A., Arenas M., Castro J.A., Massutí E., Ramon C., Terrasa B. (Submitted).



## Summary

Batoidea (rays, skates, and their relatives) is one of the largest groups included in elasmobranchs. Mediterranean Batoidea fauna is relatively diverse, characterized by a history of isolation and connectivity resulting from tectonic movements and changes in ocean circulation. The evolutionary histories of Batoidea species in the Mediterranean were provided from two mitochondrial markers (*COI* and *ND2*) through dating and Bayesian analyses. Additionally, two species delimitation analyses (Poisson Tree Process and General Mixed Yule Coalescent) were conducted to elucidate species boundaries. The relationship between *Raja* species – their ecological traits and species level phylogeny, – was also studied to understand speciation in this genus. Results concerning the phylogenetic relationship between Batoidea species were largely consistent with recent molecular studies. Divergence times showed a parallel evolutionary history of Batoidea fauna and Mediterranean history, which began from the Cretaceous. Two important speciation events were detected: i) at the Eocene-Oligocene transition, when almost all genera of Batoidea were originated; and ii) during the Miocene, when almost all current species of Batoidea appeared. These diversification events correspond to crucial paleo-climatic and paleo-geographical events that took place in the Mediterranean. Phylogenetic and species delimitation analyses revealed the presence of 18 species, including the confirmation of *Dasyatis* cf. *tortonesei* as a valid species. *Raja* species showed different ecological preferences related to depth and habitat, which indicated a genus speciation process driven by different habitat characteristics.

**Keywords**: Batoidea, phylogeography events, mtDNA, *Raja*, speciation, *Dasyatis* cf. *tortonesei*.

### 4.3.1.1 Introduction

One of the most important aims in evolutionary biology is the identification of patterns and causes of speciation and reproductive isolation among related taxa (Mayr 1947; Coyne and Orr, 2004). Speciation occurs when populations evolve as independent gene pools, due to the accumulation of enough reproductive isolating barriers (Mayr 1947). However, the scarcity of geographical barriers to gene flow in marine ecosystems poses a challenge to the traditional view of speciation, with emphasis on geographical isolation and the formation of vicariant populations (e.g. allopatric, parapatric or peripatric speciation; Palumbi, 1994). Ecological speciation with gene flow may involve divergence between macrohabitats or along environmental gradients, such as depth or partitioning of trophic resources and microhabitats (Ackerly *et al*., 2006; Ingram, 2011).

Batoidea (rays, skates and their allies; hereafter "batoids") are a monophyletic group (Douady *et al*., 2003) with around 630 species, representing more than half of chondrichthyan diversity, from a total of 1188 species (Weigmann, 2016). Like most chondrichthyans, the life history traits of batoids are characterised by late sexual maturity, slow growth rates, low fecundity, and small litters, which makes them especially vulnerable to fishing exploitation (Frisk *et al*., 2001). Batoids have long been regarded as a hotspot in the systematic of elasmobranchs (Last *et al*., 2016), as they are morphologically diverse, but all have dorso-ventrally flattened bodies and ventrally located gill slits (Ebert and Winton, 2010). Molecular analysis supports batoids originating between 200 and 230 Mya (Aschliman *et al*., 2012). The first known rays were the Rhinobatoids (guitarfishes) which appeared during the Upper Jurassic (Compagno, 1977), whereas the derivation of the rest remaining major lineages of batoids occurred at about 140 Mya, at the transition between the end of the Upper Jurassic and the beginning of the Lower Cretaceous, and their distribution should reflect the breakup of Gondwana during the latter part of the Mesozoic Era (Pitman *et al*., 1993; Aschliman *et al*., 2012). At that time, most of the shallow continental shelves were associated to the Tethys Sea, a major tropical waterway extending from the central Atlantic opening to what is today the western Pacific (Aschliman *et al*., 2012). In this sea, most groups of extant batoids appear in similar habitats, or are thought to have originated in shallow environments (McEachran and Aschliman, 2004).

The Mediterranean used to be of Tethys (Cretaceous; 145-65 million years ago "Mya"), and was connected with the Atlantic and Indo-Pacific oceans (Meynard *et al*., 2012). At present, this sea is surrounded by land, with only one opening to the Atlantic through the Strait of Gibraltar (15 km wide and 290 m deep), another to the Black Sea through the Strait of Dardanelles (7 km wide and an average depth of 55 m), and a third to the Red Sea through the Suez Canal. Geological and oceanographic processes, such as those involved with the origin of the Mediterranean, have been shown to globally affect its fish diversity (Friedman, 2010; Lasram *et al*., 2010). In this region, batoids are characterized by a relatively high diversity, with 37 species recorded, up to four of which could be considered endemic (*Leucoraja melitensis*, *Raja polystigma*, *Raja radula*, *Mobula mobular*), while the other species are also distributed through the Northeast Atlantic (Bradai *et al*., 2012).

Measurements of genetic variation of batoids, based on mitochondrial DNA (mtDNA), have contributed to know the extent of their taxonomic divergence, diversity and phylogeny (Aschliman *et al*., 2012), as well as the reproductive isolation between their species (Pasolini *et al*., 2011). It has also allowed to identify cryptic species, such as those in the *Dipturus batis* complex (Griffiths *et al*., 2010; Iglésias *et al*., 2010), and to elucidate taxonomic uncertainties between species, such as *Raja maderensis* and *R. clavata* (Ball *et al*., 2016). Particularly, mtDNA markers like cytochrome oxidase subunit 1 (*COI*, commonly denominated DNA barcoding; Hebert *et al*., 2003) and dehydrogenase subunit 2 (*ND2*) have proved to be very useful to discriminate between species in the Order Rajiformes (Serra-Pereira *et al*., 2011; Coulson *et al*., 2011; Naylor *et al*., 2012a), to delineate species relationships (Richards *et al*., 2009) and to estimate levels of divergence (Naylor *et al*., 2012b).

Few approaches have addressed the phylogeny of batoids. Valsechcchi *et al*. (2005) studied the phylogenetic relationship of Mediterranean Rajidae species, whereas Aschliman *et al*. (2012) performed a complete phylogeny of batoids using mitochondrial genomes and nuclear genes, but Mediterranean species were not included. The study of batoid phylogeny in an area with such a complex paleogeographic history as the Mediterranean can help to understand the processes underlying their diversification. Thus, the aims of the present study were i) to establish the phylogenetic relationship and divergence time in relation to historical evolutionary events of Batoidea species in the Mediterranean, and ii) to use the sympatric *Raja* species as a model of speciation, based on different habitat preferences.

## 4.3.1.2 Material and methods

*Sample collection*

Batoid samples were collected from 41 to 810 m depth during the International Bottom Trawl Survey MEDITS (see Bertrand *et al*., 2002 sampling protocol applied) carried out between 2013 and 2016 along the western Mediterranean off the Iberian Peninsula and the Balearic Islands (Figure 4.3.1.1). Batoid species were identified following Capapé (1997), Whitehead *et al*. (1983), and Ebert and Stehmann (2013). A total of 98 specimens from 17 putative species were collected (Table 4.3.1.1). Information on sampling locations, size, sex and pictures of these specimens are available in the project "Improvement in the Mediterranean Batoidea Identification" in the Barcode of Life Data System (BOLD) (http://www.boldsystems.org; Ratnasingham and Hebert, 2007; Supplementary Table S4.3.1.1).

*Laboratory procedures*

A small piece of muscular tissue from the pectoral or dorsal fins was excised from each specimen and stored at 4°C in absolute ethanol. Genomic DNA was extracted by a standard phenol-chloroform method (Terrasa *et al*., 2009). *COI* and *ND2* mtDNA genes were amplified by polymerase chain reaction (PCR). Primers and PCR conditions are described in Supplementary Table S4.3.1.2. PCR products were purified using the QIAquick® PCR Purification Kit (QIAGEN). Both heavy and light strands were sequenced on an automated ABI 3130 sequencer using the ABI Prism Terminator BigDye® Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City CA, USA).

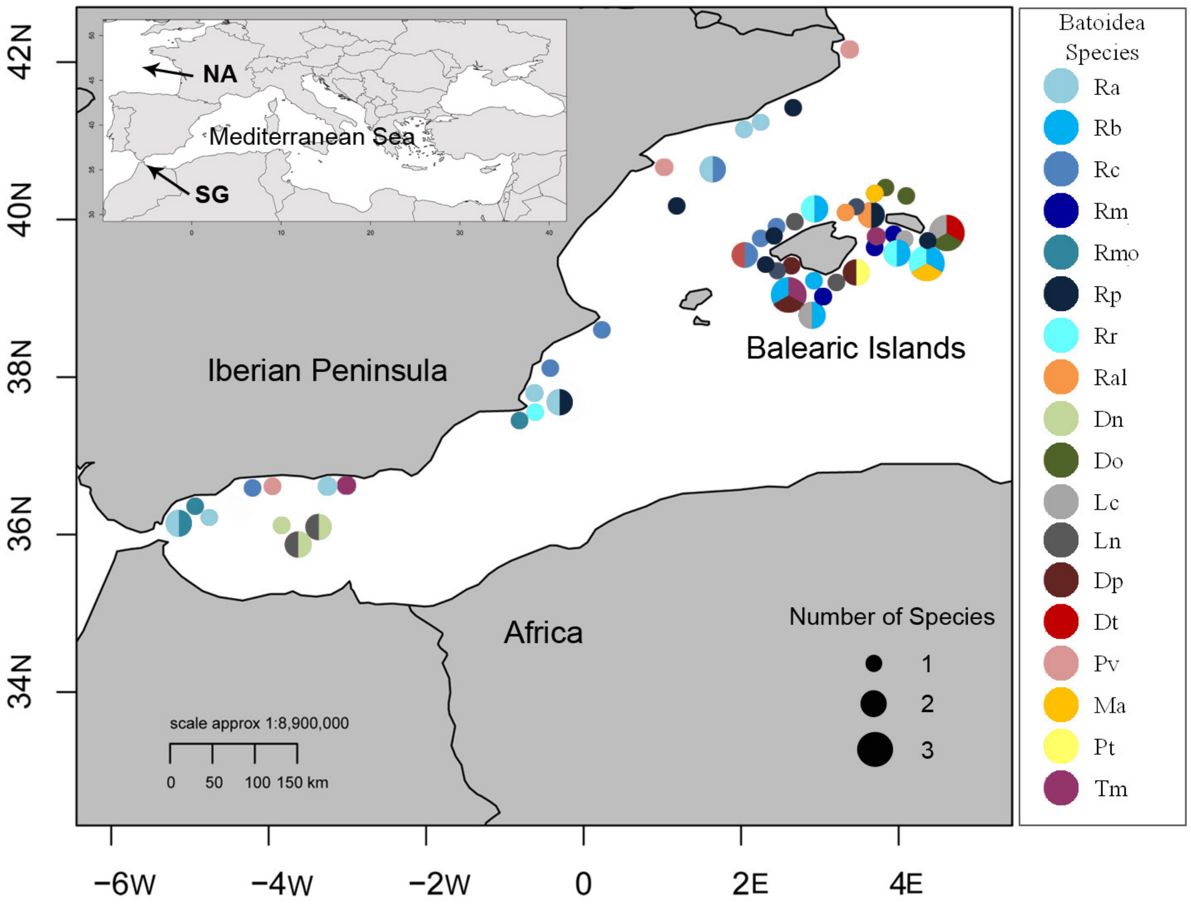


Figure 4.3.1.1. Location of Batoidea samples obtained during the MEDITS bottom trawl survey, carried out from 2013 to 2016 along the western Mediterranean. Species nomenclature is indicated in Table 4.3.1.1. Arrows indicate the Strait of Gibraltar (SG) and Northeast Atlantic (NA).

Sequences were imported into BioEdit 7.0.5.2. (Hall, 1999) and checked for quality and accuracy with nucleotide base assignment. Multiple sequence alignments (MSA) were obtained with ClustalW (Thompson *et al*., 1994). Both fragments were concatenated for data analysis.

### *Phylogenetic analysis, haplotype network and genetic diversity*

A phylogenetic analysis was performed using MrBayes v.3.2 (Ronquist *et al*., 2011). The general time reversible plus gamma distribution and proportion of invariable sites model (GTR+I+G) was assigned as the optimal substitution model of molecular evolution. This model was selected followed Bayesian criteria (BIC) using jModelTest v.2 (Darriba *et al*., 2012). Bayesian MCMC analysis was conducted in parallel with random starting trees, run for 10 M generations and sampled every 1000 generations. Chain was estimated by stable split-standard deviations (between the two runs) and stable sampled log likelihood values. Burn-in data sampled from generations preceding this point were discarded. Bayes factors were calculated with TRACERv.1.5 (Rambaut and Drummond, 2009). Posteriori analyses were performed according to Bayesian phylogenetic results.

Additionally, to compare the Dasyatis samples of this study with other species belonging to Dasyatidae from the Mediterranean and Northeast Atlantic, *COI* sequences from GenBank were used to perform a Bayesian analysis. Further, a median joining network for the mtDNA haplotypes was inferred for all batoids with Network v.5.0 (Bandelt *et al*. 1999).

Nucleotide diversity indices, number of segregating sites (S), number of haplotypes (Nh), haplotype diversity (h), and nucleotide diversity (π) were calculated with DnaSP v5 (Librado and Rozas, 2009). Evolutionary divergence between pair of species was calculated using the maximum composite method, with MEGAv.6 (Tamura *et al*., 2013). Identity percentage and numbers of base differences between pair of sequences of species were also estimated with Geneious v. 7.1.3 software (Biomatters Ltd.; www.geneious.com).

### *Divergence time estimation*

Both data sets, *COI* and *ND2*, were tested for nucleotide substitution saturation using the test by Xia *et al*. (2003) implemented in DAMBE5 (Xia, 2013). The test revealed no significant saturation, so both fragments were used for the subsequent steps. Divergence times were estimated using a multispecies coalescent model (*beast) in a Bayesian framework with *BEAST v1.8.3 (Heled and Drummond, 2010; Drummond *et al*., 2012). Concatenated mtDNA fragment was analysed using a relaxed clock (Drummond *et al*., 2006), GTR+I+G substitution model, and the Yulee process tree prior. We provided a prior on node a calibration time of the Myliobatiformes (Myliobatidae/Dasyatidae) proposed by Aschliman *et al*. (2012), with a mean of 70 +/-5.0 Mya. The species were joined at genus level. The analysis started with a random tree, with the MCMC chain being run for 600 M generations, and sampled every 10000 steps. Convergence was assessed by effective sample size (ESS) calculation and was visualized using TRACERv.1.5. TreeAnnotator v1.8.3 (BEAST package) was used to combine the maximum sum of clades credibility criteria and for analysing the posterior tree samples.

### *Species delimitation*

To elucidate species boundaries within Mediterranean batoids, species delimitation analyses were performed using the Poisson Tree Process (PTP; Zhang *et al*., 2013) and the General Mixed Yule Coalescent (GMYC; Pons *et al*., 2006) methods. The PTP method was implemented using the Bayesian tree performed in MrBayes as input data, and calculations were conducted on the bPTP webserver (http://species.h-its.org/ptp/), with 250.000 MCMC generations, thinning set to 100 and burning at 25% and performing a Bayesian search. For the GMYC method the Bayesian tree performed in BEAST was used as input data. This method was carried out in R 3.3.1 (R Development Core Team) using the splits (Ezard *et al*., 2009) and ape (Paradis *et al*., 2004) packages. Numbers of clusters were estimated by single threshold optimisation.

*Speciation and habitat preferences*

In order to understand the speciation process of batoid species, the bathymetric distribution and habitat preferences of the sympatric species of the genus *Raja* were analysed. The bathymetric distribution of each *Raja* species was modelled through generalized linear models (GLM) and linear regression, using the standardised abundance (individuals/km$^2$) as the dependent variable, and depth of the sampling station as the explanatory variable. The response type of a particular species, uni-modal (bell-shape) or linear, was chosen on the basis of the lowest value of the Akaike information criterion (AIC), as a measure of the parsimony of each regression model (Ter Braak and Smilauer, 2002).

In the Balearic Islands, the highest abundance and diversity of *Raja* species (Ordines *et al*., 2011; Ramírez-Amaro *et al*., 2015), along with the presence of well-characterised macro-epibenthic assemblages (Ordines and Massutí, 2009) enabled their habitat preferences to be studied. *R. montagui* and *R. asterias* were not included in this analysis, because they were not captured in the area; the only samples of these species were obtained off the Iberian Peninsula. For this, a canonical Redundancy analysis (RDA) was carried out using the CANOCO v.4.5 package (Ter Braak and Smilauer, 2002). In this analysis, the standardised abundance (individuals/km$^2$) of *Raja* species was set as the dependent variable, whereas type of habitat (categorical variable) and depth (continuous variable) of the sampling station were set as the explanatory variables. To reduce the effect of abundant species, the standardised abundance was log-transformed. The benthic habitats considered were those identified by Ordines and Massutí (2009) in the circalittoral soft bottoms around the Balearic Islands: *Peyssonnelia* beds (PB), maërl beds (MB), shallow shelf sandy-mud bottom (SSM), deep shelf sandy-mud bottom (DSM) and crinoid beds (CB). Significance of environmental variables was tested using a Monte Carlo permutation test (with 1000 permutations).

## 4.3.1.3 Results

Two mitochondrial fragments were sequenced for all individuals, producing a concatenated fragment of 1545 bp (954 and 591 bp for *ND2* and *COI*, respectively). GenBank ID's for each fragment are indicated in Table 4.3.1.1. The alignments contained 734 polymorphic sites (47%) 712 of which were parsimony informative (46%). A total of 57 haplotypes were defined.

*Sequences divergence*

Genetic divergence between pair of species within genera ranged from 0.093 (90% identity) for P*teroplatytrygon* and *Dasyatis* to 0.283 (72% identity) for *Torpedo*-*Dipturus*, and *Torpedo*-*Myliobatis* (Supplementary Table S4.3.1.3). Comparing average divergence in batoids, the

Table 4.3.1.1. List of species of Mediterranean Batoidea studied and genetic parameters for the 98 specimens analyzed, based on mitochondrial concatenated fragment, indicating their abbreviation (ID's), number of samples (n), number of segregating sites (S), number of haplotypes (Nh), haplotype diversity (h), nucleotide diversity (π) and the GenBank accession number for *COI* and *ND2*. IUCN Category for each species is also indicated: least concern (LC), near threatened (NT), vulnerable (VU), endangered (EN), critically endangered (CR), not evaluated (NE).

| Species | ID's | Common name | IUCN category | n | S | Nh | h | π (%) | GenBank number | |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Order Rajiformes*** | | **Skates** | | | | | | | *COI* | *ND2* |
| *Family Rajidae* | | | | | | | | | | |
| *Raja asterias* | Ra | Starry skate | NT | 8 | 7 | 6 | 0.93 | 0.17 | KY949072-KY949079 | KY949160-KY949167 |
| *Raja brachyura* | Rb | Blonde ray | NT | 5 | 1 | 2 | 0.40 | 0.026 | KY949096-KY949100 | KY949184-KY949188 |
| *Raja clavata* | Rc | Thornback ray | NT | 8 | 6 | 5 | 0.86 | 0.11 | KY949088-KY949095 | KY949176-KY949183 |
| *Raja miraletus* | Rm | Brown ray | LC | 3 | 1 | 2 | 0.67 | 0.043 | KY949085-KY949087 | KY949173-KY949175 |
| *Raja montagui* | Rmo | Spotted ray | LC | 6 | 1 | 2 | 0.53 | 0.035 | KY949048-KY949053 | KY949136-KY949141 |
| *Raja polystigma* | Rp | Speckled ray | LC | 18 | 7 | 5 | 0.41 | 0.13 | KY949054-KY949071 | KY949142-KY949159 |
| *Raja radula* | Rr | Rough ray | EN | 5 | 2 | 3 | 0.80 | 0.065 | KY949080-KY949084 | KY949168-KY949172 |
| *Rostroraja alba* | Ral | White skate | EN | 2 | 1 | 2 | 1 | 0.065 | KY949112-KY949113 | KY949210-KY949211 |
| *Leucoraja circularis* | Lc | Sandy ray | CR | 5 | 1 | 2 | 0.4 | 0.026 | KY949106-KY949110 | KY949194-KY949198 |
| *Leucoraja naevus* | Ln | Cuckoo ray | NT | 5 | 8 | 5 | 1 | 0.27 | KY949101-KY949105 | KY949189-KY949193 |
| *Dipturus oxyrinchus* | Do | Longnosed skate | NT | 3 | 0 | 1 | 0 | 0 | KY949111/KU761956-57* | KY949199-KY949201 |
| *Dipturus nidarosiensis* | Dn | Norwegian skate | NT | 8 | 3 | 4 | 0.64 | 0.049 | KX783029-KX783036* | KY949202-KY949209 |
| ***Order Myliobatiformes*** | | **Stingrays** | | | | | | | | |
| *Family Dasyatidae* | | | | | | | | | | |
| *Dasyatis* cf. *tortonesei* | Dt | | NE | 6 | 3 | 4 | 0.80 | 0.10 | KY949119-KY949125 | KY949218-KY949223 |
| *Dasyatis pastinaca* | Dp | Common stingray | VU | 6 | 14 | 5 | 0.93 | 0.32 | KY949126-KY949131 | KY949224-KY949229 |
| *Pteroplatytrygon violacea* | Pv | Violet stingray | LC | 3 | 6 | 3 | 1 | 0.26 | KY949117-KY949118 | KY949215-KY949217 |
| *Family Myliobatidae* | | | | | | | | | | |
| *Pteromylaeus bovinus* | Pt | Bull ray | CR | 1 | 0 | 1 | | | KY949132 | KY949230 |
| *Myliobatis aquila* | Ma | Common eagle ray | VU | 3 | 1 | 2 | 0.67 | 0.043 | KY949114-KY949116 | KY949212-KY949214 |
| ***Oder Torpediniformes*** | | **Electric rays** | | | | | | | | |
| *Family Torpedinidae* | | | | | | | | | | |
| *Torpedo marmorata* | Tm | Marbled electric ray | LC | 3 | 8 | 3 | 1 | 0.35 | KY949133-KY949135 | KY949231-KY949233 |

(*) Sequences ID's from Ramírez-Amaro *et al*. (2017a).

maximum value was obtained between *Torpedo marmorata* and *Raja radula* 0.386 (71% identity; 438 fixed nucleotide differences), whereas the lowest values were obtained between *Raja montagui* and *Raja polystigma*, with 0.029 (97% identity; 43 fixed nucleotide differences), and between *Raja clavata* and *R. radula* with 0.041 (96% identity; 61 fixed nucleotide differences) (Table 4.3.1.2; Supplementary Table S4.3.1.4). The morphologically similar skates (*Raja brachyura*, *R. montagui* and *R. polystigma*) showed a low genetic divergence between each other, ranging between 0.029 and 0.062 (Table 4.3.1.2).

In the seven species in which a greater number of specimens were analyzed (>5), intra-specific variation was also detected (Table 4.3.1.1). Three of them, *R. polystigma*, *R. montagui* and *Dipturus nidarosiensis*, exhibited low haplotype diversity ($h < 0.65$), whereas *Dasyatis* cf. *tortonesei*, *Dasyatis pastinaca*, *Raja asterias* and *R. clavata* showed high haplotype diversity ($h < 0.80$).

*Phylogenetic and network analysis*

The Bayesian phylogeny produced a tree topology with high posterior probabilities (Figure 4.3.1.2). This phylogenetic tree was made up of three monophyletic major clades corresponding to the main evolutionary lineages (Orders): Rajiformes, Myliobatiformes and Torpediniformes.

Rajiformes, which was only represented in our samples by the Rajidae family, can be seen as a sister clade of Myliobatiformes (Figure 4.3.1.2). Within Rajidae, two major sub-clades can be distinguished, the first one composing the Rajini tribe, whereas the second was a tribe complex made up of Amblyrajini and Rostrorajini. Amblyrajini were composed of two *Leucoraja* species, and Rostrorajini by only one species (*Rostroraja alba*). Within the Rajini, two sub-clades can be differentiated, which corresponded to the genera *Dipturus* and *Raja*. Two groups consisting of *Dipturus oxyrinchus* and *D. nidarosiensis* were distinguished within the *Dipturus* genus.

*Raja* genus was a monophyletic clade, consisting of seven species (Figure 4.3.1.2). The morphologically similar skates *R. montagui* and *R. polystigma* exhibited a close phylogenetic relationship forming terminal clades and, more recently, evolved lineages. In this sense, five juvenile skates specimens (ID's: Rb163, Rb170, Rb192, Rmo152, Rp169) including both species and also *R. brachyura*, which shared a very similar morphotype, were initially misidentified using morphological trait form identification keys, but could be correctly identified with the genetic results presented here.

Within the Myliobatiformes clade, two major sub-clades were observed, which corresponded to Myliobatidae and Dasyatidae families (Figure 4.3.1.2). Myliobatidae were clearly divided into two lineages, made up of *Myliobatis aquila* and *Pteromylaeus bovinus*. Within Dasyatidae, three species were identified with a basal separation of Pteroplatytrygon violacea and the monophyletic *Dasyatis* genus, in which the two Bayesian analyses supported *D. pastinaca* and *D.* cf. *tortonesei* as distinct species (Figure 4.3.1.2 and Supplementary Figure S4.3.1.1).

Table 4.3.1.2. Estimates of average evolutionary divergence (below the diagonal) and identity percentage (above the diagonal) between Mediterranean Batoidea species, based on mitochondrial concatenated fragment. Species nomenclature is indicated in Table 4.3.1.1.

| | **Rmo** | **Rp** | **Ra** | **Rr** | **Rm** | **Rc** | **Rb** | **Ln** | **Lc** | **Do** | **Dn** | **Ral** | **Ma** | **Pv** | **Dt** | **Dp** | **Pb** | **Tm** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rmo | | 97.20 | 92.85 | 93.12 | 91.52 | 94.30 | 94.06 | 84.41 | 84.89 | 89.86 | 89.78 | 85.94 | 76.10 | 77.28 | 77.35 | 76.74 | 75.88 | 72.58 |
| Rp | 0.029 | | 92.78 | 93.49 | 91.64 | 94.29 | 94.49 | 84.77 | 85.36 | 89.64 | 89.56 | 86.23 | 75.95 | 76.88 | 77.18 | 76.80 | 75.96 | 72.39 |
| Ra | 0.075 | 0.076 | | 93.16 | 90.92 | 94.73 | 92.49 | 84.71 | 85.12 | 89.59 | 89.38 | 85.46 | 75.55 | 76.45 | 76.66 | 75.92 | 75.00 | 71.87 |
| Rr | 0.072 | 0.068 | 0.072 | | 90.69 | 96.04 | 93.07 | 84.62 | 84.56 | 89.15 | 88.66 | 85.16 | 74.66 | 75.98 | 76.69 | 75.61 | 75.29 | 71.62 |
| Rm | 0.090 | 0.089 | 0.097 | 0.099 | | 92.04 | 91.55 | 84.71 | 84.95 | 88.01 | 87.60 | 85.96 | 76.74 | 77.80 | 77.91 | 76.68 | 75.32 | 72.73 |
| Rc | 0.059 | 0.059 | 0.055 | 0.041 | 0.084 | | 94.59 | 85.11 | 84.93 | 89.55 | 89.43 | 86.35 | 76.46 | 77.32 | 77.49 | 76.56 | 76.36 | 72.33 |
| Rb | 0.062 | 0.057 | 0.079 | 0.073 | 0.090 | 0.056 | | 84.76 | 84.75 | 84.75 | 89.59 | 89.60 | 75.45 | 76.55 | 77.39 | 76.48 | 75.98 | 72.14 |
| Ln | 0.177 | 0.172 | 0.173 | 0.174 | 0.173 | 0.168 | 0.172 | | 93.90 | 85.40 | 85.59 | 85.85 | 75.71 | 75.58 | 77.25 | 75.71 | 74.72 | 72.15 |
| Lc | 0.170 | 0.165 | 0.168 | 0.175 | 0.170 | 0.170 | 0.172 | 0.064 | | 86.20 | 86.26 | 85.97 | 75.89 | 75.86 | 76.68 | 75.38 | 75.35 | 72.82 |
| Do | 0.109 | 0.111 | 0.112 | 0.117 | 0.131 | 0.112 | 0.112 | 0.164 | 0.154 | | 93.45 | 86.31 | 76.14 | 76.12 | 76.87 | 76.48 | 75.08 | 71.72 |
| Dn | 0.110 | 0.112 | 0.114 | 0.123 | 0.136 | 0.114 | 0.112 | 0.161 | 0.153 | 0.068 | | 86.50 | 76.04 | 75.83 | 76.54 | 75.76 | 74.95 | 71.78 |
| Ral | 0.157 | 0.153 | 0.163 | 0.166 | 0.157 | 0.152 | 0.151 | 0.158 | 0.156 | 0.152 | 0.149 | | 76.82 | 76.22 | 76.95 | 75.85 | 75.63 | 72.61 |
| Ma | 0.305 | 0.307 | 0.313 | 0.327 | 0.296 | 0.300 | 0.314 | 0.313 | 0.310 | 0.305 | 0.306 | 0.295 | | 82.18 | 82.36 | 83.32 | 83.28 | 71.65 |
| Pv | 0.287 | 0.293 | 0.300 | 0.307 | 0.280 | 0.287 | 0.298 | 0.313 | 0.309 | 0.305 | 0.309 | 0.304 | 0.206 | | 90.83 | 90.63 | 81.19 | 72.99 |
| Dt | 0.285 | 0.288 | 0.296 | 0.296 | 0.277 | 0.283 | 0.284 | 0.288 | 0.297 | 0.293 | 0.298 | 0.293 | 0.203 | 0.098 | | 92.80 | 80.93 | 72.67 |
| Dp | 0.295 | 0.295 | 0.309 | 0.314 | 0.297 | 0.299 | 0.299 | 0.312 | 0.317 | 0.300 | 0.311 | 0.310 | 0.191 | 0.100 | 0.076 | | 81.38 | 73.09 |
| Pb | 0.308 | 0.306 | 0.321 | 0.318 | 0.317 | 0.301 | 0.306 | 0.328 | 0.318 | 0.320 | 0.321 | 0.312 | 0.191 | 0.222 | 0.225 | 0.219 | | 72.94 |
| Tm | 0.368 | 0.372 | 0.381 | 0.386 | 0.365 | 0.375 | 0.377 | 0.377 | 0.365 | 0.384 | 0.384 | 0.365 | 0.383 | 0.361 | 0.367 | 0.359 | 0.364 | |

The Torpediniformes clade showed the most basal position in the phylogenetic tree and was strongly supported as a group different from the rest of batoids (Figure 4.3.1.2). This clade was composed of only one species (*Torpedo marmorata*).

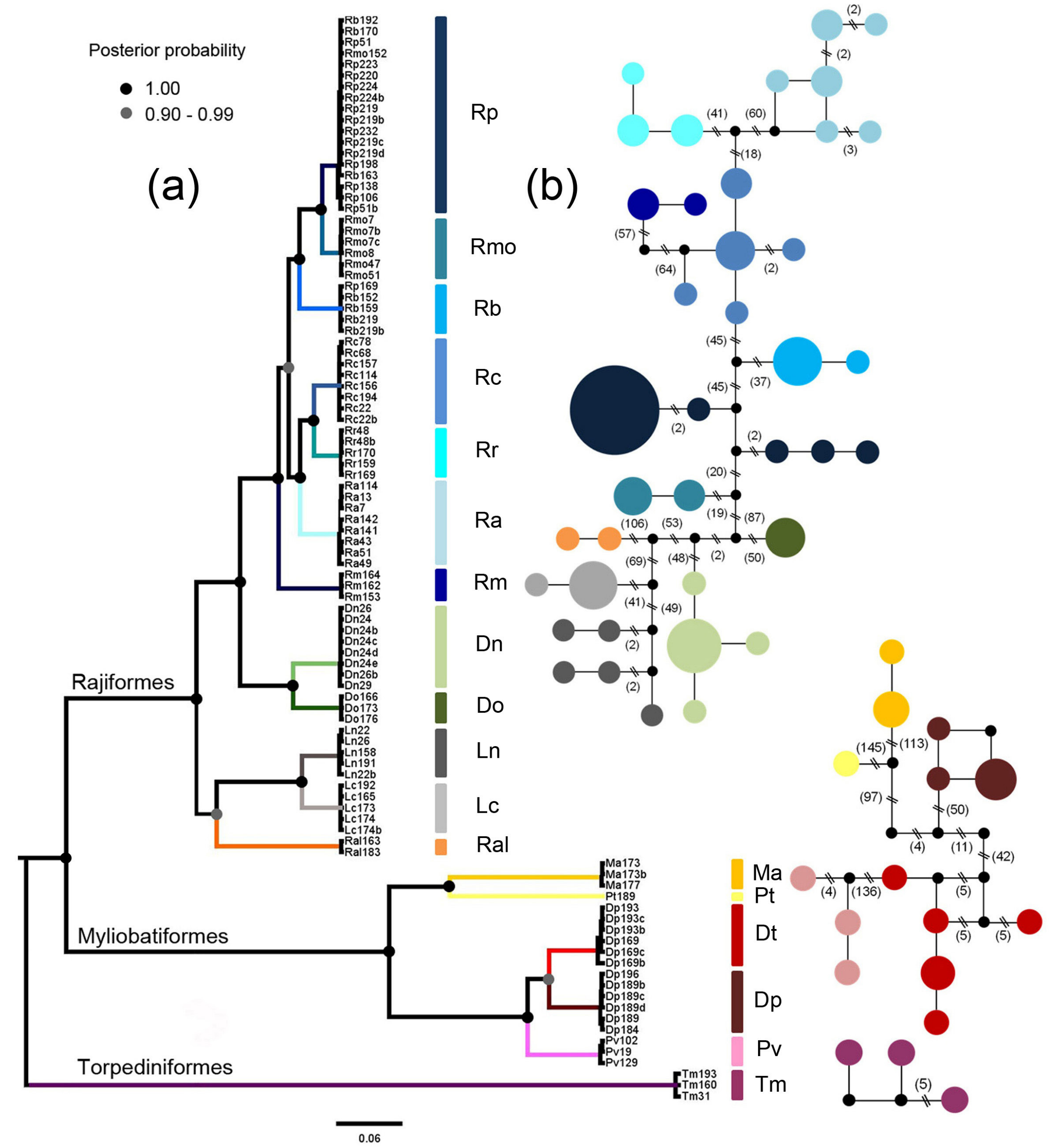


Figure 4.3.1.2. Bayesian phylogeny (a) and network analysis (b) of Batoidea species from the western Mediterranean, based on mitochondrial concatenated fragment (*COI* and *ND2*). For the network, relative area of circles reflects sample size, and mutational steps are indicated in brackets. Species nomenclature is indicated in Table 4.3.1.1.

In agreement with phylogenetic analyses, the haplotype network showed no haplotype common to all species (Figure 4.3.1.2). Many mutational steps can be observed between genera and species, with their association related to the phylogenetic tree. The fewest mutational steps was observed between *R. polystigma* and *R. montagui* and between *D.* cf. *tortonesei* and *D. pastinaca* for Rajiformes and Myliobatiformes, respectively (Supplementary Table S4.3.1.4).

*Divergence times*

Bayesian divergence times, estimated in Beast, showed that Mediterranean batoids diverged during the Early Cretaceous (143 Mya), with the separation of Torpediniformes and Rajiformes/Myliobatiformes (Figure 4.3.1.3). At the end of this period (112 Mya), subsequent lineages of batoids, Myliobatiformes and Rajiformes, diverged. The greatest Myliobatiformes sub-clade diversification (Myliobatidae/Dasyatidae) was estimated to have occurred during the Paleocene (62 Mya), with subsequent radiation of each species from the Eocene to the Middle Miocene (between 42 and 13 Mya). At the end of this radiation occurred the dichotomy between *D. pastinaca* and *D.* cf. *tortonesei*, which corresponded to 13 Mya. Otherwise, the date of the phylogeny suggested that the divergence of the Rajiformes clade started during the Late Eocene (36 Mya), but the main skate species radiation was estimated to have occurred after the Middle Miocene (from 12 to 4 Mya). During this time, the Mediterranean endemic skate species *R. radula* (6.6 Mya) originated, followed by the occurrence of *R. polystigma* (4.4 Mya).

*Molecular species delimitation*

PTP analyses identified up to 18 clusters (Supplementary Figure S4.3.1.2), which correspond to the same numbers of clades found from phylogenetic analysis, while 19 entities consisting of 17 clusters were recorded by the single threshold GMYC (Cl= 18-19, InL of null model = 308.77, ML of GMYC model= 344.15, $p < 0.001$; Supplementary Figure S4.3.1.3). Only one specimen of *P. bovinus* was sequenced, so it was not considered in this analysis. Therefore, the number of clusters and species identified were the same. The GMYC lineage through time plot also indicated an exponential increase in breaching rate near the tip of the tree, indicating a recent speciation process (Supplementary Figure S4.3.1.3).

*Ecological traits*

Bathymetric distribution models showed clear differences between the *Raja* species (Figure 4.3.1.4). For the phylogenetic closely related *R. radula* and *R. clavata*, the optimum depth was clearly greater in *R. clavata* (162 m) than *R. radula* (51 m). By contrast, the other closely related species *R. montagui* and *R. polystigma*, showed a very similar optimum depth (144 and 147 m, respectively), whilst their sister clade *R. brachyura* had a distinct optimum depth (70 m). The phylogenetically distant species *R. asterias* and *R. miraletus* showed a shallow optimum depth (49 and 79 m, respectively).

RDA analysis revealed different habitat preferences for *Raja* species in the Balearic Islands (Figure 4.3.1.5). *R. clavata* showed a clear preference for deeper bottoms with the presence of crinoid beds, whereas *R. miraletus* and *R. radula* showed similar preferences, being more abundant in shallow shelf bottoms with the presence of *Peyssonnelia* beds, but also maërl and sandy bottoms. Similarly, *R. brachyura* and *R. polystigma* showed different habitat preferences, the former being more abundant in shallow shelf *Peyssonnelia* and maërl beds, but also sandy bottoms, whereas the latter preferred deep shelf sandy-mud bottoms.

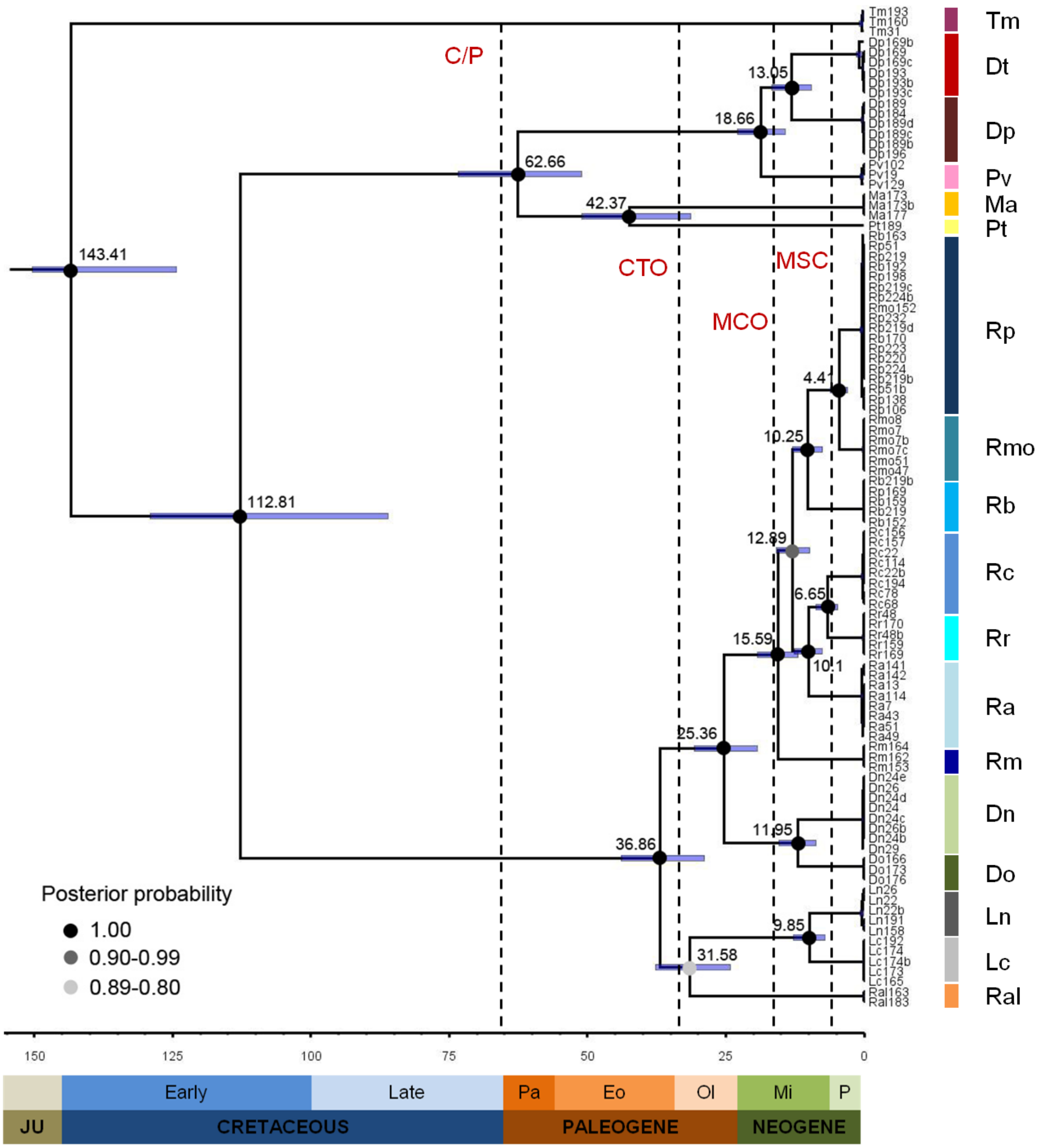


Figure 4.3.1.3. Estimated divergence times of Mediterranean Batoidea. Dates (Mya) were estimated from BEAST, based on mitochondrial concatenated fragment. Confidence intervals (95%) are indicated as blue bars on the tree. Dotted lines showed geological division. C/P: Cretaceous/Paleogene Boundary; CTO: Closure of Tethys Ocean; MCO: Miocene Climatic Optimum; MSC: Messinian Salinity Crisis; JU: Jurassic Period; Pa: Paleocene; Eo: Eocene; Ol: Oligocene; Mi: Miocene; P: Pliocene. Species nomenclature is indicated in Table 4.3.1.1.

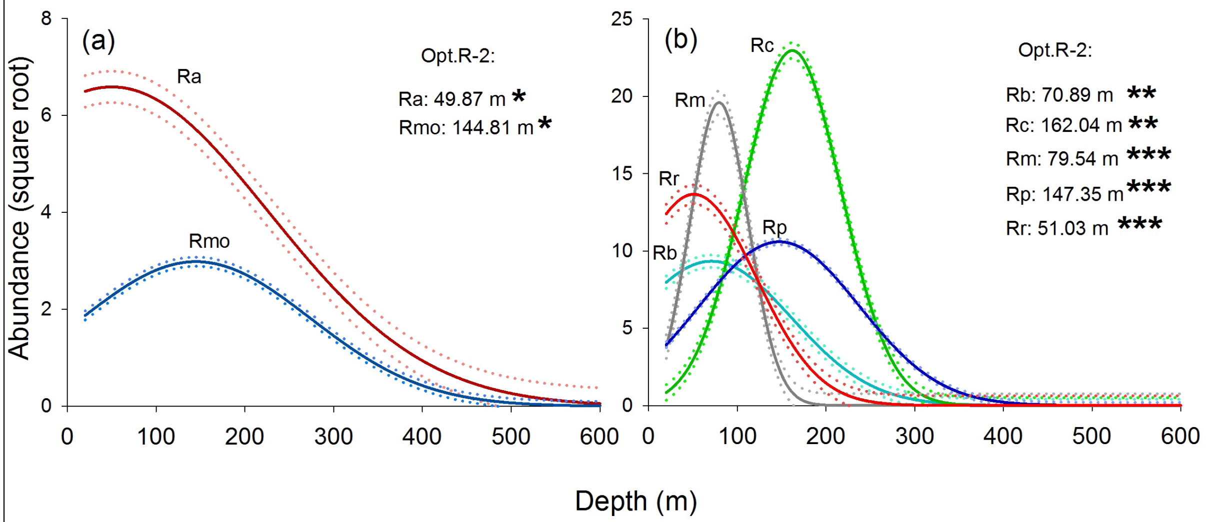


Figure 4.3.1.4. Bathymetric distribution models for *Raja* species in the western Mediterranean: a) Iberian Peninsula; b) Balearic Islands. Optimum depth (Opt.R$^{-2}$) is also indicated. Statistical significance of a unimodal response: (*) $P < 0.05$; (**) $P < 0.01$, (***) $P < 0.001$. Species nomenclature is indicated in Table 4.3.1.1.

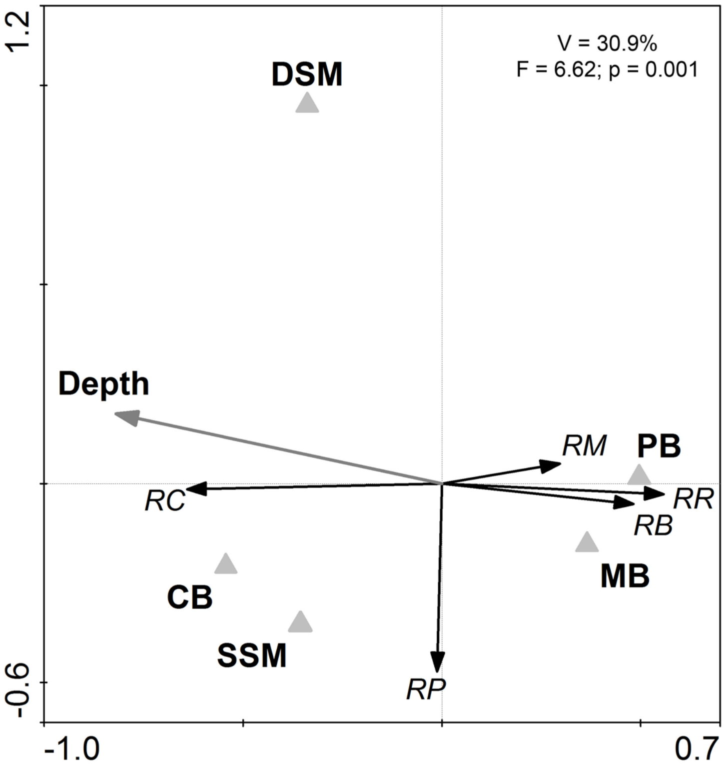


Figure 4.3.1.5. Redundancy analysis (RDA) biplot for *Raja* species and macro-epibenthic assemblages (as proxy of benthic habitats; from Ordines and Massutí, 2009) off the Balearic Islands. Species nomenclature is indicated in Table 4.3.1.1. PB: *Peyssonnelia* beds; MB: maërl beds; SSM: shallow shelf sandy-mud bottom; DSM: deep shelf sandy-mud bottom; CB: crinoid beds. The graph also indicates the percentage of variance explained by benthic habitats (V), F-ratio of the regression model (F) and significance value (p).

### 4.3.1.4 Discussion

The origin and radiation of batoids have been affected by a succession of biogeographic events. The opening of the South Atlantic, during the Early Cretaceous, caused a northeastward movement of the African plate relative to the Eurasian plate, which gave rise to drastic changes in Mediterranean shape and connectivity (Voigt *et al*., 1999). Phylogenetic analysis supports the diversification of the current Mediterranean batoids from ancestral forms existing at the beginning of the Cretaceous. The high number of ecological niches existing in the Tethyan domain during this period could have promoted this diversification, as has been shown by the increase in numbers of families of ammonites (Fabrizio, 1998). The first cladogenetic event for Mediterranean batoids (Torpediniformes and Myliobatiformes-Rajiformes) was estimated during the Early Cretaceous (143 Mya), which coincides with the proposal of Aschliman *et al*. (2012) for batoids on a global scale (~140 Mya). This time of divergence is also supported by fossil records, which reveal a significant neoselachian diversification through the Cretaceous, resulting in the first appearance of many new orders (Carvalho 1996; Underwood, 2006).

During the Middle Cretaceous (112 Mya), a second major cladogenetic event originated the Orders Myliobatiformes and Rajiformes. This period was also the origin of the major orders of teleost fishes in the Mediterranean (Meynard *et al*., 2012) and is coincident with the global diversification of acanthomorphs (Friedman, 2010). Based on fossil data, the origin of Rajiformes has been estimated at 97 Mya (Cappetta *et al*., 1993) and the fossil records of Myliobatiformes in Europe have been documented during the Late Cretaceous (Cappetta, 1987).

The combination of the massive extinction of some shark groups in the Cretaceous-Paleogene (Guinot *et al*., 2012), and the appearance of new ecological niches due to paleoclimatic changes probably promoted the diversification of batoids in the Mediterranean. The Myliobatiformes clade branched into two sub-clades, the Dasyatidae and Myliobatidae families at 62 Mya, and the Rajiformes clade was divided into two sub-clades at 36 Mya: the Rajini tribes proposed by Ebert and Compagno (2007), and Amblyrajini-Rostrorajini proposed by McEachran and Dunn (1998) and Chiquillo *et al*. (2014), respectively. Both diversification events occurred during the Paleogene, between 66 and 23 Mya. This speciation is concurrent with the Paleogene diversification of elasmobranchs at global level (Guinot *et al*., 2012) and the radiation of most Perciformes families in the Mediterranean (Meynard *et al*., 2012). The first fossil records of Myliobatidae (around 70 Mya; Claeson *et al*., 2004) and Rajidae (early Paleogene; Long, 1994) support the appearance date estimated here for both sub-clades.

Some inconsistencies have been detected, particularly within Amblyrajini-Rostrorajini tribes. The phylogenetic position of *Rostroraja alba* has been historically confused and remains under discussion. McEachran and Dunn (1998) and Compagno and Ebert (2007) placed this species in the Rajini tribe, but recently Chiquillo *et al*. (2014) proposed a new Rajidea tribe named Rostrorajini, in which *R. alba* is included. Our results agree with those by Naylor *et al*. (2012 a, 2012b) and Ball *et al*. (2016) and confirm that *R. alba* has no close phylogenetic relationship with the Rajini tribe, neither should it belong to the Rostrorajini tribe. This species must be included in the Amblyrajini tribe, along with the genus *Leucoraja*.

During the Eocene-Oligocene transition (around 33.5 Mya) occurred the deepest climatic change in the last 50 Mya, associated with dramatic oceanographic reorganization and the largest climatic cooling (Miller *et al*., 2009). The connection between the Mediterranean and the Indian Ocean was closed at around the Middle Miocene (Rögl, 1998; Hamon *et al*., 2013), with significant impact on climate and ocean circulation and changes in the sedimentary environment of the Mediterranean, which influenced the composition of its flora and fauna (Rögl, 1998; Kocsis *et al*., 2009), and increased its productivity (Miller *et al*., 2009). It is during this period (Oligocene; between 31.5 and 18.6 Mya) when, according to our results, an important and rapid radiation process of batoids occurred in the Mediterranean, the result of which was the appearance of all their genera, with the only exceptions of Myliobatis and Pteromylaeus.

Once the Mediterranean was isolated from the Indian Ocean, a more recent interval of global warming, known as the Miocene Climatic Optimum (MCO), at 17-15 Mya (Rögl, 1998; Holburn *et al*., 2014), caused another increment in ocean productivity, especially of the calcareous algae Sporolithon (Halfar and Mutti, 2005; Baraga and Bassi, 2007; Braga *et al*., 2010). Coinciding with this period, from the mid Miocene (15.5 Mya) to late Miocene (6.6 Mya), the largest and most recent radiation process of batoid species in the Mediterranean occurred. Currently, maërl beds, also made up of calcareous algae or rhodoliths, play a key role in marine ecological processes and are associated with high marine biodiversity (Riosmena-Rodríguez *et al*., 2016). The growth of carbonate platforms, built up by calcareous algae during the Middle Miocene, could also have played a crucial role, increasing biodiversity and triggering the speciation process of batoids in the Mediterranean. Our results show that nine species originated during this period, including the one endemic species *Raja radula*. This recent speciation corresponds to that proposed by Valsecchi *et al*. (2005), who indicated that Mediterranean skates (Rajidae) underwent recent diversification, from the Middle Miocene to the Early Pleistocene, and suggested that several skate species, whose divergence times dated from 15 to 7 Mya, probably dispersed throughout the whole region via the Atlantic-Mediterranean gateway, until the temporary closure of this connection at 7 Mya. Taking into account our results, this hypothesis must be expanded to stingrays (Dasyatidae and Myliobatidae) and electric rays (Torpenidae). This dispersion of batoids was probably promoted by adverse conditions, such as the closure of the Strait of Gibraltar, the subsequent isolation of the Mediterranean, and the global fall in temperatures after the MCO, which caused a decrease in the availability of benthic habitat such as maërl beds (Braga, 2016).

Our results support the fact that *Raja* speciation in the Mediterranean could be associated to depth and benthic habitat preferences or that, alternatively, after the successive speciation events, a reduction of inter-specific competition was achieved from benthic community preferences and resource partitioning. The close phylogenetic lineages such as *Raja clavata*, *R. radula* and *R. asterias* that originated during the Miocene exhibit different preferences. The endemic species *R. radula* is distributed on shallow shelf bottoms, particularly those with a presence of Peyssonelia beds, which is a facies typical of Mediterranean soft circalittoral bottoms (Pérès, 1985). By contrast, *R. clavata*, a species widely distributed in the Mediterranean and Northeast Atlantic (Chevolot *et al*., 2006), is associated to deep shelf and shelf-break bottoms, with a presence of crinoids, a benthic habitat widely distributed in the Mediterranean and Northeast Atlantic (Lavaleye *et al*., 2002; Colloca *et al*., 2004). *R. asterias*, a species mainly distributed in the Mediterranean, but also in adjacent Atlantic waters (Ordines *et al*., 2017), is distributed at a similar depth to *R. radula*, on the shallow shelf, but little interaction is expected between both species. *R. asterias* prefers muddy and sandy bottoms (Serena *et al*., 2015), and is found in areas of the continental shelf with terrigenous sediments, such as those off the Ebro River Delta in the Iberian Peninsula (Navarro *et al*., 2013) and Po River in the Adriatic Sea (Ferrà *et al*. 2016). By contrast, *R. radula* is found on red algae beds in the Balearic Islands, where no river runoffs reduce the presence of terrigenous fine sediments, with predominant biogenic sand (Ordines and Massutí, 2009). In fact, a previous study developed by Ramírez-Amaro *et al*. (2015) in the same study area showed that *R. asterias* is mainly distributed off the north-eastern Iberian Peninsula, whereas it is extremely scarce off the Balearic Islands. Hence, although both species share the same depth range, they are distributed in different geographic areas. Moreover, both species show differences in their feeding ecology, which could also be involved in the speciation process (Ingram and Shurin, 2009). While *R. asterias* mainly feeds on crabs and shrimps (Navarro *et al*., 2013), *R. radula* is a more generalist predator (Consalvo *et al*., 2010).

Another example of *Raja* speciation through feeding ecology could be *Raja brachyura* and *R. miraletus*, also originated during the Miocene radiation. In the Balearic Islands, both species appear to share depth (shallow shelf) and habitat (red algae beds). However, while *R. miraletus*, – a relatively small skate species which maximum length is around 70 cm (Stehmann and Bürkel, 1984) – displays a narrow niche breadth, mainly preying on small crustaceans (Valls *et al*., 2011), the diet of *R. brachyura* – which attains larger size, reaching approx. 120 cm (Stehmann and Bürkel, 1984) – is a more generalist, with bony fishes as their main preys, followed by crustaceans (Farias *et al*., 2006).

Considering that the ancestral Rajini tribe was probably made up pf shallow water species, our results suggest that some individuals dispersed to deeper bottoms, promoting the origin of Rajidae species belonging to the genus *Dipturus* during the Miocene. Currently, *Dipturus oxyrinchus* shows an optimum depth of 294 m off the Balearic Islands (Ramírez-Amaro *et al*., 2015), whereas *D. nidarosiensis*, its co-generic species whose existence in the Mediterranean was discovered a few years ago (Cannas *et al*., 2010; Ramírez-Amaro *et al*., 2017a), is distributed from 600 m to over 1400 m depth.

The divergence of the genus *Dasyatis* into *D. pastinaca* and *D*. cf. *tortonesei* also occurred during the Miocene. The lack of a common mtDNA haplotype between both clades and the reciprocal monophyly support the hypothesis that this speciation is complete. However, both species show a strong morphological similarity, which makes their correct identification difficult based only on external characteristics. *D.* cf. *tortonesei* was described by Capapé (1975) from specimens caught off Tunisia (central Mediterranean), according to some morphological and meristic characters which differentiated it from *D. pastinaca* (Capapé, 1977b). Nevertheless, Séret and McEachran (1986) and Compagno (1999), considered *D.* cf. *tortonesei* as a doubtful species, and it was finally catalogued as a synonym of *D. pastinaca* by Tortonese (1987). More recently, parasitological studies have distinguished monogeneans specific for "two" groups of Dasyatis, supporting a taxonomic distinction of both species (Neifar *et al*., 2000) and again validating *D.* cf. *tortonesei* as a different species. Our results add phylogenetic information that reinforces this validation and points out the need for a major taxonomic revision between these species, and the consideration of *D.* cf. *tortonesei* in the scientific monitoring of chondrichthyan in the Mediterranean, as well as in the conservation status assessment of these species, developed by the International Union by the Conservation of the Nature (IUCN; Cavanagh and Gibson, 2017; Dulvy *et al*., 2016).

The Messinian Salinity Crisis (MSC), which occurred between 5.96 and 5.33 Mya (Ivanovic *et al*., 2014), resulted in a reduction of the Mediterranean and an increment in its salinity, causing an extinction crisis among its fish fauna. According to fossil records, some relict taxa of batoids disappeared: e.g., Dasyatis cavernosa, *Dipturus* aff. olisiponensis and Myliobatis crassus (Marsili, 2008). However, some other batoids, such as *R. clavata* are likely to have survived in refuge areas across the Mediterranean (Chevolot *et al*., 2006). The abrupt collapse of the Gibraltar sill and the re-flooding of Atlantic waters into the Mediterranean (Garcia-Castellanos *et al*., 2009; Roveri *et al*., 2014) contributed to the deposition of sediments in its deep basins during the Pliocene at 4.9 Mya (Müler, 1993), and to the occurrence of rhodolith beds during the early Pliocene (Aguirre *et al*., 2012). The MSC coincides with recent speciation events described for Mediterranean teleosts (Hanel *et al*., 2002; Meynard *et al*., 2012) and could also have promoted the most recent speciation between *Raja montagui* and *Raja polystigma* (4.4 Mya). Similarly to what happened with other species of the genus *Raja* in the Mediterranean, these two species show similar optimum depth on the deep shelf, but their different distribution (mainly off the Iberian Peninsula and the Balearic Islands, respectively) and feeding ecology (crabs, polychaetes and teleosts vs. natantia crustaceans, teleosts, isopods and mysids, respectively; Farias *et al*., 2006; Valls *et al*., 2011) could have benefited their differentiation.

Our results highlight the relevance of combining phylogeny, geological history and current ecology to disentangle the possible drivers during the evolution of speciation of marine fishes in the Mediterranean. Theses finding show a parallel between cladogenetic events in Batoidea and geological events in Mediterranean history, which began from the Cretaceous. Two key cladogenesis events were detected: genera originated during the Eocene-Oligocene transition, whilst almost all current species appeared during the Miocene Climatic Optimum. Our findings also support the hypothesis that *Raja* species undergo ecological speciation on an environmental gradient, with ecological factors such as depth and habitat being of equal importance as physical distance and geographical barriers. Beyond the establishment of a phylogenetic relationship between Mediterranean batoids, advances in the comprehension of their evolutionary history illustrate the assembly of regional fauna through origination and immigration, where dispersal and isolation by environment gradients have shaped the emergence of relatively high biodiversity in the western Mediterranean (mainly in the Balearic Islands).

# Supplementary material

## Tables

Table S4.3.1.1. Biological database from the genetic samples of Mediterranean Batoidea species of *COI* sequences available at http://www.boldsystems.org/. F: female; M: male; Lat: latitude; Long: longitude.

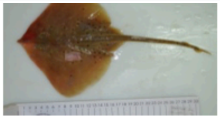
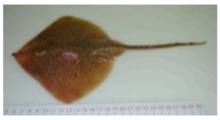
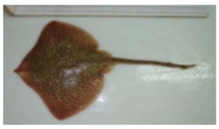
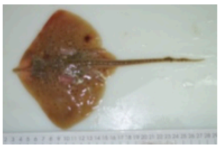
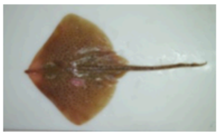
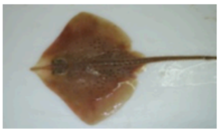
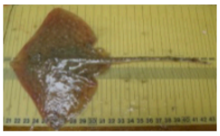
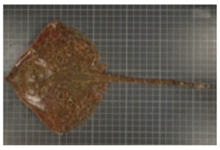
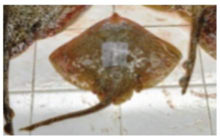
| Species name | ID's | Depth (m) | Length (cm) | Sex | Lat | Long | GenBank ID's | Picture |
|---|---|---|---|---|---|---|---|---|
| *Raja montagui* | Rmo47 | 86 | 33 | F | 36.723 | -3.292 | KY949048 |  |
| *Raja montagui* | Rmo51 | 247 | 23 | M | 37.663 | -0.356 | KY949049 |  |
| *Raja montagui* | Rmo7 | 143 | 42 | M | 36.358 | -5.123 | KY949050 |  |
| *Raja montagui* | Rmo7b | 143 | 24 | F | 36.358 | -5.123 | KY949051 |  |
| *Raja montagui* | Rmo7c | 143 | 30 | F | 36.358 | -5.123 | KY949052 |  |
| *Raja montagui* | Rmo8 | 117 | 32 | F | 36.398 | -5.033 | KY949053 |  |
| *Raja polystigma* | Rp138 | 284 | 22 | F | 41.424 | 2.781 | KY949054 |  |
| *Raja polystigma* | Rp106 | 94 | 30 | M | 39.824 | 0.697 | KY949055 |  |
| *Raja polystigma* | Rb192 | 109 | 33.5 | F | 39.021 | 2.850 | KY949056 |  |

Table S4.3.1.1. (continued)

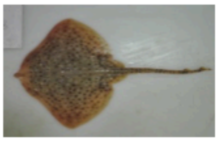
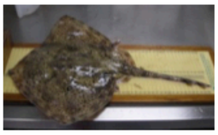
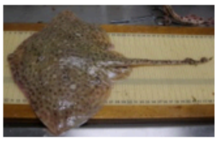
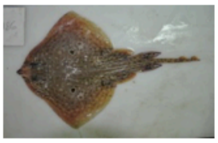
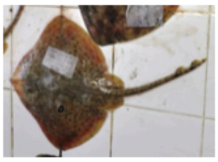
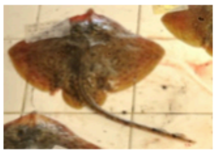
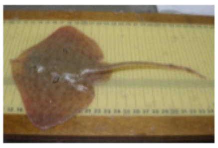
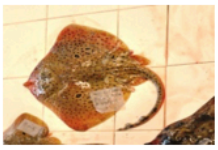
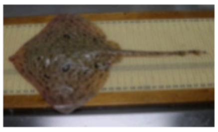
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Raja polystigma* | Rb170 | 63 | 24 | F | 39.851 | 4.094 | KY949057 |  |
| *Raja polystigma* | Rp51 | 247 | 50 | F | 37.663 | -0.356 | KY949058 |  |
| *Raja polystigma* | Rmo152 | 57 | 28 | F | 39.227 | 2.993 | KY949059 |  |
| *Raja polystigma* | Rp223 | 170 | 47 | M | 39.797 | 2.359 | KY949060 |  |
| *Raja polystigma* | Rp220 | 67 | 29 | F | 39.818 | 2.640 | KY949061 |  |
| *Raja polystigma* | Rp224 | 173 | 25.5 | F | 40.121 | 3.720 | KY949062 |  |
| *Raja polystigma* | Rp224b | 173 | 18.5 | F | 40.121 | 3.720 | KY949063 |  |
| *Raja polystigma* | Rp219 | 61 | 34 | F | 39.857 | 2.759 | KY949064 |  |
| *Raja polystigma* | Rp219b | 61 | 37.5 | F | 39.857 | 2.759 | KY949065 |  |
| *Raja polystigma* | Rp232 | 142 | 29 | M | 39.450 | 2.254 | KY949066 |  |
| *Raja polystigma* | Rp219c | 61 | 33 | F | 39.857 | 2.759 | KY949067 |  |

Table S4.3.1.1. (continued)

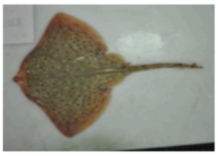
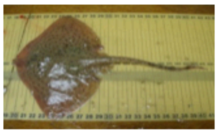
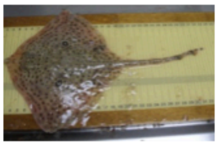
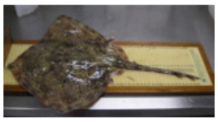
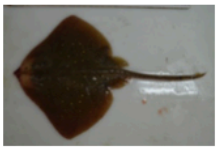
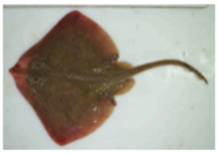
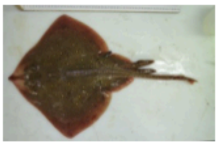
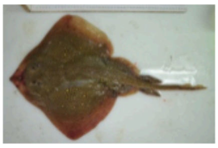
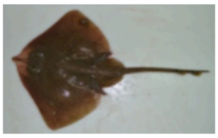
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Raja polystigma* | Rp219d | 61 | 39 | M | 39.857 | 2.759 | KY949068 |  |
| *Raja polystigma* | Rb163 | 56 | 21 | M | 39.968 | 3.758 | KY949069 |  |
| *Raja polystigma* | Rp198 | 108 | 32 | M | 37.663 | -0.356 | KY949070 |  |
| *Raja polystigma* | Rp51b | 247 | 53 | F | 40.284 | 1.117 | KY949071 |  |
| *Raja asterias* | Ra114 | 96 | 49 | F | 41.170 | 2.016 | KY949072 |  |
| *Raja asterias* | Ra142 | 85 | 57 | M | 41.170 | 2.016 | KY949073 |  |
| *Raja asterias* | Ra49 | 107 | 65 | F | 37.717 | -0.550 | KY949074 |  |
| *Raja asterias* | Ra141 | 41 | 57 | M | 41.167 | 1.850 | KY949075 |  |
| *Raja asterias* | Ra13 | 67 | 62 | F | 36.456 | -4.730 | KY949076 |  |
| *Raja asterias* | Ra7 | 119 | 65 | F | 36.332 | -5.217 | KY949077 |  |
| *Raja asterias* | Ra43 | 317 | 61.5 | F | 36.653 | -2.427 | KY949078 |  |

Table S4.3.1.1. (continued)

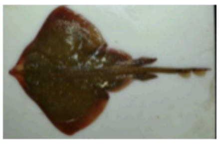
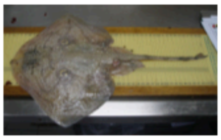
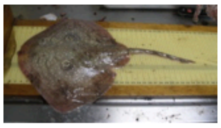
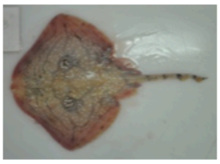
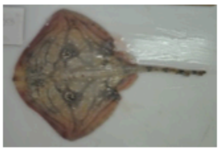
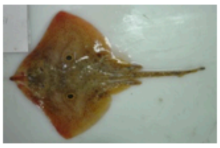
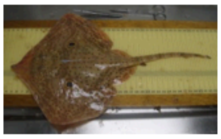
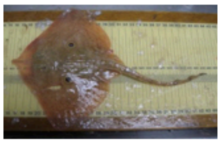
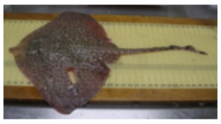
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Raja asterias* | Ra51 | 54 | 53.5 | M | 36.635 | -2.783 | KY949079 |  |
| *Raja radula* | Rr48 | 48 | 50 | F | 37.700 | -0.650 | KY949080 |  |
| *Raja radula* | Rr48b | 48 | 51 | M | 37.700 | -0.650 | KY949081 |  |
| *Raja radula* | Rr159 | 60 | 45 | M | 39.854 | 2.754 | KY949082 |  |
| *Raja radula* | Rr169 | 58 | 34 | M | 39.818 | 4.196 | KY949083 |  |
| *Raja radula* | Rr170 | 63 | 44 | M | 39.851 | 4.094 | KY949084 |  |
| *Raja miraletus* | Rm164 | 77 | 30 | F | 39.889 | 3.867 | KY949085 |  |
| *Raja miraletus* | Rm162 | 80 | 39 | F | 39.732 | 3.684 | KY949086 |  |
| *Raja miraletus* | Rm153 | 98 | 28 | F | 39.240 | 2.858 | KY949087 |  |
| *Raja clavata* | Rc157 | 145 | 54 | M | 39.771 | 2.462 | KY949088 |  |
| *Raja clavata* | Rc78 | 127 | 35 | F | 38.861 | 0.350 | KY949089 |  |

Table S4.3.1.1. (continued)

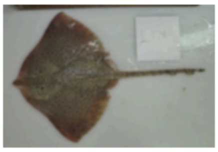
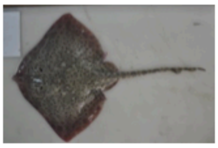
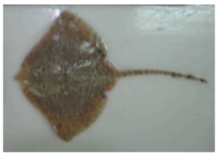
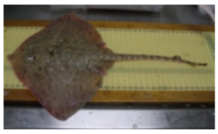
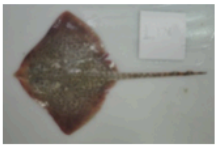
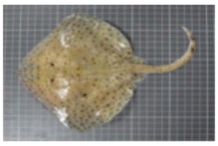
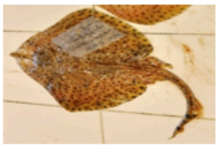
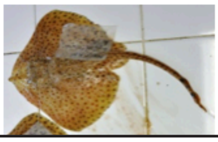
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Raja clavata* | Rc114 | 96 | 60 | F | 40.284 | 1.117 | KY949090 |  |
| *Raja clavata* | Rc156 | 369 | 37 | F | 39.744 | 2.307 | KY949091 |  |
| *Raja clavata* | Rc68 | 135 | 39 | F | 38.053 | -0.311 | KY949092 |  |
| *Raja clavata* | Rc194 | 150 | 23 | M | 39.240 | 2.637 | KY949093 |  |
| *Raja clavata* | Rc22 | 380 | 34.5 | F | 35.889 | -3.265 | KY949094 |  |
| *Raja clavata* | Rc22b | 380 | 35 | F | 35.889 | -3.265 | KY949095 |  |
| *Raja brachyura* | Rp169 | 50 | 26 | F | 39.228 | 2.992 | KY949096 |  |
| *Raja brachyura* | Rb152 | 57 | 40 | F | 39.227 | 2.993 | KY949097 |  |
| *Raja brachyura* | Rb159 | 60 | 83 | M | 39.854 | 2.754 | KY949098 |  |
| *Raja brachyura* | Rb219 | 61 | 41 | M | 39.857 | 2.759 | KY949099 |  |
| *Raja brachyura* | Rb219b | 61 | 30 | F | 39.857 | 2.759 | KY949100 |  |

Table S4.3.1.1. (continued)

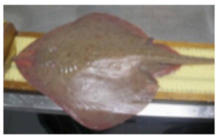
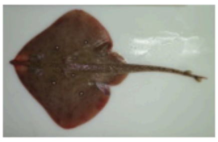
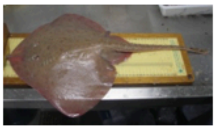
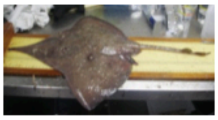
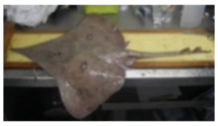
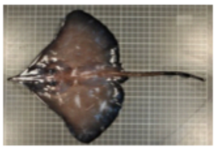
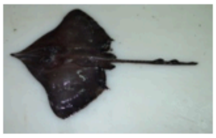
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Leucoraja circularis* | Lc165 | 385 | 62 | F | 39.812 | 3.900 | KY949107 |  |
| *Leucoraja circularis* | Lc173 | 497 | 44 | M | 39.733 | 4.521 | KY949108 |  |
| *Leucoraja circularis* | Lc174 | 134 | 54 | M | 39.784 | 4.429 | KY949109 |  |
| *Leucoraja circularis* | Lc174b | 134 | 62 | M | 39.784 | 4.429 | KY949110 |  |
| *Dipturus oxyrinchus* | Do166 | 249 | 65 | F | 40.193 | 4.109 | KU761956 |  |
| *Dipturus oxyrinchus* | Do173 | 497 | 100 | M | 39.733 | 4.521 | KY949111 |  |
| *Dipturus oxyrinchus* | Do176 | 422 | 75 | M | 40.214 | 3.699 | KU761957 |  |
| *Dipturus nidarosiensis* | Dn26 | 765 | 34.2 | M | 35.947 | -3.171 | KX783036 |  |
| *Dipturus nidarosiensis* | Dn24 | 813 | 32.1 | M | 35.940 | -3.183 | KX783035 |  |
| *Dipturus nidarosiensis* | Dn24b | 819 | 24.7 | M | 35.950 | -3.171 | KX783034 |  |
| *Dipturus nidarosiensis* | Dn24c | 819 | 27 | M | 35.950 | -3.171 | KX783033 |  |

Table S4.3.1.1. (continued)

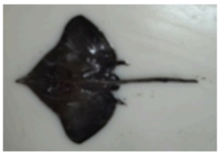
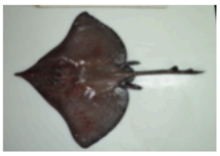
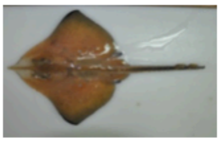
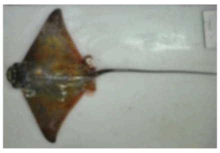
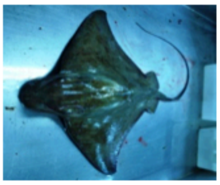
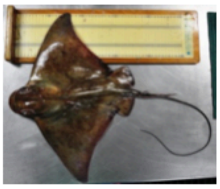
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Dipturus nidarosiensis* | Dn24d | 819 | 25.6 | F | 35.950 | -3.171 | KX783032 |  |
| *Dipturus nidarosiensis* | Dn24e | 819 | 27.9 | M | 35.950 | -3.171 | KX783031 |  |
| *Dipturus nidarosiensis* | Dn26b | 735 | 72.5 | M | 35.991 | -2.753 | KX783030 |  |
| *Dipturus nidarosiensis* | Dn29 | 620 | 24.7 | F | 35.923 | -3.194 | KX783029 |  |
| *Rostroraja alba* | Ral163 | 56 | 168 | F | 39.968 | 3.758 | KY949112 |  |
| *Rostroraja alba* | Ral183 | 48 | 29 | F | 39.230 | 2.989 | KY949113 |  |
| *Myliobatis aquila* | Ma173 | 76 | 54 | F | 39.517 | 3.507 | KY949114 |  |
| *Myliobatis aquila* | Ma173b | 76 | 81 | M | 39.517 | 3.507 | KY949115 |  |
| *Myliobatis aquila* | Ma177 | 137 | 92 | M | 39.794 | 4.437 | KY949116 |  |
| *Pteroplatitrygon violacea* | Pv129 | 124 | 155 | F | 42.133 | 3.387 | KY949117 |  |

Table S4.3.1.1. (continued)

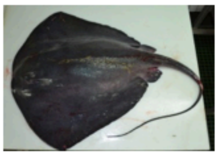
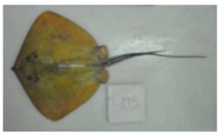
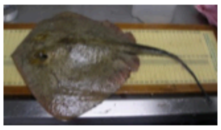
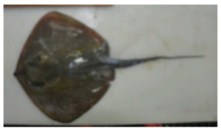
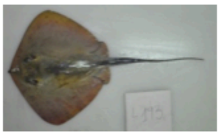
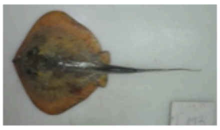
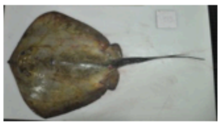
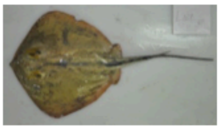
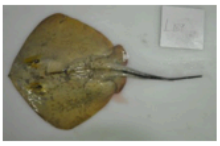
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Pteroplatitrygon violacea* | Pv102 | 51 | 157 | F | 40.127 | 0.365 | KY949118 |  |
| *Pteroplatitrygon violacea* | Pv19 | 324 | 125 | F | 36.657 | -3.824 | KY949119 |  |
| *Dasyatis tortonesei* | Dp169 | 58 | 41 | F | 39.818 | 4.196 | KY949120 |  |
| *Dasyatis tortonesei* | Dp169b | 58 | 54 | M | 39.818 | 4.196 | KY949121 |  |
| *Dasyatis tortonesei* | Dp169c | 58 | 35 | M | 39.818 | 4.196 | KY949122 |  |
| *Dasyatis tortonesei* | Dp193 | 56 | 36 | F | 39.823 | 4.182 | KY949123 |  |
| *Dasyatis tortonesei* | Dp193b | 56 | 38.2 | F | 39.823 | 4.182 | KY949124 |  |
| *Dasyatis tortonesei* | Dp193c | 56 | 86.2 | F | 39.823 | 4.182 | KY949125 |  |
| *Dasyatis pastinaca* | Dp196 | 70 | 45 | M | 39.412 | 2.523 | KY949126 |  |
| *Dasyatis pastinaca* | Dp189 | 59 | 48.6 | F | 39.675 | 3.525 | KY949127 |  |
| *Dasyatis pastinaca* | Dp189b | 59 | 40.6 | F | 39.675 | 3.525 | KY949128 |  |

Table S4.3.1.1. (continued)

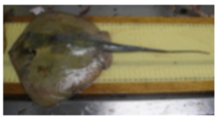
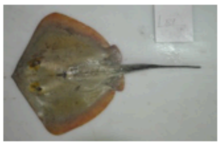
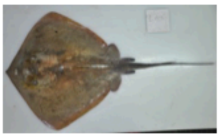
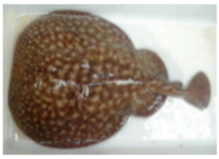
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Dasyatis pastinaca* | Dp189c | 59 | 50.4 | F | 39.675 | 3.525 | KY949129 |  |
| *Dasyatis pastinaca* | Dp189d | 59 | 43.5 | M | 39.675 | 3.525 | KY949130 |  |
| *Dasyatis pastinaca* | Dp184 | 99 | 49.3 | M | 39.243 | 2.855 | KY949131 |  |
| *Ptermylaeus bovinus* | Pt189 | 59 | 75.2 | F | 39.675 | 3.525 | KY949132 |  |
| *Torpedo marmorata* | Tm193 | 252 | 36 | M | 39.051 | 2.701 | KY949133 |  |
| *Torpedo marmorata* | Tm160 | 81 | 23 | M | 39.724 | 3.660 | KY949134 |  |
| *Torpedo marmorata* | Tm31 | 67 | 27 | F | 36.706 | -3.240 | KY949135 |  |

Table S4.3.1.2. Primers and polymerase chain reaction (PCR) conditions for each mitochondrial fragment.

| Regions | Primers | PCR condition | | |
|---|---|---|---|---|
| Dehydrogenase subunit 2 (Sandoval-Castillo and Rocha-Olivares, 2011) | *ND2*Met47: 5’-TTTTGGGCCCATACC-3’<br>*ND2*Trp18: 5’-GCTTTGAAGGCTTTTGGT-3’ | 95.0ºC | 2:00 | |
| | | 94.0ºC | 0:14 | |
| | | 54.0ºC | 1:00 | 35 cycles |
| | | 72.0ºC | 2:00 | |
| | | 72.0ºC | 6:00 | |
| Cytochrome oxidase subunit I (Ward *et al*. 2005) | FishF1: 5’-TCAACCAACCACAAAGACATTGGCAC-3’<br>FishF2: 5’-TCGACTAATCATAAAGATATCGGCAC-3’<br>FishR1: 5’-TAGACTTCTGGGTGGCCAAAGAATCA-3’<br>FishR2: 5’-ACTTCAGGGTGACCGAAGAATCAGAA-3’ | 95.0ºC | 2:00 | |
| | | 94.0ºC | 0:30 | |
| | | 54.0ºC | 0:30 | 35 cycles |
| | | 72.0ºC | 1:00 | |
| | | 72.0ºC | 10:00 | |
| Cytochrome oxidase I (Ivanova *et al*., 2007) | FF2d: 5’-TTCTCCACCAACCACAARGAYATYGG-3’<br>FR1d: 5’-CACCTCAGGGTGTCCGAARAAYCARAA-3’ | 94.0ºC | 2:00 | |
| | | 94.0ºC | 0:30 | |
| | | 52.0ºC | 1:00 | 35 cycles |
| | | 72.0ºC | 1:00 | |
| | | 72.0ºC | 10:00 | |

Table S4.3.1.3. Estimates of average evolutionary divergence (below the diagonal) and identity percentage (above the diagonal) between Mediterranean Batoidea genera, based on mitochondrial concatenated fragment.

| | ***Raja*** | ***Leucoraja*** | ***Dipturus*** | ***Rostroraja*** | ***Myliobatis*** | ***Pteroplatytrygon*** | ***Dasyatis*** | ***Pteromylaeus*** | ***Torpedo*** |
|---|---|---|---|---|---|---|---|---|---|
| *Raja* | | 84.85 | 88.90 | 86.39 | 75.84 | 76.89 | 76.82 | 75.68 | 72.24 |
| *Leucoraja* | 0.152 | | 85.86 | 85.91 | 75.80 | 75.72 | 76.26 | 75.04 | 72.49 |
| *Dipturus* | 0.108 | 0.141 | | 86.41 | 76.09 | 75.98 | 76.41 | 75.02 | 71.75 |
| *Rostroraja* | 0.141 | 0.141 | 0.136 | | 76.82 | 76.22 | 76.40 | 75.63 | 72.61 |
| *Myliobatis* | 0.242 | 0.242 | 0.239 | 0.232 | | 82.18 | 82.84 | 83.28 | 71.65 |
| *Pteroplatytrygon* | 0.231 | 0.243 | 0.240 | 0.238 | 0.178 | | 90.73 | 81.19 | 72.99 |
| *Dasyatis* | 0.232 | 0.237 | 0.236 | 0.236 | 0.172 | 0.093 | | 81.16 | 72.88 |
| *Pteromylaeus* | 0.243 | 0.250 | 0.250 | 0.244 | 0.167 | 0.188 | 0.188 | | 72.94 |
| *Torpedo* | 0.278 | 0.275 | 0.283 | 0.274 | 0.283 | 0.270 | 0.271 | 0.271 | |

Table S4.3.1.4. Estimates of number of base differences between Mediterranean Batoidea species, based on mitochondrial concatenated fragment. Species nomenclature is indicated in Table 4.3.1.1.

| | **Rmo** | **Rp** | **Ra** | **Rr** | **Rm** | **Rc** | **Rb** | **Ln** | **Lc** | **Do** | **Dn** | **Ral** | **Ma** | **Pv** | **Dt** | **Dp** | **Pb** | **Tm** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rmo | | | | | | | | | | | | | | | | | | |
| Rp | 43.33 | | | | | | | | | | | | | | | | | |
| Ra | 110.54 | 111.65 | | | | | | | | | | | | | | | | |
| Rr | 106.27 | 100.58 | 105.73 | | | | | | | | | | | | | | | |
| Rm | 131.00 | 129.28 | 140.21 | 143.73 | | | | | | | | | | | | | | |
| Rc | 88.04 | 88.15 | 81.38 | 61.18 | 122.96 | | | | | | | | | | | | | |
| Rb | 91.87 | 85.20 | 116.08 | 107.00 | 130.53 | 83.58 | | | | | | | | | | | | |
| Ln | 240.87 | 235.34 | 236.23 | 237.60 | 236.27 | 230.10 | 235.40 | | | | | | | | | | | |
| Lc | 233.47 | 226.14 | 229.83 | 238.60 | 232.53 | 232.80 | 235.60 | 94.20 | | | | | | | | | | |
| Do | 156.67 | 160.00 | 160.88 | 167.60 | 185.33 | 161.38 | 235.60 | 225.60 | 213.20 | | | | | | | | | |
| Dn | 157.92 | 161.25 | 164.13 | 175.18 | 191.58 | 163.25 | 160.80 | 222.73 | 212.33 | 101.13 | | | | | | | | |
| Ral | 217.17 | 212.78 | 224.63 | 229.30 | 216.83 | 210.88 | 160.58 | 218.70 | 216.70 | 211.50 | 208.63 | | | | | | | |
| Ma | 369.33 | 371.56 | 377.67 | 391.47 | 359.33 | 363.67 | 379.33 | 375.27 | 372.47 | 368.67 | 370.13 | 358.17 | | | | | | |
| Pv | 351.00 | 357.17 | 363.92 | 371.13 | 343.00 | 350.46 | 362.33 | 377.20 | 373.00 | 369.00 | 373.46 | 367.50 | 275.33 | | | | | |
| Dt | 350.00 | 352.50 | 360.54 | 360.13 | 341.33 | 347.79 | 349.33 | 351.53 | 360.33 | 357.33 | 362.46 | 356.17 | 272.50 | 141.67 | | | | |
| Dp | 359.33 | 358.50 | 372.08 | 376.80 | 360.33 | 362.13 | 363.33 | 375.33 | 380.33 | 363.33 | 374.46 | 373.17 | 257.67 | 144.67 | 111.33 | | | |
| Pb | 372.67 | 371.44 | 386.38 | 381.80 | 381.33 | 365.25 | 371.20 | 390.60 | 380.80 | 385.00 | 387.00 | 376.50 | 258.33 | 290.67 | 294.67 | 287.67 | | |
| Tm | 423.67 | 426.61 | 434.54 | 438.40 | 421.33 | 427.54 | 430.47 | 430.20 | 419.87 | 437.00 | 436.00 | 423.17 | 438.00 | 417.33 | 422.33 | 415.67 | 418.00 | |

# Figures

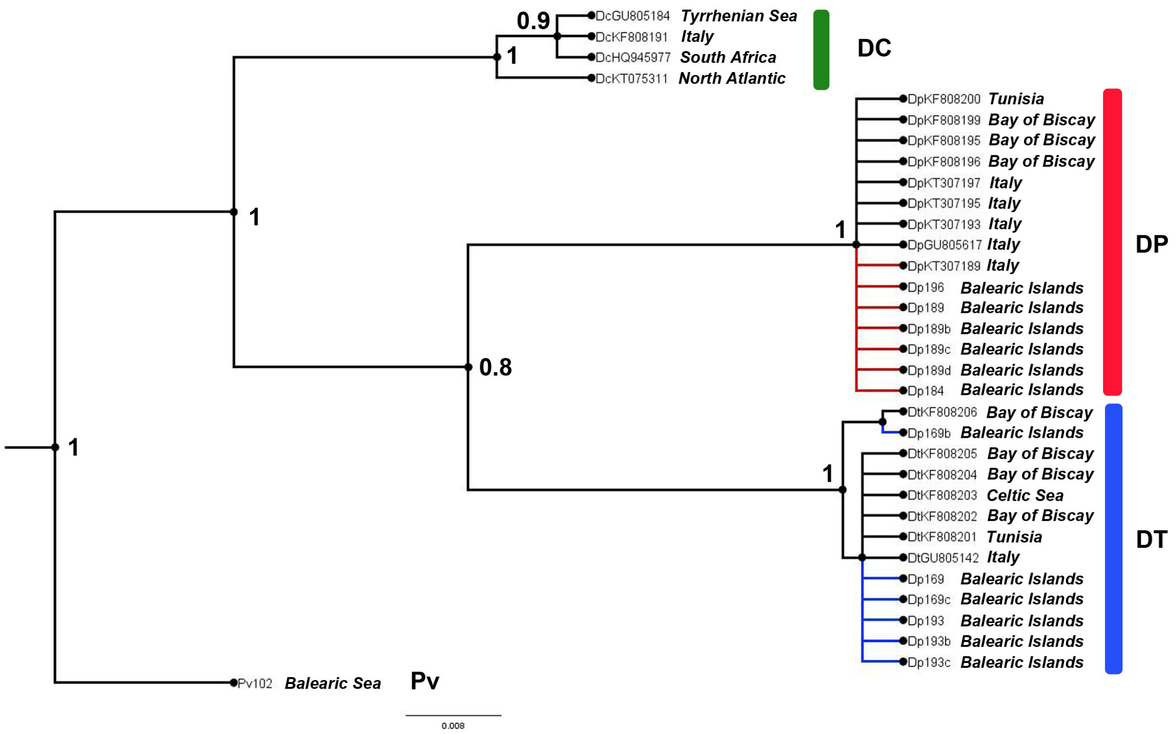


Figure S4.3.1.1. Bayesian tree based on *COI* nucleotide sequences of Dasyatidae species from Mediterranean and Northeast Atlantic, using HKY+I as substitution model. The GenBank accession numbers and sampling localities are indicated close the node. DC: *Dasyatis centraura*; DP: *Dasyatis pastinaca*; DT: *Dasyatis* cf. *tortonesei*; Pv: *Pteroplatytrygon violacea*. Numbers near the nodes are posterior probabilities.

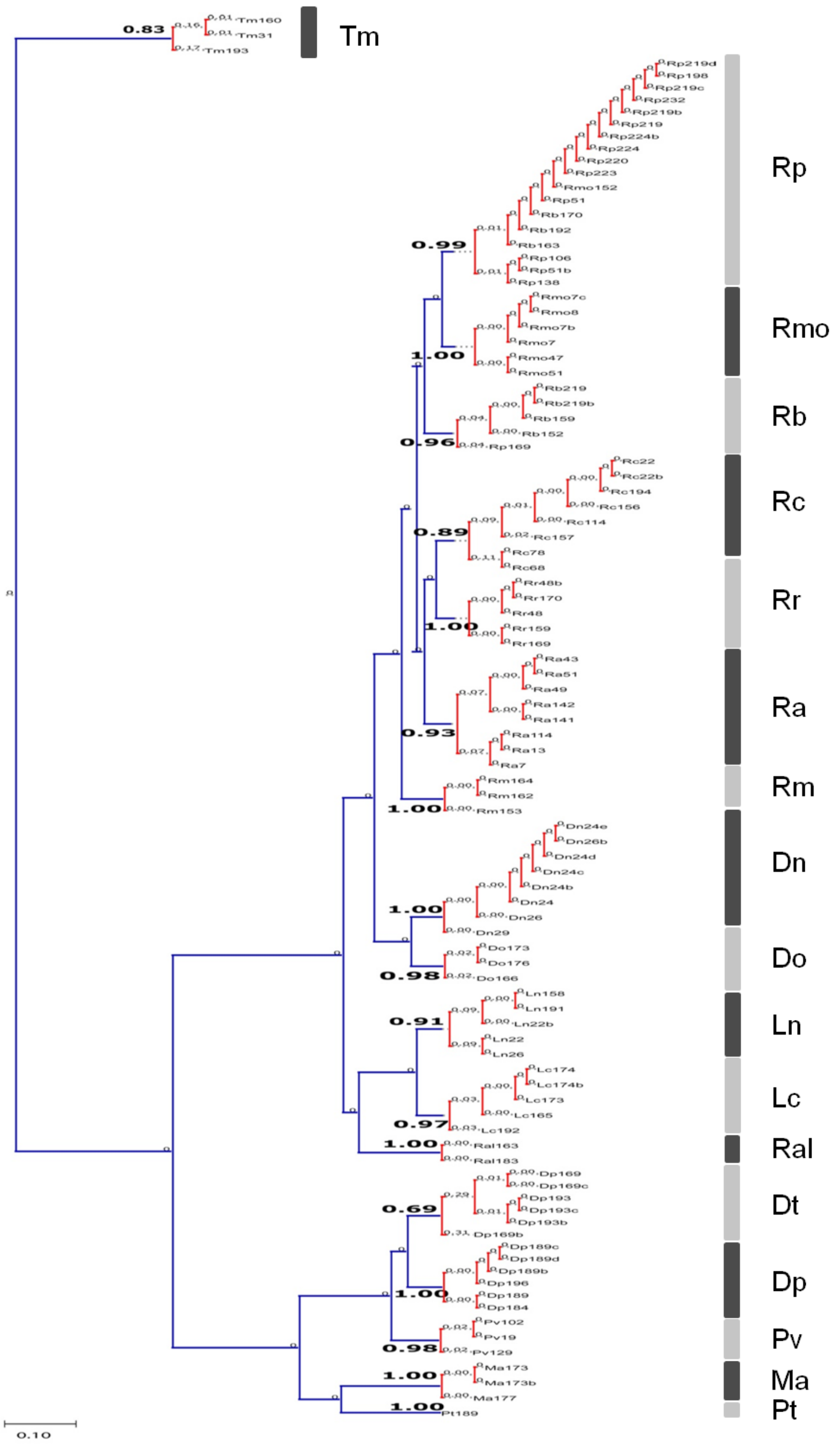


Figure S4.3.1.2. Species delimitation analyses based on mitochondrial concatenated fragment using Poisson Tree Process (PTP). Species nomenclature is indicated in Table 4.3.1.1.

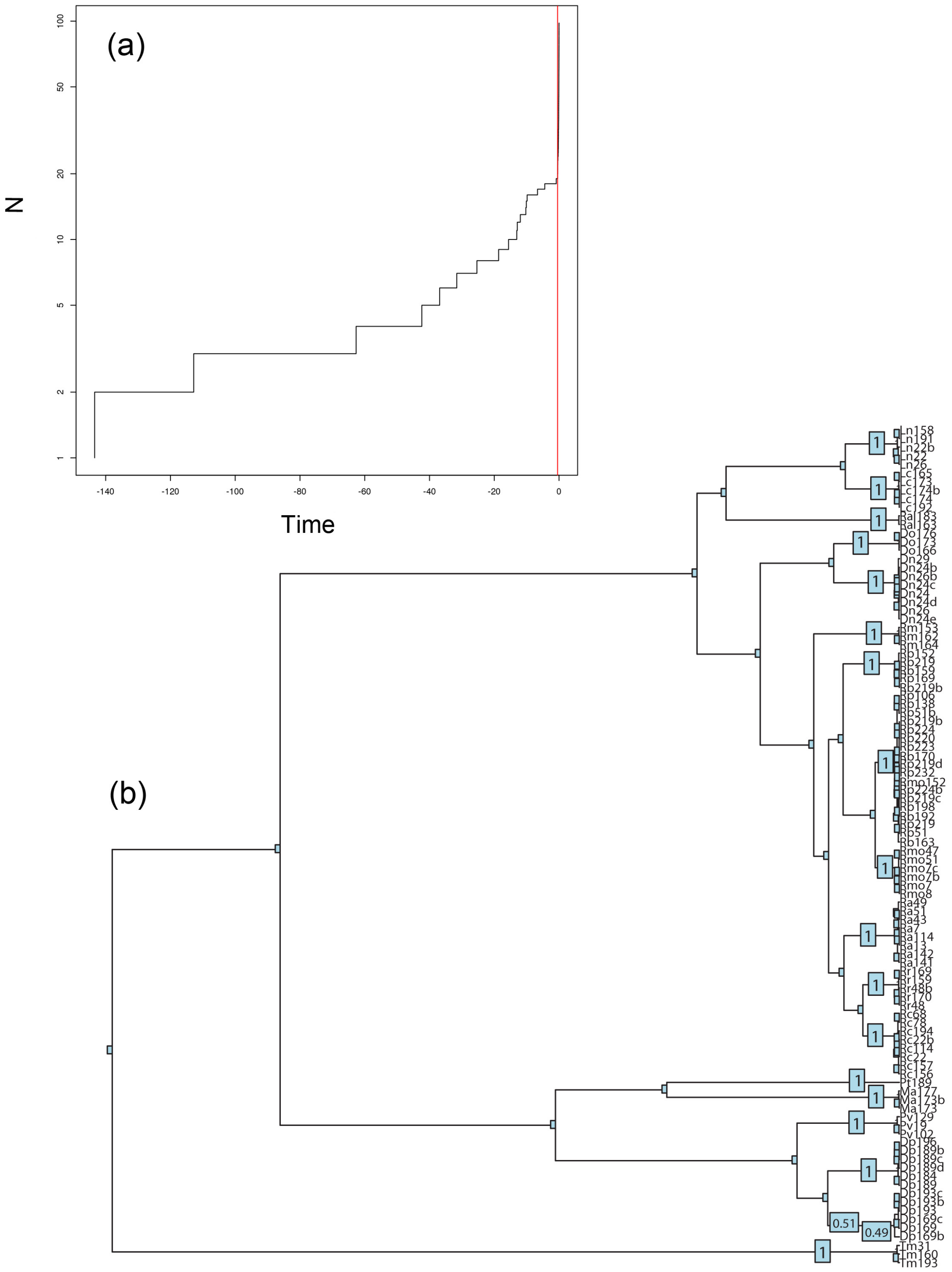


Figure S4.3.1.3. Species delimitation analyses based on mitochondrial concatenated fragment using General Mixed Yule Coalescent (GMYC): Lineages through-time plot (a) and GMYC tree (b) for single thresholds. In plot (a), N in indicates the number of reconstructed lineages. Species nomenclature is indicated in Table 4.3.1.1.

## 4.3.2 New morphological and molecular evidence confirm the presence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea and extend its distribution to the western basin

Ramírez-Amaro S., Ordines F., Puerto M.A., García C., Ramon C., Terrasa B., Massutí E. (2017)

*Mediterranean Marine Science* 18/2: 253–261

## Summary

The present study confirms the presence of the Norwegian skate *Dipturus nidarosiensis* (Storm, 1881) in the Mediterranean Sea, by means of morphological traits and molecular markers providing the first record of this species in the Alboran Sea. Cannas *et al*. (2010) reported *D. nidarosiensis* for the first time in the Mediterranean from specimens captured in the central western basin, but Ebert and Stehmann (2013) and Stehmann *et al*. (2015) considered these records "likely refer to the smaller morphotype, *Dipturus* sp.", a species not yet described. Eight specimens of the *Dipturus* genus (Rajiformes: Rajidae) were caught off the Alboran Island (western Mediterranean) in 2012, 2013 and 2016, between 620 and 819 m depth. These specimens showed morphometric diagnostic features corresponding to those of Norwegian skates from the Northeast Atlantic and the central western Mediterranean Sea. Moreover, the Alboran individuals were genetically compared to Northeast Atlantic specimens available in GenBank by means of two mitochondrial DNA fragments: cytochrome c oxidase subunit I (*COI*) and cytochrome b (*Cytb*). Analyses showed that the Northeast Atlantic Norwegian skate specimens and the Alboran Sea ones were genetically similar and shared haplotypes, corroborating the identification of the Alboran individuals as *Dipturus nidarosiensis*. However, they were different from other *Dipturus* species distributed throughout the Northeast Atlantic and the Mediterranean Sea. Our results confirm the occurrence of this deep-sea large skate species in the Mediterranean, although the IUCN Red List of Threatened Species does not consider it possible (Stehmann *et al*., 2015).

**Keywords**: Alboran Sea, deep-sea skate, *Dipturus nidarosiensis*, DNA barcoding, western Mediterranean.

## 4.3.2.1 Introduction

Skates (Rajiformes: Rajidae) are considered especially vulnerable to the impact of fishing activities because of their k-selected life-history strategy, characterized by slow growth, late attainment of sexual maturity, long life spans and low fecundity (Stevens *et al*., 2000). The Rajidae is a highly diverse family of elasmobranch fishes, including more than 285 species (Weigmann, 2016). Within this family, the genus *Dipturus* (Rafinesque, 1810) is among the most diverse, comprising at least 29 species (Ebert and Compagno, 2007). This genus includes some of the largest know skates, such as the critically endangered common skate, *Dipturus batis* (Dulvy *et al*., 2006), which was recently reported to be a species-complex in which two species were confounded: the blue skate *D.* cf. *flossada* (Risso, 1826) and the flapper skate *D.* cf. *intermedia* (Parnell, 1937) (Griffiths *et al*., 2010; Iglésias *et al*., 2010). In the Northeast Atlantic Ocean, besides the two aforementioned species, the presence of the spear-nose skate *D. oxyrinchus* (Linnaeus, 1758) and the Norwegian skate *D. nidarosiensis* (Storm, 1881) is also known, both suggested to represent species complexes similar to that reported for *D. batis* (Ebert and Stehmann, 2013).

The Norwegian skate occurs mainly off central and southern Norway, off Iceland, Scotland and Ireland (Ebert and Stehmann, 2013). Recent records include the Bay of Biscay (Rodríguez-Cabello *et al*., 2013) and the central western Mediterranean (Sardinia; Cannas *et al*., 2010). However, Ebert and Stehmann (2013) considered the records of 14 individuals from the Mediterranean (Cannas *et al*., 2010) “likely refer to the smaller morphotype, *Dipturus* sp.”, a species not yet described. This previous study did not take into account the fact that several fish and non-fish species distributed in both the Atlantic and Mediterranean exhibited a smaller morphotype and probably genetic divergence in the latter basin, even if the population genetic boundaries are not geographically and hydrogeographically homogeneous (Parternello *et al*., 2007). The inference assumed by Ebert and Stehmann (2013) has been included in the latest assessment of this species by the IUCN Red List of Threatened Species (Stehmann *et al*., 2015), where the Mediterranean records are not taken into account; even though additional records were reported also in the central western basin by Follesa *et al*. (2012). Moreover, a very recent study has documented *D. nidarosiensis* in the Rajidae fauna of the Ionian Sea also in the central western Mediterranean (Cariani *et al*., 2017).

The Norwegian skate is mainly distributed on the outer continental shelf and slope bottoms, from around 200 m to more than 1,000 m depth (Ebert & Stehmann, 2013). Although this species is not targeted by commercial fishing, it is a by-catch of the deep-water trawl and long-line fisheries in which it is occasionally caught (ICES, 2015). *D. nidarosiensis* is currently catalogued as “near threatened” in the Northeast Atlantic by the IUCN Red List of Threatened Species (Stehmann *et al*., 2015). Therefore, an improvement in knowledge regarding the distribution of this species is of significant importance for conservation purposes. The present study provides new morphological and molecular evidence that confirms the presence of *D. nidarosiensis* in the Mediterranean and enlarges its distribution range from Sardinia to the Alboran Sea, next to the Northeast Atlantic where the species is well known.

## 4.3.2.2 Materials and Methods

### *Samples*

During the MEDITS bottom trawl surveys (see Bertrand *et al*., 2002 for specification on sampling scheme and gear used) in 2012, 2013, and 2016, eight specimens of *Dipturus* sp. were collected from hauls caught off the Alboran Island (Figure 4.3.2.1) at depths ranging from 620 to 819 m. All the specimens were caught from three sampling stations (Figure 4.3.2.1). Additionally, two specimens of *D. oxyrinchus* were collected during the MEDITS survey in 2013 off the Balearic Islands. A small piece of muscular tissue of each specimen was preserved in absolute ethanol for molecular analyses.

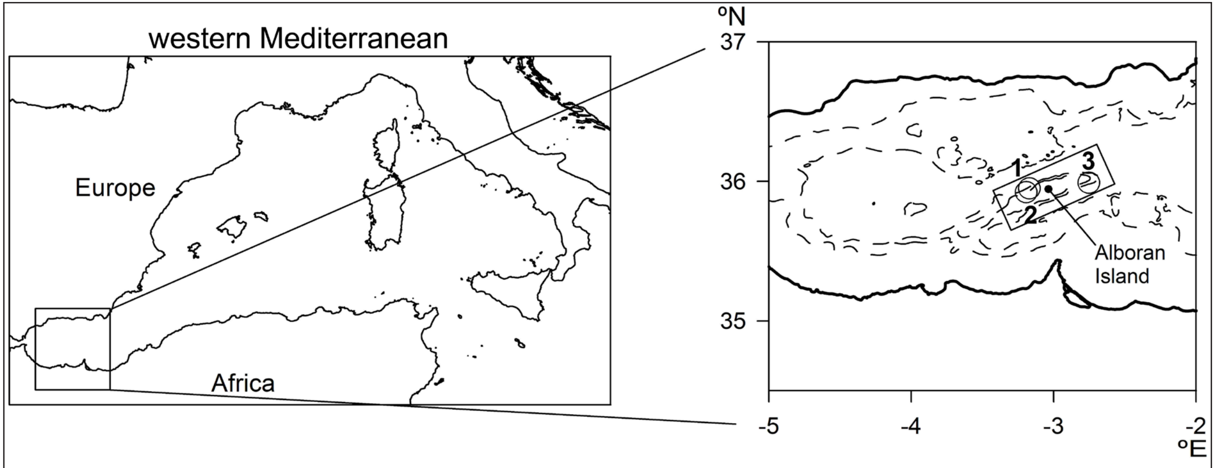


Figure 4.3.2.1. Map of the study area (Alboran Sea, western Mediterranean), indicating the sampling stations (circles) where the samples were caught: (1) CFM-IEOMA 3570, CFM-IEOMA 3472, CFM-IEOMA 6040, CFM-IEOMA 6041, CFM-IEOMA 6042, CFM-IEOMA 6043; (2) CFM-IEOMA 6044, (3) CFM-IEOMA 6045. Isobaths correspond to 600 and 1,000 m depth.

### *Morphology*

Morphometric measurements and external observations were recorded for all *Dipturus* sp. specimens according to Stehmann (1995) and Cannas *et al*. (2010). All measurements are indicated in Table 4.3.2.1. All individuals are preserved in the Marine Fauna Collection (http://www.ma.ieo.es/cfm/) based at the Centro Oceanográfico de Málaga (Instituto Español de Oceanografía). Catalogue IDs for each sample (CFM-IEOMA samples) are indicated in Table 4.3.2.2.

### *Molecular analyses*

Genomic DNA was extracted from tissue samples following the protocol proposed by Terrasa *et al*. (2009). Polymerase chain reaction (PCR) and the universal primers FF2d, FR1d and L14724, H15175 (Ivanova *et al*., 2007; Palumbi, 1996) were used to amplify two mitochondrial fragments: cytochrome c oxidase subunit I (*COI*) and the cytochrome b (*Cytb*). The thermal profile for PCR consisted of 96°C for 5 min; then 35 cycles at 94°C for 60 s, 56/52°C for 60 s, and 72°C for 60 s, followed by a final extension at 72°C for 10 min. PCR products were purified using the commercial kit QIAquick PCR Purification Kit (QIAGEN). Both heavy and light strands were sequenced on an automated ABI 3130 sequencer using ABI Prism Terminator BigDye TM Cycle Sequencing Reaction Kit (Applied Biosystems). Sequences were edited and aligned within BioEdit v. 7.0.5.2 (Hall, 1999). The DNA sequences obtained for both mitochondrial fragments were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) and, additionally, the *COI* fragments were recorded in the Barcode of Life Data (BOLD) database, http://www.boldsystems.org; Ratnasingham and Hebert, 2007).

In order to compare our *Dipturus* samples with other *Dipturus* species in the Northeast Atlantic, we downloaded from GenBank the *COI* and *Cytb* sequences provided by Griffiths *et al*. (2010), Rodríguez-Cabello *et al*. (2013) and Lynghammar *et al*. (2014) (GenBank ID's reported below).

Genetic distances (p-distance), identity percentage, and number of base differences between pair of DNA sequences were calculated with MEGAv.6 (Tamura *et al*., 2013) and Geneious v. 7.1.3 software (Biomatters Ltd., www.geneious.com). The average values of three previous genetic indices between our study samples and GenBank sequences were compared. Additionally, the haplotype network was made for both mitochondrial DNA fragments by statistical parsimony in TCS v.1.21 (Clement *et al*., 2000) and manually edited in Illustrator CS5 (Adobe Systems Inc., CA, USA). Phylogenetic reconstruction was estimated using the substitution model selected by AIC implemented in jModelTest v.2.1.7 (Darriba *et al*., 2012): Hasegawa-Kishino-Yano (1985) model with invariant sites (HKY+I) and Tamura 3-parameter (Tamura, 1992) for *COI* and *Cytb* (*Leucoraja naevus* as outgroup), respectively. Trees were made with MEGA v.6 using the maximum likelihood (ML) assessed by non-parametric bootstrapping (1,000 replicates).

## 4.3.2.3 Results

*External features*

Morphometric measures of all specimens are summarized in Table 4.3.2.1.The *Dipturus* sp. specimens (CFM-IEOMA samples) caught off the Alboran Island ranged from 24.7 to 72.5 cm and from 17.3 to 51.6 cm in total length and disc width, respectively (Table 4.3.2.1). In all, 6 males and 2 females were captured, all of which were immature, with males presenting soft claspers shorter than the length of the pelvic fins, and females with barely visible ovaries and undistinguishable ovarian follicles.

All specimens showed the main traits characterizing the genus *Dipturus*, including a long and pointed snout (internarial width less than 70% prenarial snout length), anterior margins deeply concave and thorns on disc usually absent (Ebert and Stehmann, 2013; Figure 4.3.2.2). All individuals were identified as *Dipturus nidarosiensis* due to the combination of the following diagnostic features: thorns on disc absent, 39-43tooth rows in the upper jaw, interdorsal thorns absent (which is common in juvenile specimens), dorsal side uniformly grey-brownish without dots and ventral side almost completely black, covered by a firm layer of black mucus. However, the specimens showed irregularly scattered tail spines, which were fewer (10-12) than those described for adult specimens of *D. nidarosiensis* in the Atlantic (40-50; Ebert and Stehmann, 2013) and the central western Mediterranean (30-41; Cannas *et al*., 2010), but similar to the 9-11 thorns reported by the latter authors for juvenile specimens.

*Genetics*

All specimens were sequenced for *COI* and *Cytb* mitochondrial fragments, producing 591 and 378 bp, respectively. The GenBank and BOLD accession number for *D. oxyrinchus* and CFM-IEOMA samples are summarized in Table 4.3.2.2.

The haplotype network (Figure 4.3.2.3) showed that CFM-IEOMA samples shared haplotypes of both mitochondrial fragments with samples of Northeast Atlantic *D. nidarosiensis*. The low values of genetic distance, reduced numbers of base differences, and the high percentage of identity for *COI* (0.002; 0.9 bp and 99.85, respectively, Table 4.3.2.3) and *Cytb* (0.002; 0.8 bp and 99.80, respectively, Table 4.3.2.3), between the CFM-IEOMA samples and *D. nidarosiensis* from the Northeast Atlantic and the Bay of Biscay supported the morphological identification of the CFM-IEOMA samples as *D. nidarosiensis*. Meanwhile, larger values for genetic distance and lower percentage identity were recorded when comparing the CFM-IEOMA samples and the other *Dipturus* species: *D. oxyrinchus* (0.059/94.0 and 0.040/95.9 for *COI* and *Cytb*, respectively), *D*. cf. *intermedia* (0.056/94.3 for *COI*), and *D*. cf. *flossada* (0.052/94.8 for *COI*), respectively (Table 4.3.2.3).

Phylogenetic reconstruction for *COI* (Figure 4.3.2.4), indicated two strongly supported monophyletic clades. The first corresponds to *D. nidarosiensis* and the CFM-IEOMA samples. The second clade includes the other *Dipturus* species (*D. oxyrinchus*, *D.* cf. *intermedia* and *D.* cf. *flossada*).

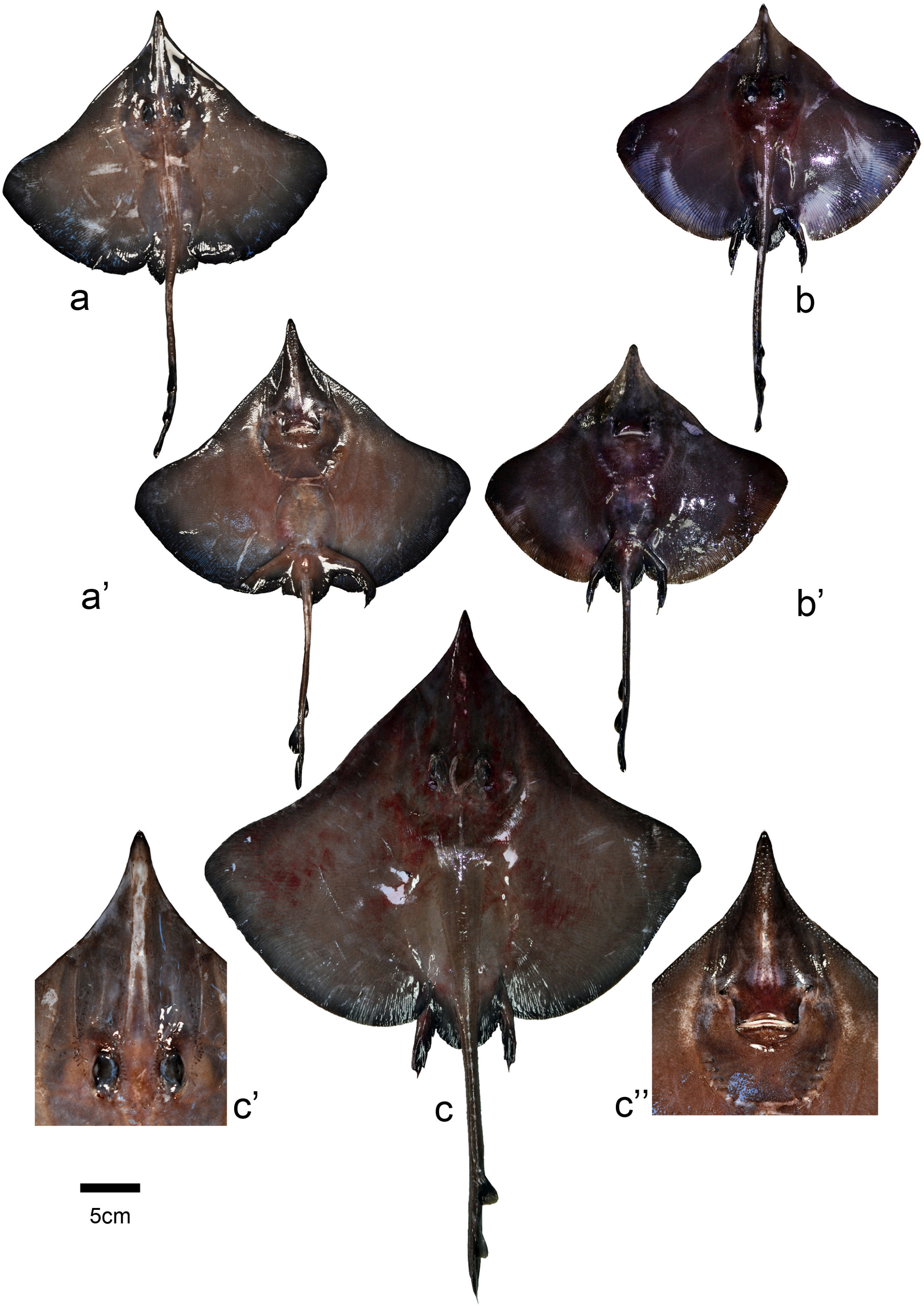


Figure 4.3.2.2. Pictures of *Dipturus nidarosiensis* specimens caught in the Alboran Sea (western Mediterranean). Dorsal (a, b) and ventral (a’, b’) view of male (CFM-IEOMA 3472: a, a’) and female (CFM-IEOMA 6042: b, b’) specimens. Dorsal view of largest specimen (CFM-IEOMA 6044: c) with close view to the side (c’, c’’).

Table 4.3.2.1. Measurements (cm) and percentage of total length (%) of CFM-IEOMA samples caught in the Alboran Sea (western Mediterranean). Sex of each specimen (M, male; F, female) is indicated in brackets.

| Measurements | CFM-IEOMA 3472 (M) | | CFM-IEOMA 3570 (M) | | CFM-IEOMA 6040 (M) | | CFM-IEOMA 6041 (M) | | CFM-IEOMA 6042 (F) | | CFM-IEOMA 6043 (M) | | CFM-IEOMA 6044 (M) | | CFM-IEOMA 6045 (F) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | cm | % | cm | % | cm | % | cm | % | cm | % | cm | % | cm | % | cm | % |
| Total Length | 34.2 | | 32.1 | | 24.7 | | 27 | | 25.6 | | 27.9 | | 72.5 | | 24.7 | |
| Disc width | 24.9 | 72.81 | 22.9 | 71.34 | 17.5 | 70.85 | 19.1 | 70.74 | 18.4 | 71.88 | 19.3 | 69.18 | 51.6 | 71.17 | 17.3 | 70.04 |
| Disc length | 19.7 | 57.60 | 18.2 | 56.70 | 13.3 | 53.85 | 14.9 | 55.19 | 14.4 | 56.25 | 15.5 | 55.56 | 43 | 59.31 | 13 | 52.63 |
| Snout tip to max disc width | 16.5 | 48.25 | 15.2 | 47.35 | 12.9 | 52.23 | 13 | 48.15 | 12.9 | 50.39 | 13.4 | 48.03 | 39.8 | 54.90 | 12.8 | 51.82 |
| Preorbital snout length | 7.6 | 22.22 | 6.4 | 19.94 | 4.7 | 19.03 | 5.7 | 21.11 | 5.3 | 20.70 | 5.8 | 20.79 | 16.1 | 22.21 | 4.7 | 19.03 |
| Eyeball length (left) | 0.7 | 2.05 | 0.8 | 2.49 | 0.7 | 2.83 | 0.8 | 2.96 | 0.7 | 2.73 | 0.8 | 2.87 | 2 | 2.76 | 0.8 | 3.24 |
| Orbit diameter | 1.2 | 3.51 | 1 | 3.12 | 1 | 4.05 | 1.1 | 4.07 | 1 | 3.91 | 1.2 | 4.30 | 2.2 | 3.03 | 1 | 4.05 |
| Orbit + spiracle length | 1.6 | 4.68 | 1.4 | 4.36 | 1.2 | 4.86 | 1.4 | 5.19 | 1.3 | 5.08 | 1.5 | 5.38 | 3.1 | 4.28 | 1.1 | 4.45 |
| Spiracle length | 0.6 | 1.75 | 0.6 | 1.87 | 0.6 | 2.43 | 0.4 | 1.48 | 0.6 | 2.34 | 0.5 | 1.79 | 1.2 | 1.66 | 0.4 | 1.62 |
| Interorbital width | 1.3 | 3.80 | 1.1 | 3.43 | 1.1 | 4.45 | 1.2 | 4.44 | 1.2 | 4.69 | 1.2 | 4.30 | 3.2 | 4.41 | 1.1 | 4.45 |
| Distance between spiracles | 2.1 | 6.14 | 1.9 | 5.92 | 2.1 | 8.50 | 1.9 | 7.04 | 1.9 | 7.42 | 2.1 | 7.53 | 4.5 | 6.21 | 2.2 | 8.91 |
| Precaudal length | 17.2 | 50.29 | 15.2 | 47.35 | 12 | 48.58 | 13 | 48.15 | 12.2 | 47.66 | 13.2 | 47.31 | 37.2 | 51.31 | 11.4 | 46.15 |
| Tail length to first dorsal fin | 8.3 | 24.27 | 8.7 | 27.10 | 6.7 | 27.13 | 7.5 | 27.78 | 6.8 | 26.56 | 7.5 | 26.88 | 19.7 | 27.17 | 7.2 | 29.15 |
| Tail length to second dorsal fin | 11.4 | 33.33 | 11.5 | 35.83 | 8.8 | 35.63 | 9.7 | 35.93 | 9.3 | 36.33 | 9.4 | 33.69 | 25.2 | 34.76 | 9.2 | 37.25 |
| Tail length | 15.7 | 45.91 | 14.8 | 46.11 | 13.1 | 53.04 | 13.2 | 48.89 | 12.9 | 50.39 | 13.6 | 48.75 | 35.5 | 48.97 | 12.2 | 49.39 |
| Preoral length | 7.9 | 23.10 | 7.2 | 22.43 | 5.1 | 20.65 | 6.1 | 22.59 | 5.6 | 21.88 | 6.1 | 21.86 | 17.5 | 24.14 | 5.1 | 20.65 |
| Prenarial length | 6.9 | 20.18 | 6.1 | 19.00 | 4.3 | 17.41 | 5.3 | 19.63 | 5 | 19.53 | 5.5 | 19.71 | 16.5 | 22.76 | 5.4 | 21.86 |
| Mouth width | 3 | 8.77 | 2.5 | 7.79 | 2 | 8.10 | 2.2 | 8.15 | 2.1 | 8.20 | 2.3 | 8.24 | 5.4 | 7.45 | 2 | 8.10 |
| Internarial distance | 3.3 | 9.65 | 3.2 | 9.97 | 2.1 | 8.50 | 2.6 | 9.63 | 2.3 | 8.98 | 2.5 | 8.96 | 6.5 | 8.97 | 2.2 | 8.91 |
| Width of the first gill slits | 0.5 | 1.46 | 0.3 | 0.93 | 0.5 | 2.02 | 0.5 | 1.85 | 0.5 | 1.95 | 0.5 | 1.79 | 1.1 | 1.52 | 0.5 | 2.02 |
| Width of the fifth gill slits | 0.35 | 1.02 | 0.2 | 0.62 | 0.4 | 1.62 | 0.4 | 1.48 | 0.4 | 1.56 | 0.3 | 1.08 | 0.9 | 1.24 | 0.4 | 1.62 |
| Distance between first gill slit | 5 | 14.62 | 4 | 12.46 | 3.4 | 13.77 | 3.5 | 12.96 | 3.3 | 12.89 | 3.6 | 12.90 | 9.3 | 12.83 | 3.3 | 13.36 |
| Distance between fifth gill slit | 3.7 | 10.82 | 3.5 | 10.90 | 2.4 | 9.72 | 2.4 | 8.89 | 2.2 | 8.59 | 2.5 | 8.96 | 6.3 | 8.69 | 2.3 | 9.31 |
| Tail width at axil of pelvic fins | 0.7 | 2.05 | 0.6 | 1.87 | 0.5 | 2.02 | 0.5 | 1.85 | 0.5 | 1.95 | 0.6 | 2.15 | 1.6 | 2.21 | 0.5 | 2.02 |
| Tail height at axil of pelvic fins | 0.75 | 2.19 | 0.7 | 2.18 | 0.65 | 2.63 | 0.65 | 2.41 | 0.64 | 2.50 | 0.76 | 2.72 | 1.5 | 2.07 | 0.6 | 2.43 |
| First dorsal fin base | 1.9 | 5.56 | 1.8 | 5.61 | 1.5 | 6.07 | 1.8 | 6.67 | 1.5 | 5.86 | 1.5 | 5.38 | 4.3 | 5.93 | 1.5 | 6.07 |
| First dorsal fin height | 0.8 | 2.34 | 0.9 | 2.80 | 0.6 | 2.43 | 0.6 | 2.22 | 0.7 | 2.73 | 0.8 | 2.87 | 2.2 | 3.03 | 0.6 | 2.43 |
| Second dorsal fin base | 2.3 | 6.73 | 1.8 | 5.61 | 1.5 | 6.07 | 1.8 | 6.67 | 14.3 | 55.86 | 1.5 | 5.38 | 4.8 | 6.62 | 1.6 | 6.48 |
| Second dorsal fin height | 1 | 2.92 | 0.9 | 2.80 | 0.65 | 2.63 | 0.65 | 2.41 | 0.64 | 2.50 | 0.8 | 2.87 | 2 | 2.76 | 0.7 | 2.83 |
| First dorsal fin to caudal fin tip | 5.5 | 16.08 | 4.5 | 14.02 | 5.1 | 20.65 | 4.5 | 16.67 | 4.4 | 17.19 | 4.7 | 16.85 | 11.5 | 15.86 | 4.5 | 18.22 |
| Second dorsal fin to caudal fin tip | 3.5 | 10.23 | 2.5 | 7.79 | 2.7 | 10.93 | 2.4 | 8.89 | 2.3 | 8.98 | 2.6 | 9.32 | 5.1 | 7.03 | 2.5 | 10.12 |
| Interdorsal distance | 0.7 | 2.05 | 0.8 | 2.49 | 0.8 | 3.24 | 0.5 | 1.85 | 0.7 | 2.73 | 0.8 | 2.87 | 1.7 | 2.34 | 0.6 | 2.43 |
| Teeth in upper jaw | 43 | | 39 | | 43 | | 43 | | 43 | | 43 | | 44 | | 43 | |
| Tail thorns | 12 | | 10 | | 12 | | 12 | | 12 | | 12 | | 10 | | 11 | |

Table 4.3.2.2. GenBank and BOLD accession numbers for CFM-IEOMA samples and *Dipturus* oxyrhinchus specimens of *COI* and *Cytb* sequences.

| **Species** | **Catalogue ID** | **Year collected** | *COI* | | *Cytb* |
|---|---|---|---|---|---|
| | | | **GenBank no** | **BOLD no** | **GenBank no** |
| *Dipturus nidarosiensis* | CFM-IEOMA 3472 | 2012 | KX783036 | AAEES003-16 | KX463276 |
| | CFM-IEOMA 3570 | 2013 | KX783035 | AAEES004-16 | KX463275 |
| | CFM-IEOMA 6040 | 2016 | KX783034 | AAEES005-16 | KX686016 |
| | CFM-IEOMA 6041 | 2016 | KX783033 | AAEES006-16 | KX686017 |
| | CFM-IEOMA 6042 | 2016 | KX783032 | AAEES007-16 | KX686018 |
| | CFM-IEOMA 6043 | 2016 | KX783031 | AAEES008-16 | KX686019 |
| | CFM-IEOMA 6044 | 2016 | KX783030 | AAEES009-16 | KX686020 |
| | CFM-IEOMA 6045 | 2016 | KX783029 | AAEES010-16 | KX686021 |
| *Dipturus oxyrhinchus* | 166 | 2013 | KU761956 | AAEES001-16 | KX463277 |
| | 176 | 2013 | KU761957 | AAEES002-16 | KX463278 |

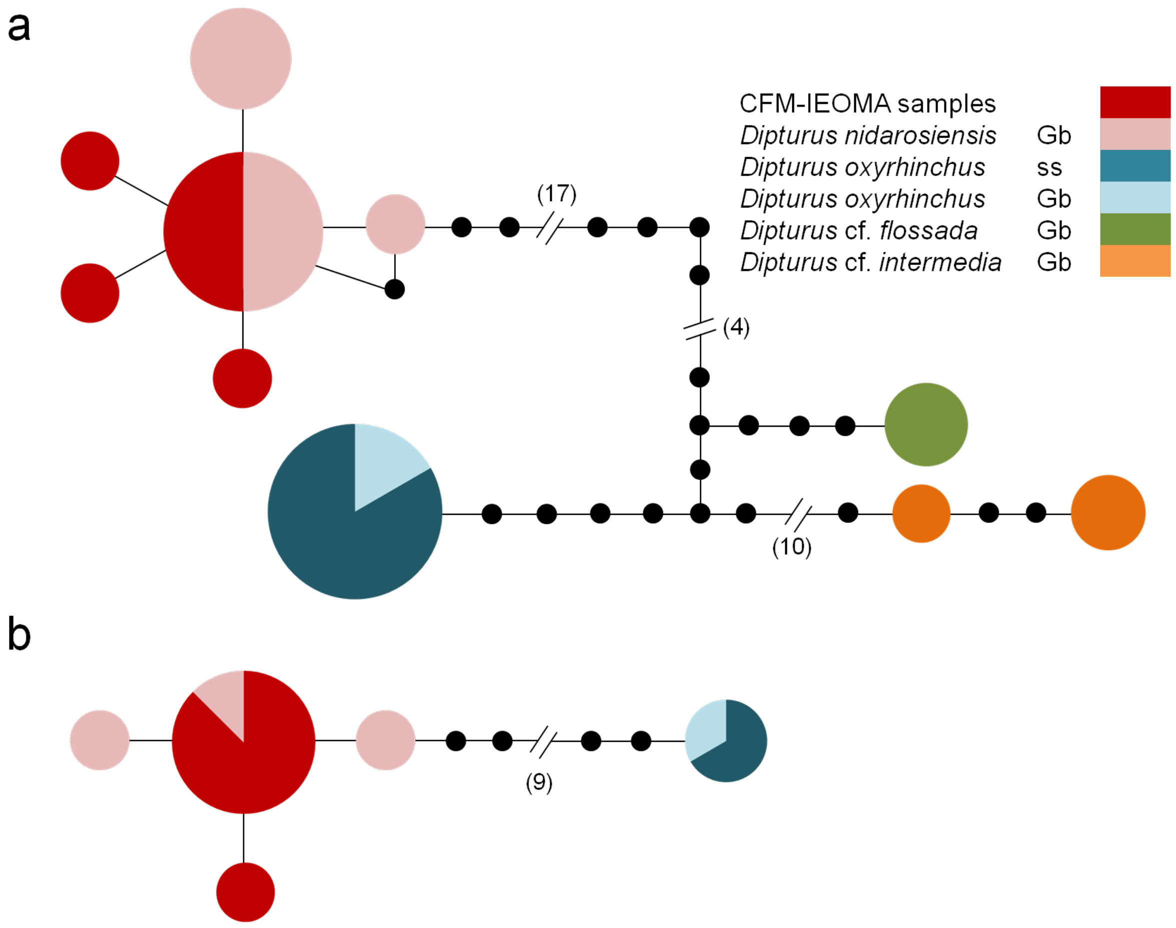


Figure 4.3.2.3. Haplotype networks derived for (a) *COI* and (b) *Cytb* fragments of *Dipturus* species from the Northeast Atlantic and the Mediterranean. Sizes of circles correspond to the number of individuals displaying each haplotype. Numbers in brackets indicate the total mutational steps separating each haplotype. Black circles represent mutational steps, separated by oblique lines when necessary for the shortening of the steps connecting the largest differentiated haplotypes. ss: study samples; Gb: GenBank samples.

Table 4.3.2.3. Average genetic distances and percentage of identity for *COI* and *Cytb* fragments from *Dipturus* species below and above the diagonal, respectively. Average numbers of base differences for both fragments are also indicated in brackets. Dn: *Dipturus nidarosiensis*; Do: *Dipturus* oxyrhinchus; Df: *Dipturus* cf. *flossada*; Di: *Dipturus* cf. *intermedia*; ss: study samples; Gb: GenBank samples.

| *Dipturus* **samples** | **CFM-IEOMA samples** | | **Dn Gb** | | **Do ss** | | **Do Gb** | | **Df Gb** | **Di Gb** |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***COI*** | ***Cytb*** | ***COI*** | ***Cytb*** | ***COI*** | ***Cytb*** | ***COI*** | ***Cytb*** | ***COI*** | ***COI*** |
| CFM-IEOMA samples | | | 99.85 | 99.80 | 94.03 | 95.98 | 94.03 | 95.98 | 94.82 | 94.37 |
| Dn Gb | 0.002 (0.9) | 0.002 (0.8) | | | 94.02 | 96.03 | 94.02 | 96.03 | 94.77 | 94.36 |
| Do ss | 0.059 (35.1) | 0.040 (15.1) | 0.060 (35.5) | 0.040 (15) | | | 97.04 | 100 | 95.74 | 96.28 |
| Do Gb | 0.059 (35.1) | 0.040 (15.1) | 0.060 (35.5) | 0.040 (15) | 0.0 (0.0) | 0.0 (0.0) | | | 96.61 | 98.14 |
| Df Gb | 0.052 (30.7) | ------ | 0.52 (30.7) | ------ | 0.034 (20) | ------ | 0.034 (20) | ------ | | 97.34 |
| Di Gb | 0.056 (33.1) | ------ | 0.57 (33.5) | ------ | 0.019 (11) | ------ | 0.019 (11) | ------ | 0.031 (18.3) | |

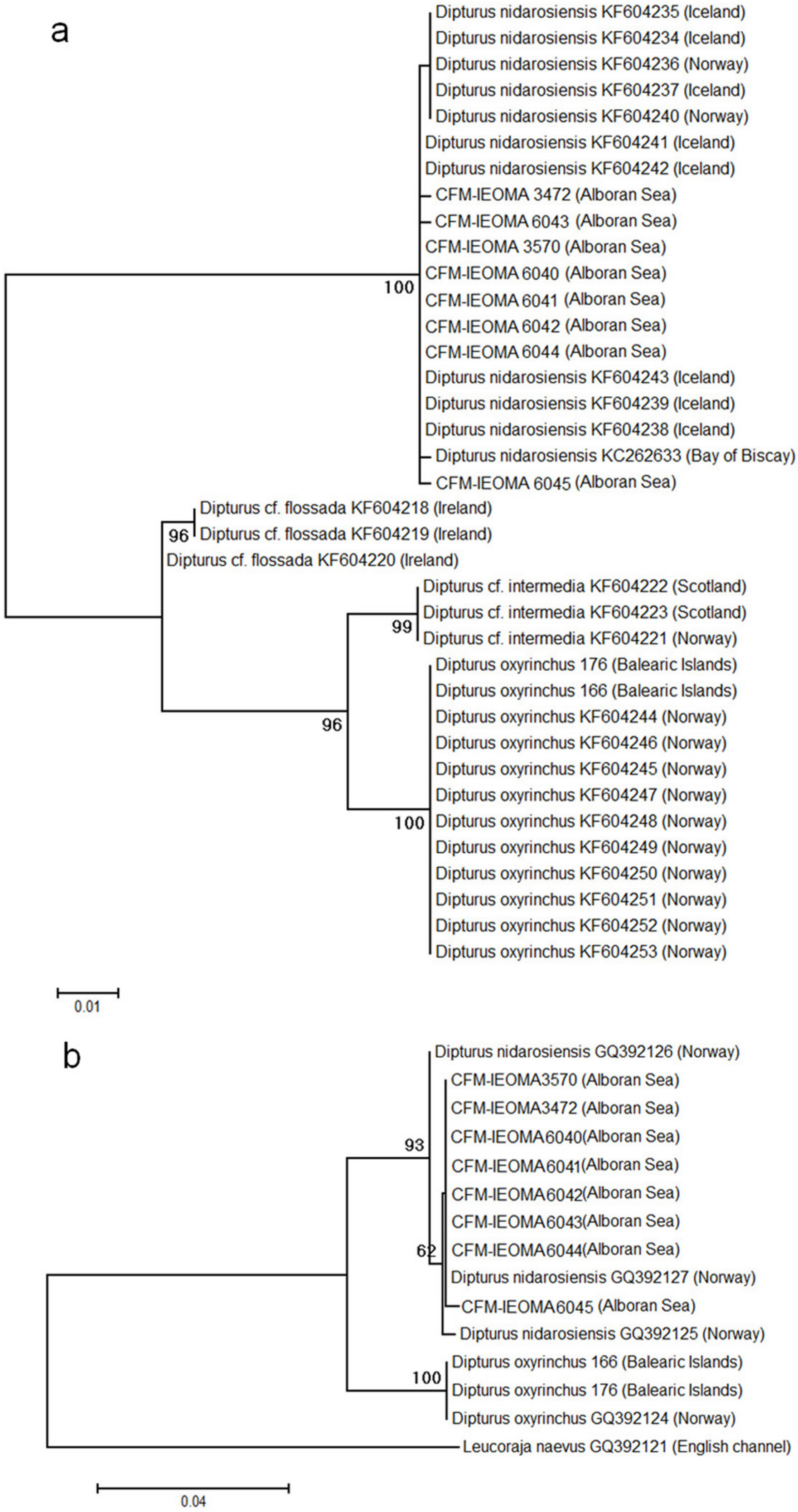


Figure 4.3.2.4. Maximum likelihood tree based on *COI* (a) and *Cytb* (b) fragments of *Dipturus* species from the Northeast Atlantic and the Mediterranean. Bootstrap values and GenBank accession numbers are indicated near the nodes and the species name, respectively. Sampling localities are also shown in brackets. Nodes with bootstrap values of <50 are not shown.

### 4.3.2.4 Discussion

The present study uses morphological traits and molecular markers to identify 8 specimens of *Dipturus nidarosiensis* (Storm, 1881) captured in the Alboran Sea (western Mediterranean) to provide new evidence of its questioned presence in the Mediterranean Sea. These specimens had the external diagnostic features described by Storm (1881) and showed similar morphometric measurements to those present in the immature specimens of *D. nidarosiensis* collected in Sardinia. All *D. nidarosiensis* individuals from the Alboran Sea were juveniles and, similarly to the juvenile specimens reported by Cannas *et al*. (2010), showed a reduced number of median thorns on the tail. As has been recorded in other skate species, the median thorns could increase progressively as maturity approaches (e.g. *Raja eglanteria* and *Raja montagui*; Ebert and Stehmann, 2013). Little is known about juveniles of *D. nidarosiensis*, so in order to have adequate identification in the future, the characterization of these morphological changes is important.

Molecular analysis confirmed the morphological identification of the CFM-IEOMA samples as *D. nidarosiensis*. Sequence divergence in both mitochondrial fragments (*COI* and *Cytb*) indicated that the Alboran specimens were genetically similar to Northeast Atlantic specimens, and shared haplotypes with them. Phylogenetic analyses revealed that the Alboran and Northeast Atlantic specimens of *D. nidarosiensis* do form a monophyletic group that is markedly differentiated with respect to the other *Dipturus* species. Besides, phylogenetic analyses show a close relationship with *D. oxyrinchus* and *D.* cf. *intermedia*, similarly to the results described by Griffiths *et al*. (2010), Iglésias *et al*. (2010) and Lynghammar *et al*. (2014).

The morphometric and molecular analyses presented here, along with the comparisons with the DNA sequences of Northeast Atlantic Norwegian skates available in the GenBank, add evidence concerning the presence of this species in the Mediterranean Sea, corroborating the first record of this species in the Mediterranean made by Cannas *et al*. (2010).

The new records of the Norwegian skate, which in the Mediterranean was only known from Sardinia, suggest a wider Mediterranean distribution than previously thought. The Strait of Gibraltar and the adjacent Alboran Sea are transition zones between the Atlantic and the Mediterranean, and are influenced by the inflow of Atlantic water through the Strait and the interchange of these waters with Mediterranean waters through the Balearic channels. The Alboran Sea is characterized by complex bottom topography, where the presence of numerous submarine canyons provides a favorable environmental scenario to the enrichment of deep-water ecosystems (Millot, 1999; Baro *et al*., 2012). These conditions would be favorable to the presence of *D. nidarosiensis*, as well as to other deep-water Atlantic chondrichthyan species, such as *Galeus atlanticus*, also present in this area but unknown in other parts of the Mediterranean (Rey *et al*., 2010; Ramírez-Amaro *et al*., 2015).

The depth at which *D. nidarosiensis* has been found in the Mediterranean, from 600 to 1,420 m off Sardinia (Cannas *et al*., 2010) and from 620 to 819 m in the present work, points out that this species is one of the deepest living skates in the area. This bathymetric distribution could probably be one of the reasons for the little knowledge on the biology of this species. The background inference by Ebert and Stehmann (2013) and Stehmann *et al*. (2015) together with all the recent findings reported by Cariani *et al*. (2017) and the present study launches the challenge to resolve this still-open question by merging all Mediterranean *D. nidarosiensis* specimens (meaning those collected from Sardinia, Ionian and the Alboran Seas) in a unique specimen set and genetically comparing them against the Northeast Atlantic skate using appropriate molecular markers (e.g. microsatellites or SNPs) in order to detect intraspecific differences and assess gene flow. In addition, tagging experiments would contribute to resolve population connectivity between the Atlantic and the Mediterranean and within the Mediterranean.

The Alboran Sea has been reported as a hot spot of marine biodiversity, highlighting its ecological importance for many threatened or endangered vertebrate species (Coll *et al*., 2010; Danovaro *et al*., 2010). Despite the ecological and economic interest of the area, there is a lack of studies on Alboran deep fauna. Moreover, the deep-water trawl is not well developed in this area, and its western part and Alboran Island showed a low level of fishing exploitation mainly between depths of 500 and 800 m depth (Moranta *et al*., 2007). Current records allow an improvement of chondrichthyan biodiversity in the Alboran Sea and support the importance of this area for the biodiversity conservation of deep-sea species of these vulnerable fishes, as has been suggested in previous studies (Ramírez-Amaro *et al*., 2015).

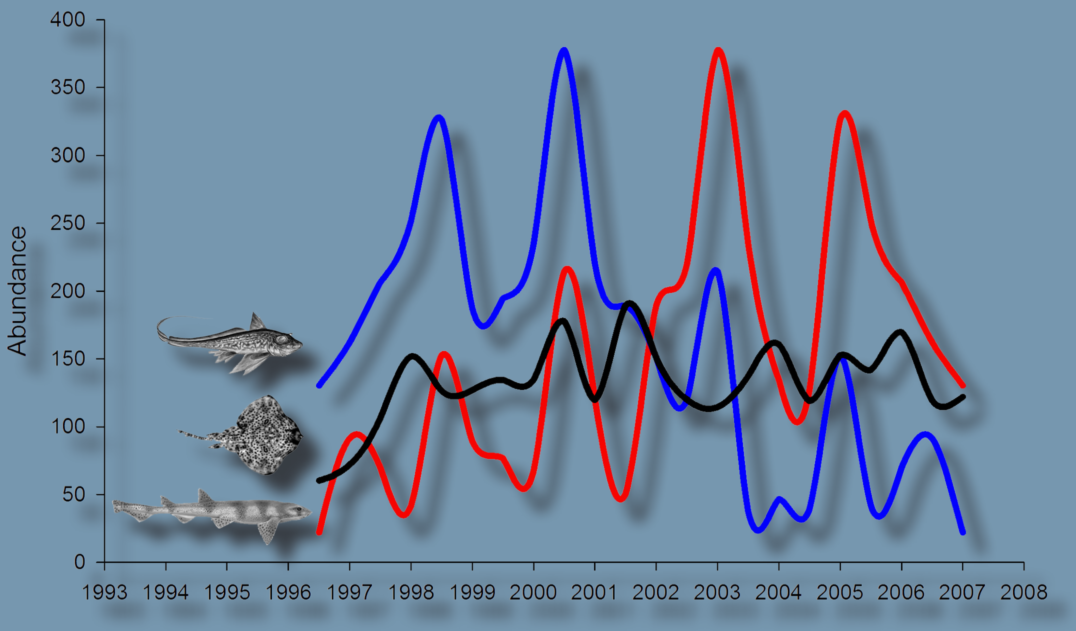
Our results represent an important contribution to the knowledge of the distribution of *D. nidarosiensis* in the Mediterranean Sea. The confirmation of the presence of this species in the area and the new records in the most western part of the basin should finally be included in the future updates of the IUCN Red List. Moreover, special attention is recommended during the periodic activities devoted to the scientific monitoring of fisheries (e.g. European Commission's Data Collection Framework). In fact, the occurrence of *D. nidarosiensis* could potentially be even more widespread within the Mediterranean, but still unnoticed because of the possible misidentification with *D. oxyrinchus*, despite their various morphological differences, as has also been reported in the past for *D. oxyrinchus vs*. *D. batis* (e.g. Dulvy *et al*., 2006).

# CHAPTER IV

## 4.4 TRENDS

Spatio-temporal trends in demersal chondrichthyans exploited by bottom trawling in the western Mediterranean: some resist, but others do not.

Ramírez-Amaro S., Ordines F., Esteban A., García C., Guijarro B., Salmerón F., Terrasa B., Massutí E. (In preparation).



## Summary

In the Mediterranean, there is international concern regarding changes in the diversity and abundance of chondrichthyans because of their particular life history traits, which make them vulnerable to fishing impact. However, a lack of accurate landings data on these species and scarcity of biological information have restricted the assessment of their populations. Here, we assess, at community and population levels, the spatio-temporal trends of demersal chondrichthyans in the western Mediterranean, where these species are an important by-catch of bottom trawl fishery. Fishery-independent data were obtained from scientific surveys carried out annually between 1994 and 2015 in the three adjacent geographic sub-areas (GSAs), established by the General Fisheries Commission for the Mediterranean (GFCM) in the western Mediterranean: Northern Alboran (GSA01), Balearic Islands (GSA05) and Northern Spain (GSA06). Our results reflect fairly stable populations in terms of species richness and density (abundance and biomass) levels with some increment in the abundance and biomass of the most abundant species dwelling on the continental shelf (*Scyliorhinus canicula*, *Raja clavata* and *Torpedo marmorata*), and even for the most abundant species dwelling on the slope (*Galeus melastomus*). In contrast, decreasing trends in some deep water species (Etmopterus spinax and *Dipturus oxyrinchus*) were observed. These findings can be explained by the reduction of the trawling effort on the continental shelf during the last decades, due to the decreased size of the trawling fleet and to its displacement towards deep waters, along with the higher resilience displayed by some of these species. Meanwhile, while size composition of the most abundant demersal chondrichthyans remained stable over the period analysed, a decreasing trend in length at first maturity of *S. canicula* was detected, indicating a likely evolutionary response to the general overfishing of demersal resources exploited since 1980s. These results can be useful for implementing management measures that strengthen or initiate the recovery in the Mediterranean of these vulnerable species, whose populations play a very important role in marine ecosystems, in addition to developing true adaptive management.

**Keywords**: Chondrichthyans, vulnerable species, diversity, abundance, biomass, length at first maturity, size, temporal trends, assessment, conservation status, management, recovery, fisheries, bottom trawl, by-catch, scientific survey, GFCM, Mediterranean.

## 4.4.1 Introduction

Chondrichthyans are highly vulnerable species to fishing exploitation, due to their life-history characteristics such as a slow growth rate, late maturity and low fecundity (Stevens *et al*., 2000; Lucifora *et al*., 2012). Human impact like overfishing and habitat degradation has profoundly altered and reduced chondrichthyan populations in many parts of the world (Stevens *et al*., 2000; Lotze *et al*., 2006; Dulvy *et al*., 2014), calling for the application of effective management strategies to ensure their conservation (Lucifora *et al*., 2012; Barausse *et al*., 2014; Dulvy *et al*., 2014; Davidson *et al*., 2015).

The Mediterranean fishery is one of the oldest on the planet, and has experienced long exploitation histories (Bas *et al*., 1985; Ferretti *et al*., 2005; Lotze *et al*., 2011). Most demersal and pelagic stocks have been overfished for the entire area over many decades (Palomera *et al*., 2007; Colloca *et al*., 2013; Quetglas *et al*., 2013; Tsikliras *et al*., 2015). Moreover, the decline in chondrichthyan populations has been documented throughout the Mediterranean (Ferretti *et al*., 2008, 2013; Maravelias *et al*., 2012; Ligas *et al*., 2013; Barausse *et al*., 2014). Due to these anthropogenic impacts, the International Union for Conservation of Nature (IUCN) has recently revealed the Mediterranean region as a key hotspot of extinction risk of chondrichthyans, due to the fact that more than half of the species assessed, 39 out of a total of 73, are threatened at different degrees: 20 of them are critically endangered, 11 endangered, and 8 are considered vulnerable (Dulvy *et al*., 2016).

In the western Mediterranean, chondrichthyans are mainly bottom-dwelling species, inhabiting demersal ecosystems on continental shelves and slopes, making them especially vulnerable to bottom trawl fishing (Cavanagh and Gibson, 2007). This fishery has been well developed in this area since the middle of the 20th century (Bas *et al*., 1985; Gil de Sola, 1993; Quetglas *et al*., 2013) and in the present century trawling is the main fishery in terms of catch, fleet power, and fishing capacity (Lleonart and Maynou, 2003). In this area, bottom trawling operates over a wide bathymetric range, between 50 and 800 m, exploiting different biological communities and their corresponding species (Moranta *et al*., 2008). It results in a high number of species in the catches, not only fishes but also decapod crustaceans and cephalopods, and with chondrichthyans representing an important fraction of the by-catch and discards (Moranta *et al*., 2000; Carbonell *et al*., 2003).

The temporal evolution of chondrichthyans in the western Mediterranean has mainly been studied through fishery-dependent data. Based on long data series of fishing landings, Guijarro *et al*. (2012) showed a clear decreasing trend in elasmobranch landings from the Balearic Islands from 1965 to 2009 as a consequence of the fishing exploitation exerted since the middle of the 20th century. Quetglas *et al*. (2013) also assessed the population dynamics of demersal elasmobranchs (mainly *Scyliorhinus canicula* and *Raja clavata*) in the same area, based on landings statistics during the early 1980s. In the Gulf of Lions, a study based on bottom trawl surveys and fishing landings also pointed towards a clear decrease in diversity and abundance of demersal elasmobranchs since the 1960s (Aldebert, 1997; Bertrand *et al*., 1998). Likewise, a decline in chondrichthyan species in terms of abundance and landings, such as the thornback ray (*R. clavata*; Bertrand *et al*., 1998), velvet belly lanternshark (Etmopterus spinax; Ordines *et al*., 2011), and starry ray (*Raja asterias*; Coll *et al*., 2013; Navarro *et al*., 2013), has been detected in the western Mediterranean.

Understanding spatio-temporal changes in exploited fish populations requires the consideration of several factors, such as their intrinsic vulnerability to exploitation, biological interactions (predator-prey relationship and competition, among many others) exposure to fishing, and susceptibility to other stressors such as habitat changes, pollution, and climate warming (Coll *et al*., 2010; Ferretti *et al*., 2013). These factors can shift the species-specific response to exploitation; generating complex community changes over time and space (Ferretti *et al*., 2010). Detecting temporal trends can be helpful for devising effective management strategies in at least three ways (see Wagner *et al*., 2013 and references therein): i) management actions often have time-oriented objectives; ii) aquatic ecosystems may respond in a complex way to natural and anthropogenic factors; and iii) recognition of previous system dynamics might help to identify more realistically the possible outcomes of suitable management.

There is an urgent need for improvement of fisheries assessment and management in the Mediterranean because of the general overfishing, the alarming decline of stocks (Vasilakopoulos *et al*., 2014) and the status of chondrichthyan populations, which may face an extinction crisis (Dulvy *et al*., 2016). It is therefore necessary to make compatible the sustainability of fisheries and the conservation of these vulnerable species. The objective of the present study was to assess the spatio-temporal evolution of demersal chondricthyan populations in three different areas of the western Mediterranean from fishery-indepedent data. Particularly, we explored the largest database of bottom trawl surveys existing in the area, between 1994 and 2015, in order to detect trends in species richness, abundance and biomass, and key biological parameters such as length at first maturity and size structure of the main species.

## 4.4.2 Material and methods

### *Data source*

Data were obtained from the MEDITS International Bottom Trawl Survey in the Mediterranean. These surveys are developed annually, during spring and early summer, along the continental shelf and slope off the Mediterranean Iberian Peninsula and Balearic Islands, from 30 to 800 m depth. The sampling methodology was the one applied throughout the Mediterranean within the framework of the MEDITS program (Bertrand *et al*., 2002). During the survey, a stratified random sampling scheme is carried out considering five bathymetric strata (A: 30-50 m; B: 51-100; C: 101-200 m; D: 201-500; E: 501-800) and the four geographic sub-areas (GSAs) defined by the General Fisheries Commission for the Mediterranean (GFCM) in Spain (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain). The GSA02 (Alboran Island) is also included within the Spanish western Mediterranean, but since it has not been sample every year it was excluded from the analyses. The experimental bottom trawl used during all temporal series was a GOC-73. Sampling hauls were conducted during daylight hours, with an effective duration of 30’ at depths shallower than 200 m and 60’ below 200 m depth, at an average towing speed around 3 knots. Arrival and departure of the net to and from the bottom, in addition to the horizontal and vertical openings of the net (on average, 16.4 and 2.8 m, respectively), were measured using a SCANMAR system.

The catch of each sample was sorted on board and classified to species level. Then, species were counted, weighed, and individuals were measured (total length, TL; in the case of elasmobranch, and anal length for holocephali). The abundance and biomass was standardized to one km$^2$ using the surface of bottom sampled during the haul, calculated from the effective distance covered (obtained from GPS system) and the horizontal opening of the gear (obtained from SCANMAR data). For chondrichthyans, sex was determined and maturity of the gonads was assigned from macroscopic examination, following a four-stage scale based on Holden and Raitt (1974). For females, the four maturity stages were: i) immature, narrow or not appreciable uterus, white ovary with no apparent follicles; ii) maturing, clear capsule gland and developed follicles in ovary; iii) spawning, capsules in uterus or in the glands; and iv) post-spawning, irrigated glands, expanded uterus and ovary reducing. For males, the four maturity stages were: i) immature, unformed seminal vesicles, narrow testes, and soft claspers; ii) maturing, no sperm in vesicle, wide testes and developing soft claspers passing pelvic fins; iii) spawning, calcified claspers and abundant sperm in vesicle; iv) post-spawning, expanded empty vesicle and wide testes.

The time series of data on abundance and biomass spanned from 1994 to 2015 for GSA01 and GSA06, and from 2001 to 2015 for GSA05. Due to changes in the MEDITS surveys sampling protocol over the years, data series on size and maturity were shorter. In GSA01 and GSA06 they comprised the period 2000-2015, whereas in GSA05 the available periods were 2001-2015 and 2007-2015 for size and maturity, respectively.

### *Data analysis*

Temporal trends were analysed for several parameters and by applying different methods. Because stratum A was scarcely sampled in all GSAs, it was excluded from the analysis. According to the general classification proposed by Nelson *et al*. (2016), the chondrichthyan community-level groups taking into account for the analyses were the sharks (Subdivision Selachii), the batoids (Subdivision Batomorphi) and the chimaeras (Subclass Holocephali), the latter composed by a single species in the Mediterranean, Chimaera monstrosa. The annual mean standardized abundance and biomass of these groups were calculated by year and GSA, but within each depth stratum. At population level, the annual mean standardized abundance (individuals km$^{-2}$) and biomass (kg km$^{-2}$) was estimated by GSA for the most abundant species within their bathymetric distribution range.

Dynamic factor analysis (DFA) is a multivariable method applied to detect common trends used mainly on density indices such as abundance and biomass (e.g. Keller *et al*., 2017; Peterson *et al*., 2017), for relative short and non-stationary time-series data (Zuur *et al*., 2003a). Here this method was used to identify common trends for each bathymetric stratum in mean standardized abundance and biomass data series between GSAs for community-level groups. Since batoid abundances were almost null below 500 m depth, stratum E was not included. DFA was also applied at species-level for the most important species (i.e. those with an average percentage per year≥85% in at least two GSAs).

In DFA, the time-series data are modelled as a linear combination of underlying common trends and factor loading indicates the strength of the influence of each GSA time series on the corresponding common trend. Factor loading values higher than 0.2 denote a strong effect of the given GSA on the detected common trend, whereas a negative factor loading values indicates inverse relationship between factor and variable (Zuur *et al*., 2003).Correlation of observation errors was modelled using different covariance matrices: i) same variance and no covariance (diagonal-equal); ii) different variances and no covariance (diagonal-unequal); iii) same variance and covariance (equalvarcov); and iv) different variances and covariances (unconstrained). The corrected Akaike information criterion (AICc) was used a measure of goodness of fit, with the best model having the lowest AICc (Zuur *et al*., 2003; Zuur and Pierce, 2004). Model implementation was performed using the Multivariate Autoregressive State-Space "MARSS" package (Holmes *et al*., 2013) in R 3.3.2.

Linear regression analysis was applied to detect temporal trends at species-level, for the most important species within a particular GSA (i.e. those species with an average percentage of occurrence in its depth range of distribution ≥30%). Regression models were performed using SigmaPlot 11.0 (Systat software).

Likewise, in order to know the evolution of diversity and some of the main biological parameters of the most important species, linear regression analysis was used to detect trends in species richness, size structure and maturity (analyzing both length t first maturity, $L_{50}$; for females and males, and smaller size of mature females). Mean chondrichthyan species richness per haul (S), was modelled by year and GSA. Size structure was calculated for those species with more than 35 specimens measured per GSA and year. To do that, sexes were joined and grouped into different size categories according to the total length of the species, and the percentage contribution of each size category was estimated and graphed. Maturity was assessed to those species with at least 50 per year. To do this, maturation stages were converted into binary data (stages i and ii as immature and stages iii and iv as mature). Length at first maturity ($L_{50}$) for females and males, and for each year and GSA, was estimated by logistic regression using the glm (generalized linear model) function considering a binomial error distribution in R 3.3.2 statistical software (Crawley, 2007). Additionally, the $L_{50}$ was also estimated to smallest mature females.

### 4.4.3 Results

A total of 3158 hauls were analysed, 757 for GSA01, 819 for GSA05 and 1582 for GSA06 (Figure 4.4.1). The number of hauls per year, GSA, and bathymetric strata are given in Table S4.4.1. Up to 33 species of chondrichthyans belonging to 13 families were caught throughout the western Mediterranean over the last two decades, of which 26, 28 and 19 species were caught in GSA01, GSA05, and GSA06, respectively (Table 4.4.1).

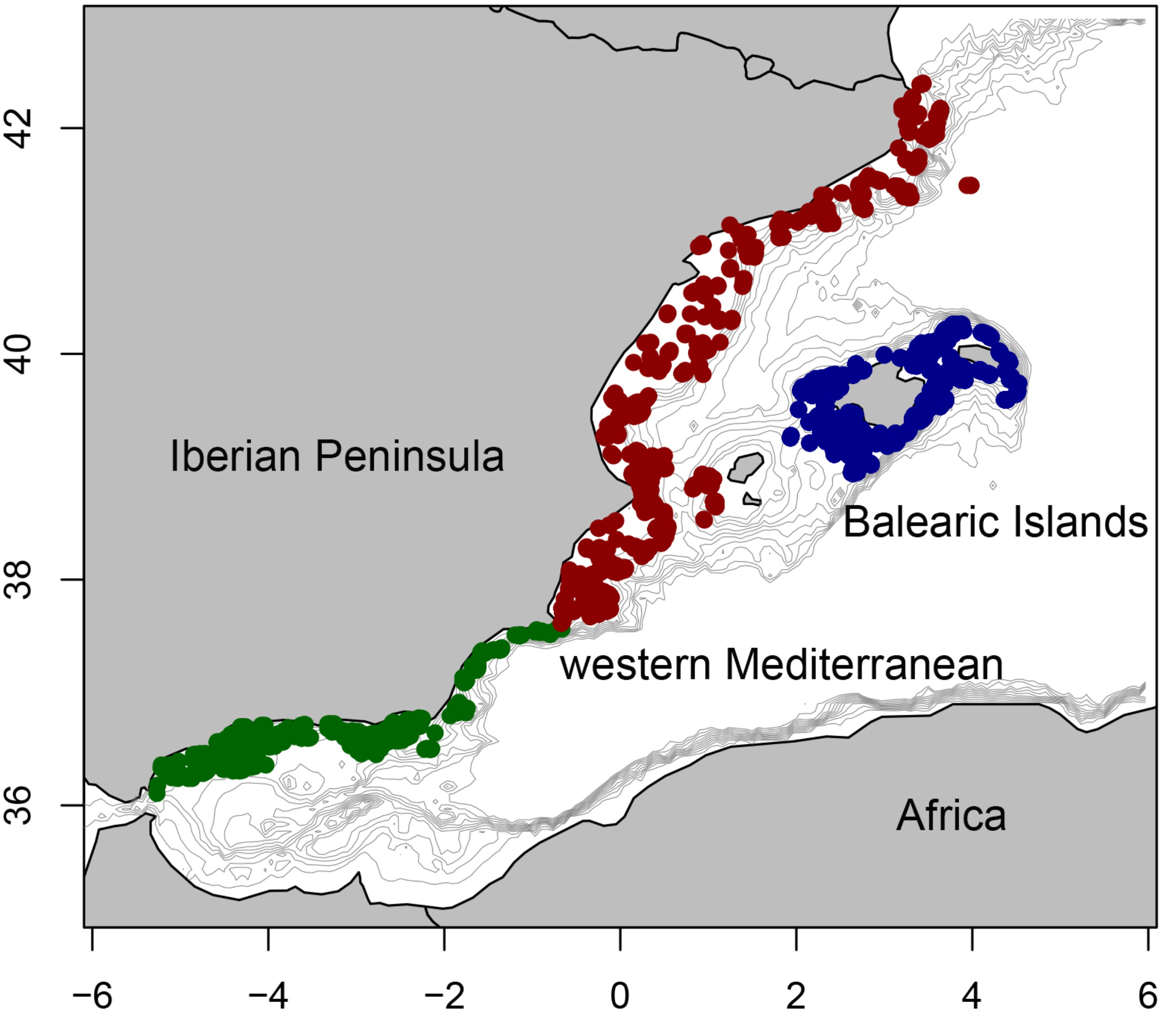


Figure 4.4.1. Map of the western Mediterranean showing the MEDITS stations between 1994 and 2015 analysed for the present study. Colors correspond to the three geographic sub-areas (GSAs) considered by the General Fisheries Commission for the Mediterranean (GFCM) in the study area: green GSA01 (Northern Alboran Sea); blue GSA05 (Balearic Islands) and red GSA06 (Northern Spain). Depth isobaths between 50 and 2000 m (by each 200 m) are also shown.

Table 4.4.1. Chondrichthyan species captured by geographic sub-area (GSA) considered by the General Fisheries Commission for the Mediterranean (GFCM) in the study area (1: GSA01 or Northern Alboran Sea; 5: GSA05 or Balearic Islands; 6: GSA06 or Northern Spain) during the MEDITS surveys in the western Mediterranean during the periods 1994-2015 for GSA01 and GSA06 and 2001-2015 for GSA05. D: depth range; %Y: average percentage of years in which the species were caught during the study period; %D: percentage of appearance within the depth range in which the species was caught for all years; N: total number of specimens caught; B: total weight of catches. IUCN indicates the regional status of species from the International Union for the Conservation of the Nature regional assessments performed in 2007 (Cavanagh and Gibson, 2017) and 2016 (Dulvy *et al*., 2016): DD (Data Deficient); LC (Least Concern); NT (Near Threatened); VU (Vulnerable); EN (Endangered); CR (Critically Endangered).

| **Family** | **Species** | **D (m)** | | | **%Y** | | | **%D** | | | **N** | | | **B** | | | **IUCN** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **5** | **6** | **1** | **5** | **6** | **1** | **5** | **6** | **1** | **5** | **6** | **1** | **5** | **6** | **2007** | **2016** |
| Chimaeridae | *Chimaera monstrosa* | 325-831 | 440-654 | 440-856 | 95.4 | 53.3 | 50 | 55.3 | 5.6 | 4.2 | 1871 | 29 | 25 | 1407 | 2.25 | 1.95 | NT | NT |
| Scyliorhinidae | *Galeus melastomus* | 301-831 | 145-755 | 232-788 | 100 | 100 | 100 | 94.8 | 93.5 | 83.6 | 84127 | 45050 | 17340 | 17253 | 1539 | 1960 | LC | LC |
| | *Galeus atlanticus* | 324-821 | -- | -- | 93.7 | -- | -- | 41.7 | -- | -- | 3238 | -- | -- | 1007 | -- | -- | NT | NT |
| | *Scyliorhinus canicula* | 40-664 | 38-626 | 42-661 | 100 | 100 | 100 | 73.4 | 95.5 | 51.7 | 9634 | 82441 | 27945 | 1876 | 5701 | 3853 | LC | LC |
| Triakidae | *Galeorhinus galeus* | 238-593 | -- | -- | 59.1 | -- | -- | 8.4 | -- | -- | 38 | -- | -- | 528 | -- | -- | VU | VU |
| | *Mustelus mustelus* | 60 | 54-141 | -- | 9.1 | 80 | -- | <0.1 | 11.2 | -- | 4 | 207 | -- | 4.4 | 80.4 | -- | EN | VU |
| | *Mustelus asterias* | -- | 107 | -- | -- | 13.3 | -- | -- | -- | -- | -- | 2 | -- | -- | 1.2 | -- | EN | VU |
| Hexanchidae | *Heptranchias perlo* | 428-556 | -- | -- | 9.1 | -- | -- | <0.1 | -- | -- | 2 | -- | -- | 8.3 | -- | -- | VU | DD |
| Centrophoridae | *Centrophorus granulosus* | 377-796 | 594-753 | -- | 81.8 | 33.3 | -- | 10.7 | 3.2 | -- | 52 | 9 | -- | 184 | 36.35 | -- | VU | CR |
| Dalatiidae | *Dalatias licha* | 325-831 | 590-743 | 411-748 | 100 | 86.6 | 63.6 | 15.8 | 4.4 | 5.2 | 124 | 8 | 23 | 500 | 19.24 | 8.7 | DD | VU |
| Etmopteridae | *Etmopterus spinax* | 314-831 | 362-755 | 236-856 | 100 | 100 | 100 | 88.7 | 58 | 41.2 | 8546 | 882 | 983 | 874 | 59.57 | 61.24 | LC | LC |
| Oxynotidae | *Oxynotus centrina* | 87-812 | 98 | -- | 68.2 | 6.6 | -- | 0.23 | -- | -- | 30 | 2 | -- | 88.35 | 0.25 | -- | CR | CR |
| Squalidae | *Squalus acanthias* | 77 | 104-654 | 124-582 | 4.5 | 46.6 | 9.1 | -- | 4.6 | 2.1 | 1 | 53 | 56 | 1.5 | 65.6 | 13.46 | EN | EN |
| | *Squalus blainvillei* | -- | 47-667 | 124-130 | -- | 73.3 | 13.6 | -- | 12.1 | 3.5 | -- | 185 | 134 | -- | 778 | 118.55 | EN | DD |
| Dasyatidae | *Dasyatis centraura* | -- | 58 | -- | -- | 6.6 | -- | -- | -- | -- | -- | 1 | -- | -- | 4.4 | -- | NT | VU |
| | *Dasyatis pastinaca* | -- | 39-98 | 54-79 | -- | 100 | 13.6 | -- | 47.3 | <0.1 | -- | 364 | 5 | -- | 169.5 | 22.29 | NT | VU |
| | *Pteroplatytrygon violacea* | 43-317 | -- | -- | 18.2 | -- | -- | <0.1 | -- | -- | 5 | -- | -- | 52.9 | -- | -- | NT | LC |
| Myliobatidae | *Mylibatis aquila* | 44 | 39-144 | 73-81 | 4.5 | 80 | 13.6 | -- | 29.2 | <0.1 | 2 | 200 | 8 | 0.61 | 322 | 24.32 | NT | VU |
| | *Pteromylaeus bovinus* | -- | 44-60 | -- | -- | 6.6 | -- | -- | <0.1 | -- | -- | 2 | -- | -- | 7.57 | -- | EN | CR |
| Rajidae | *Dipturus oxyrinchus* | 357-769 | 134-699 | -- | 9.1 | 100 | -- | <0.1 | 32.3 | -- | 2 | 509 | -- | 9.82 | 315 | -- | NT | NT |
| | *Leucoraja circularis* | 354-710 | 131-499 | 245 | 36.4 | 46.6 | 4.5 | <0.1 | 4.7 | -- | 13 | 139 | 1 | 269.5 | 29 | 0.043 | EN | CR |
| | *Leucoraja naevus* | 60-565 | 52-540 | 82-320 | 81.8 | 100 | 59 | 17.2 | 40.9 | 3.5 | 287 | 969 | 94 | 39.28 | 236 | 38.92 | NT | NT |
| | *Raja asterias* | 40-180 | 44-168 | 40-255 | 81.8 | 30 | 95.5 | 2.4 | 3.4 | 10.2 | 90 | 59 | 270 | 114.47 | 32.89 | 138 | NT | NT |
| | *Raja brachyura* | -- | 48-162 | -- | -- | 33.3 | -- | -- | 23.2 | -- | -- | 417 | -- | -- | 95.87 | -- | DD | NT |
| | *Raja clavata* | 47-420 | 57-590 | 52-582 | 18.2 | 100 | 84.4 | 0.16 | 64.7 | 4.3 | 16 | 4994 | 289 | 11.06 | 2369 | 20.9 | NT | NT |
| | *Raja miraletus* | 81-207 | 40-174 | 85-225 | 9.1 | 100 | 50 | <0.1 | 38.9 | 1.2 | 3 | 2044 | 49 | 0.53 | 115.1 | 13.02 | LC | LC |
| | *Raja montagui* | 44-160 | 52-357 | 57-406 | 22.7 | 30 | 72.7 | 0.24 | 8.6 | 5.7 | 11 | 252 | 114 | 6.33 | 120 | 32.96 | LC | LC |
| | *Raja polystigma* | 70 | 48-424 | 85-316 | 4.5 | 93.3 | 31.8 | -- | 28.5 | <0.1 | 1 | 1574 | 39 | 0.79 | 146.7 | 12.53 | NT | LC |
| | *Raja radula* | 40-53 | 40-97 | 124-211 | 9.1 | 100 | 13.6 | <0.1 | 35.2 | <0.1 | 3 | 664 | 9 | 1.98 | 257 | 2.22 | DD | EN |
| | *Rostroraja alba* | -- | 56-251 | -- | -- | 40 | -- | -- | <0.1 | -- | -- | 13 | -- | -- | 71.2 | -- | CR | EN |
| Torpedinidae | *Torpedo marmorata* | 40-290 | 51-258 | 45-297 | 100 | 86.6 | 100 | 26.4 | 5.2 | 21.2 | 187 | 71 | 253 | 116.8 | 9.5 | 71 | LC | LC |
| | *Torpedo nobiliana* | 170-777 | 368 | 144-431 | 81.8 | 6.6 | 22.7 | | -- | <0.1 | 56 | 1 | 14 | 61.2 | 0.22 | 13.17 | DD | LC |
| | *Torpedo torpedo* | 40 | -- | -- | 13.6 | -- | -- | -- | -- | -- | 11 | -- | -- | 6.92 | -- | -- | LC | LC |

*Species richness and composition*

The highest values of mean species richness per haul were obtained in GSA05, followed by GSA01, and GSA06, with average and standard error values for all time series of 3.1±0.21, 2.6±0.2 and 1.7±0.11, respectively. None of the three GSAs showed any temporal trend (Figure 4.4.2).

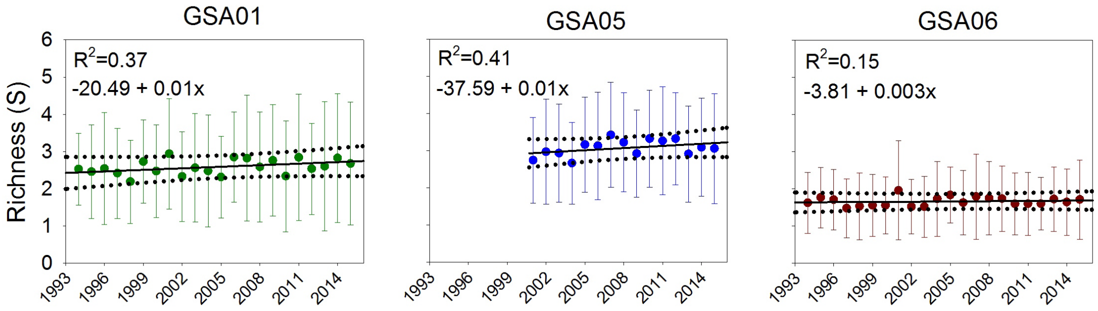


Figure 4.4.2. Mean and standard values of species richness of chondrichthyans captured in MEDITS surveys developed throughout the western Mediterranean during the period 1994-2015, by year and geographic subareas (GSAs) in the study area: GSA01 (Northern Alboran Sea in green); GSA05 (Balearic Islands in blue) and GSA06 (Northern Spain in red). Linear regression models are included, with dashed lines showing 95% confidence intervals for regression lines and asterisks indicating statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

In all GSAs, *Scyliorhinus canicula* was the most important species of sharks in term of relative abundance (>90%) and biomass (>70%) in bathymetric strata B and C, while *Galeus melastomus* was the most important sharks in stratum E (>85% both in relative abundance and biomass; Table S4.4.2). Both species showed differences between GSAs in stratum D, where *G. melastomus* was most important than *S. canicula* in GSA01 (>75% and ≤10% both in relative abundance and biomass, respectively) and GSA05 (63 vs. 36% and 52 vs. 45% in relative abundance and biomass, respectively), whereas in GSA06 *S. canicula* was most important than *G. melastomus* (55 vs. 45% and 82 vs. 18% in relative abundance and biomass, respectively).

The most important species of batoids both in relative abundance and biomass showed differences not only by depth strata but also by GSA (Table S4.4.3). In GSA01, the most important species were *Torpedo marmorata* (>40% both in relative abundance and biomass) and *Raja asterias* (14 and 55% both in relative abundance and biomass, respectively) in stratum B, whilst *Leucoraja naevus* predominated in strata C and D (>70% both in relative abundance and biomass). In GSA05, the most important species in stratum B were *Raja miraletus* (25% and 5% in relative abundance and biomass, respectively), *Raja radula* (19 and 13%, respectively), and *Raja clavata* (19 and 34%, respectively), while *R. clavata* (56 and 78%, respectively), *Raja montagui* (18 and 4%, respectively), and L. naevus (17 and 12%, respectively) predominated in stratum C, and *R. clavata* (59 and 62%, respectively) and *Dipturus* oxyrhinchus (29 and 28%, respectively) in stratum D. *R. clavata* predominated in all depth strata in GSA06 (≥28% and ≥40% in relative abundance and biomass, respectively), while the second most important species was *R. asterias*, with ≥22% in both relative abundance and biomass in stratum B, ≥9% in stratum C, and 33 and 15% in relative abundance and biomass, respectively, in stratum D.

### *Abundance and biomass*

The DFA models showed a single common temporal trend in the abundance and biomass of sharks in most depth strata (Figure 4.4.3) with different covariance matrix structure (Table S4.4.4). In strata B and C, trends exhibited low density values during the first years with an increment during the last 10 years. Stratum D exhibited an increment until 2008 with a stable trend in the last 5 years. DFA models in stratum E exhibited unstable trends with cyclic changes in abundance and biomass both in abundance and biomass over the study period. In this stratum, negative factor loading were observed in the case of GSA01, following an inverse trend compared to GSA05 and GSA06.

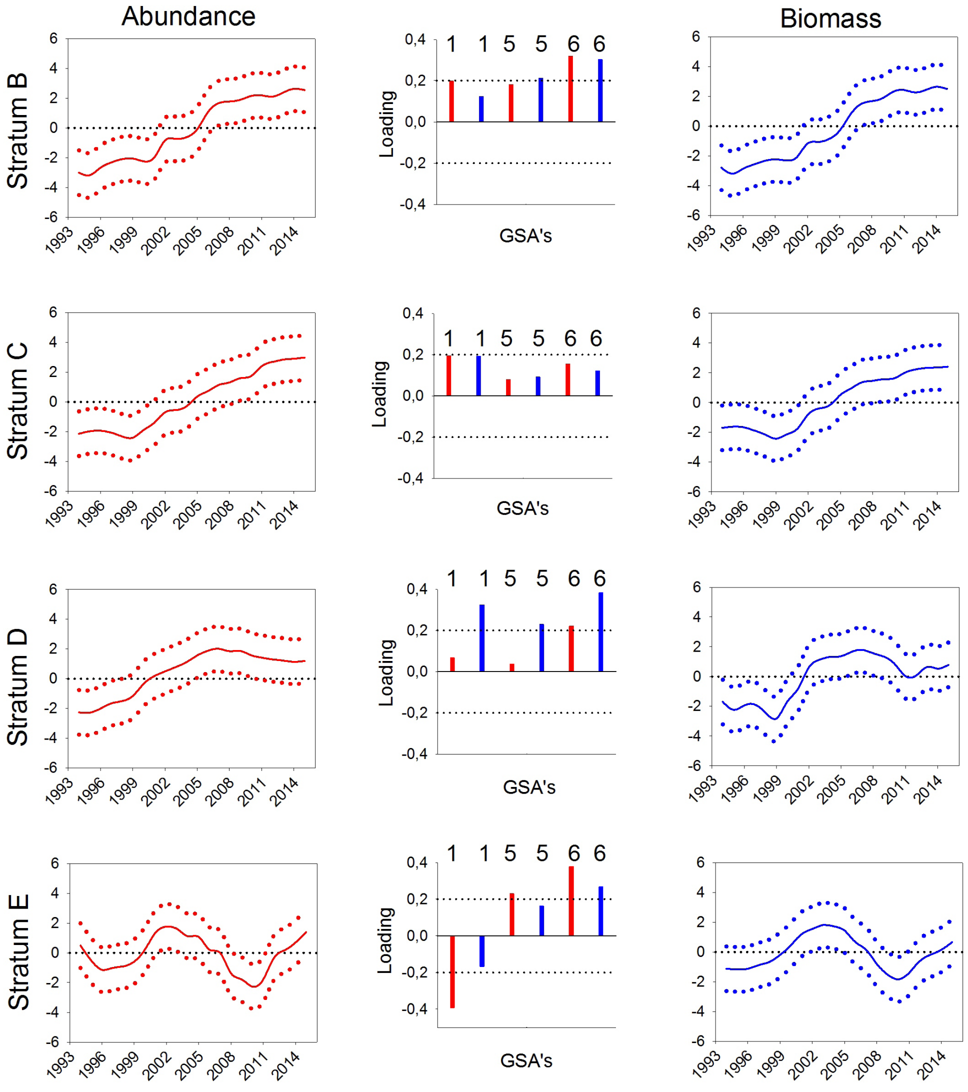


Figure 4.4.3. Common temporal trends and factor loadings from dynamic factor analysis (DFA) applied to standardized abundance (in red) and biomass (in blue) indices of sharks by geographic sub-areas (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) and depth strata (B: 50-100 m; C: 101-200 m; 201-500 m; E: 501-800 m) during the period 1994-2015 in GSA01 and GSA06, and 2001-2015 in GSA05. Factor loading greater than 0.2 (dotted lines) correspond to indices that had a relative strong influence on the resulting common trend. Dashed lines indicate the 95% confidence intervals.

Results of DFA models for the three most abundant shark species in all GSAs are shown in Figure 4.4.4 and Table S4.4.5. The common trend modelled for *S. canicula* revealed an increase in both abundance and biomass, especially over the last 10 years, which was followed by each GSA. DFA models for *G. melastomus* also showed a general increasing trend, with an inverse trend in term of biomass in GSA05, but, since it showed lowest factor loading (-0.1), this trend was not considered. DFA results for E. spinax showed two common trends in abundance, where the highest factor loading were observed in the second trend (Figure 4.4.4, in gray), showing an unstable trends with a slight decrease over the last 5 years. In this trend, GSA05 followed an inverse trend, but with low factor loading values. Moreover, DFA model for biomass revealed a common trend in all GSAs, showing a decreasing trend over the last 15 years.

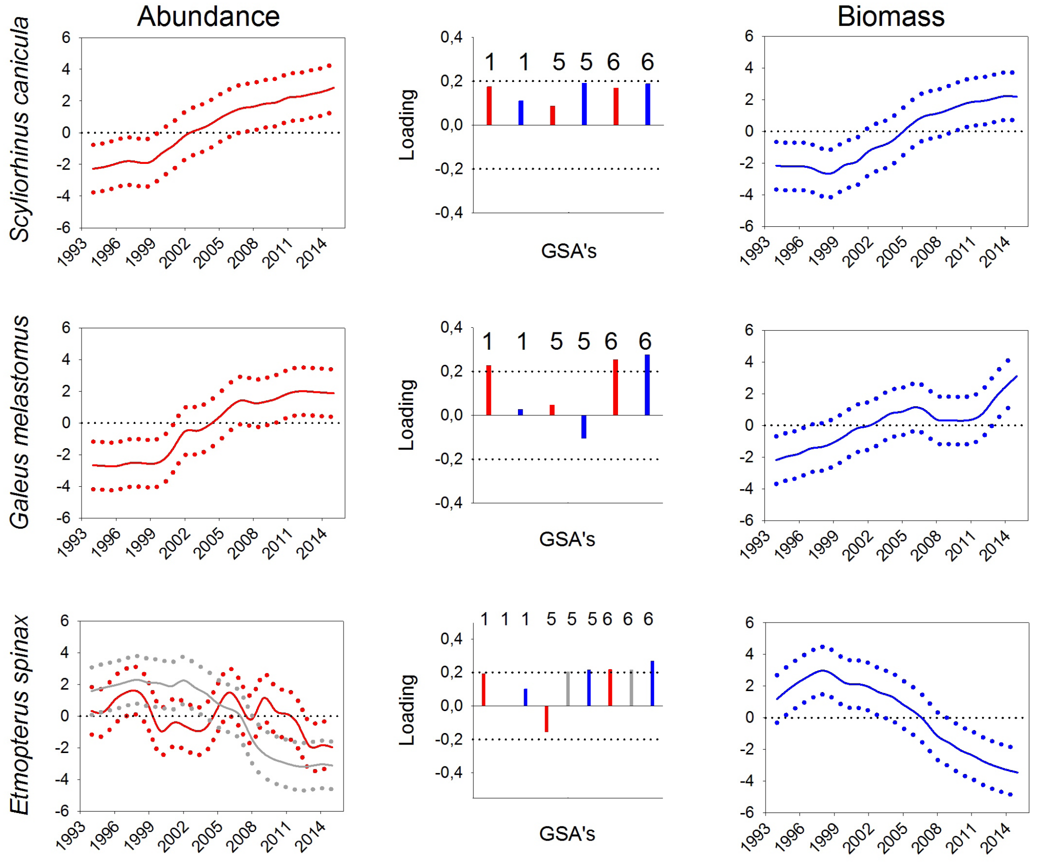


Figure 4.4.4. Common temporal trends and factor loadings from dynamic factor analysis (DFA) applied to standardized abundance (in red) and biomass (in blue) indices for the most abundant sharks species, *Scyliorhinus canicula*, *Galeus melastomus* and Etmopterus spinax (gray lines correspond to the second common trend identified), by geographic sub-areas (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) during the period 1994-2015 in GSA01 and GSA06, and 2001-2015 in GSA05. Factor loading greater than 0.2 (dotted lines) correspond to indices that had a relative strong influence on the resulting common trend. Dashed lines indicate 95% confidence intervals.

For batoids, the best DFA models had a single common trend both in abundance and biomass in all depth strata (Figure 4.4.5) with different observation covariance structure (Table S4.4.6). The common trends in strata B and C showed an initial decrease since 2002, which remained until recent years. Stratum D exhibited stable trends in abundance and biomass over the last 10 years, and an inverse trend was observed in GSA01. DFA models were only developed for one batoid species, *T. marmorata*, where a common trend for GSA01 and GSA06, both in abundance and biomass was observed, with an increase over the last 10 years (Figure 4.4.6 and Table S4.4.7).

Linear regression analysis was applied to seven chondrichthyan species, of which two and five species were caught more frequently in GSA01 and GSA05, respectively (Table 4.4.1). In GSA01 the two most important species were *Galeus atlanticus* and *Chimaera monstrosa*. Linear regression models for both species showed stable trends in term of abundance and biomass, with a slight decrease over the study period in *C. monstrosa* (Figure 4.4.7). In GSA05 the most important species were *Raja clavata*, *Raja miraletus*, *Raja radula*, *Leucoraja naevus* and *Dipturus oxyrinchus* (Figure 4.4.8). Linear regressions results for *R. clavata* showed a significant increasing trend for abundance and biomass. A similar trend for abundance and biomass, with stability during the study period, was exhibited for *R. miraletus*, *R. radula* and L. naevus. *D. oxyrinchus* showed a stable trend for biomass, while for abundance was observed a significant decreasing trend.

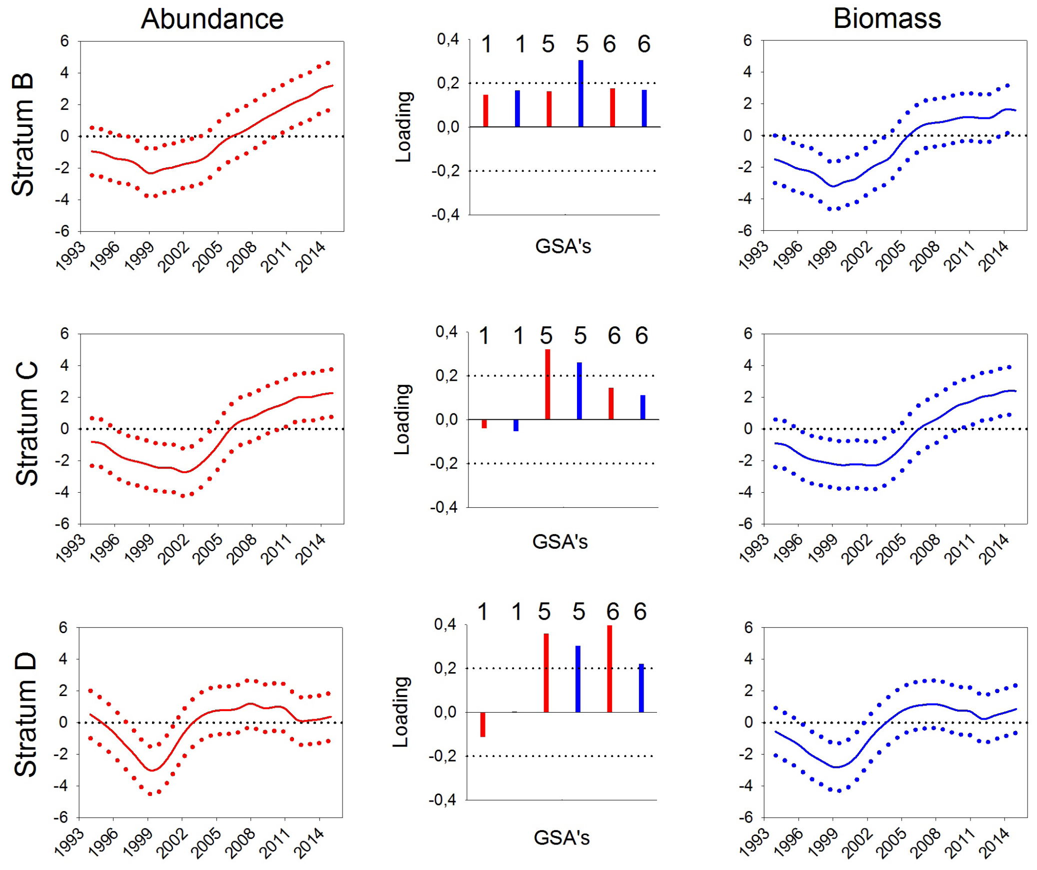


Figure 4.4.5. Common temporal trends and factor loadings from dynamic factor analysis (DFA) applied to standardized abundance (in red) and biomass (in blue) indices for batoids by geographic sub-areas (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) and depth strata (B: 50-100 m; C: 101-200 m; 201-500 m; E: 501-800 m) during the periods 1994-2015 in GSA01 and GSA06, and 2001-2015 in GSA05. Factor loading greater than 0.2 (dotted lines) correspond to indices that had a relative strong influence on the resulting common trend. Dashed lines indicate 95% confidence intervals.

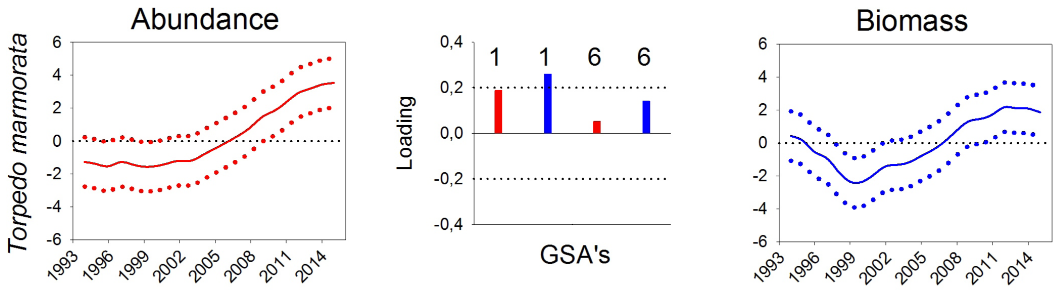


Figure 4.4.6. Common temporal trends and factor loadings from dynamic factor analysis (DFA) applied to standardized abundance (in red) and biomass (in blue) indices for *Torpedo marmorata* in the geographic sub-areas GSA01 (Northern Alboran Sea) and GSA06 (Northern Spain) during the period 1994-2015. Factor loading greater than 0.2 (dotted lines) correspond to indices that had a relative strong influence on the resulting common trend. Dashed lines indicate 95% confidence intervals.

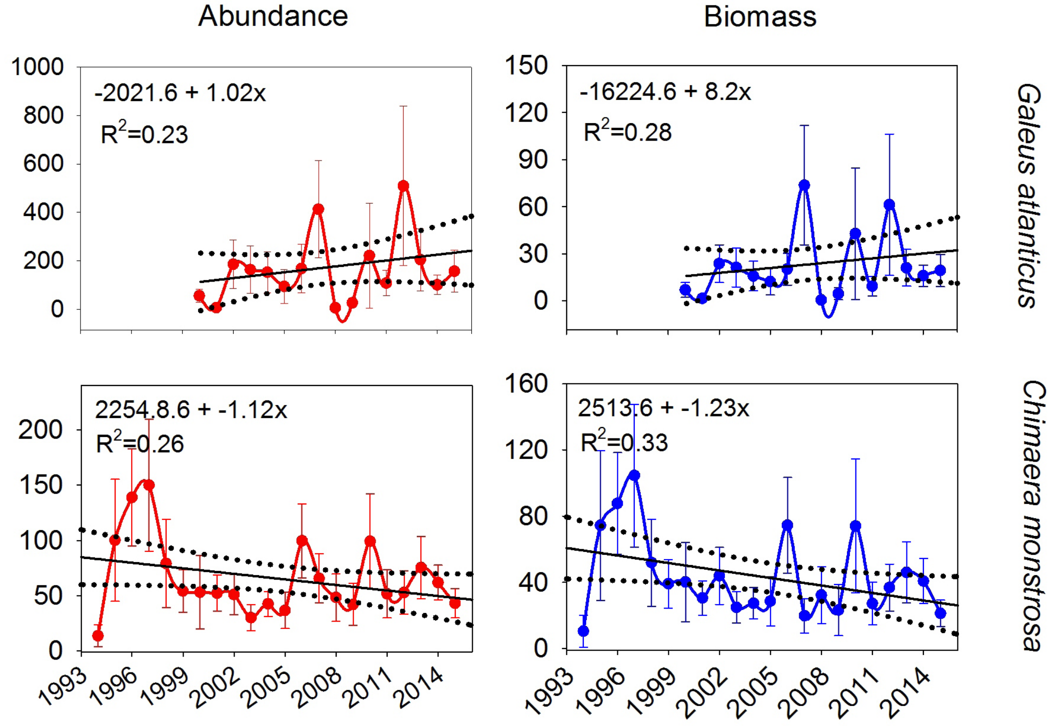


Figure 4.4.7. Linear regression models of standardized abundance (ABU, in red) and biomass (BIO, in blue) indices for *Galeus atlanticus* and *Chimaera monstrosa* in geographic sub-area GSA01 (Northern Alboran Sea) during the periods 2000-2015 for *G. atlanticus* and 1994-2015 for *C. monstrosa*. Dashed lines show 95% confidence intervals for regression lines and asterisks indicate statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

### *Size*

Relative length frequencies were estimated by three species of sharks, *S. canicula*, *G. melastomus* and *E. spinax*, in all GSAs. However, in the case of batoids, due to the low number of specimens captured, the only species that could be estimated was *R. clavata* in GSA05.

Linear regression models for *S. canicula* in GSA01 showed a significant increase in the relative frequency of the small and intermediate categories (<20, 20-30, and 30-40 cm TL), causing a decrease in the largest ones (40-50, and >50 cm TL) (Figure 4.4.9). GSA05 and GSA06 showed, on the contrary, a significant decrease in the relative frequency of the smallest category (<20 cm TL), but similar results for the intermediate (30-40 cm TL), and the largest (>50 cm TL) ones, whose relative frequencies increased and decreased, respectively.

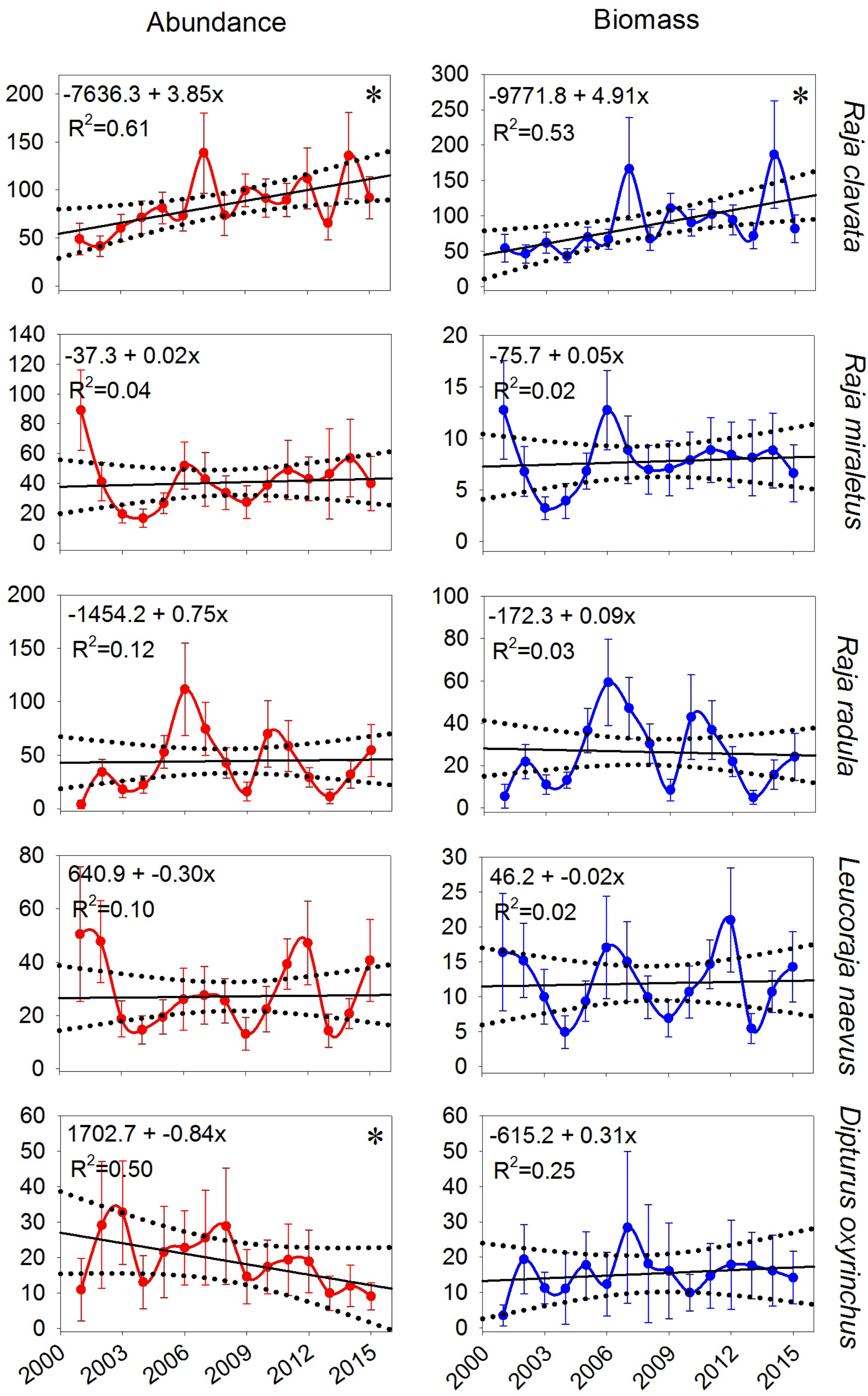


Figure 4.4.8. Linear regression models of standardized abundance (ABU, in red) and biomass (BIO, in blue) indices for the most abundant batoids in GSA05 (Balearic Islands): *Raja clavata*, *Raja miraletus*, *Raja radula*, *Leucoraja naevus* and *Dipturus oxyrinchus*. Dashed lines show 95% confidence intervals for regression lines and asterisks indicate statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

Relative length frequency of *G.melastomus* did not reveal a similar trend for all GSAs (Figure 4.4.10). In GSA01 a significant increase in the relative frequency was detected in small and intermediate categories (20-30 and 30-40 cm TL). In GSA05 the significant models were obtained for the smallest (<20 cm TL) and the largest (>50 cm TL) categories, which increased and decreased in the relative frequency, respectively. GSA06 exhibited a significant decrease in the relative length frequencies in the smallest category (<20 cm TL) and an increase in the intermediate category (30-40 cm TL).

*E. spinax* did not show significant trends in relative length frequency, except in the smallest category (<15 cm TL) of GSA01, which decreased (Figure 4.4.11). *R. clavata* in GSA05 showed a significant decrease relative length frequency in the smallest category (<15 cm TL) and an increase in the largest category (50-60 cm TL) (Figure 4.4.12).

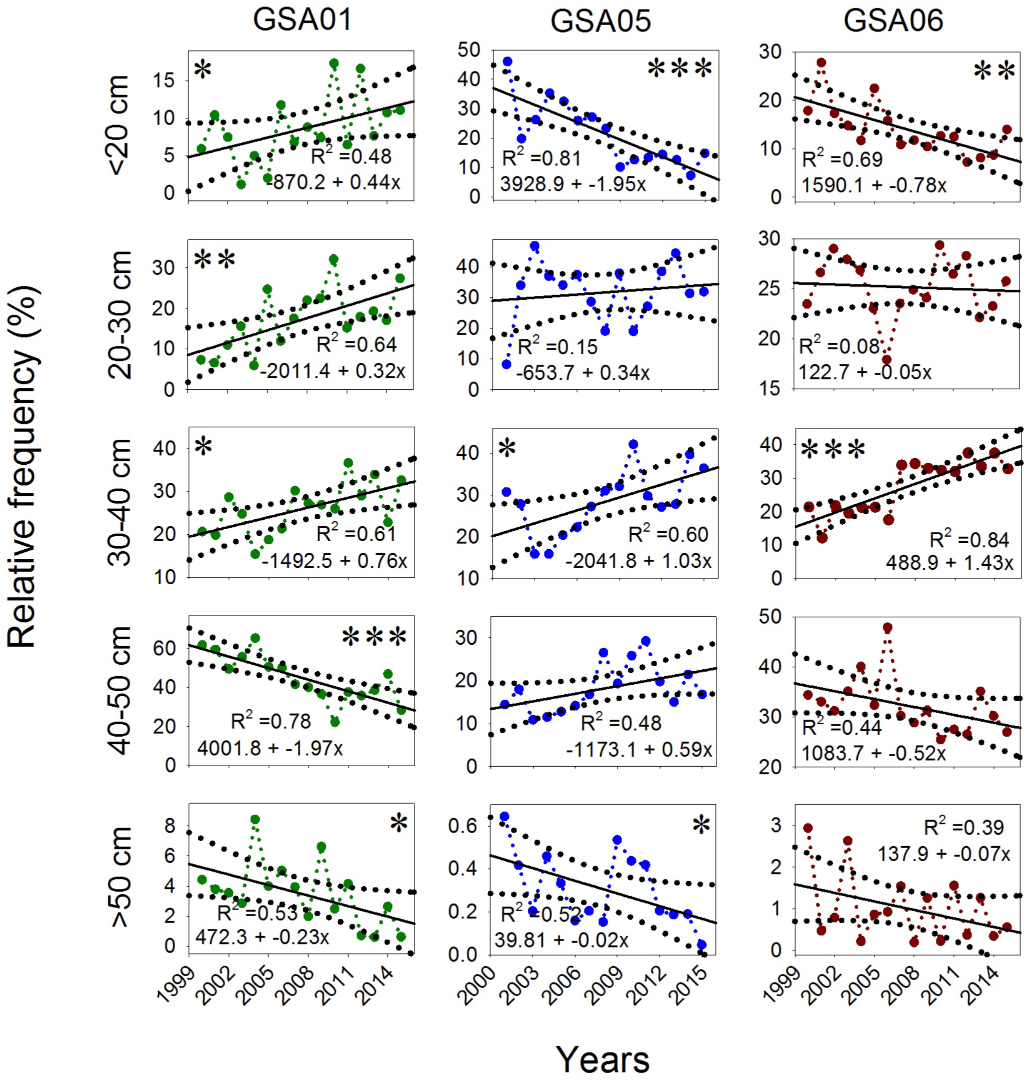


Figure 4.4.9. Linear regression models of length frequency composition for *Scyliorhinus canicula* by geographic sub-areas (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) during the periods 2000-2015 in GSA01 and GSA06, and 2001-2015 in GSA05. Dashed lines show 95% confidence intervals for regression lines, and asterisks indicate statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

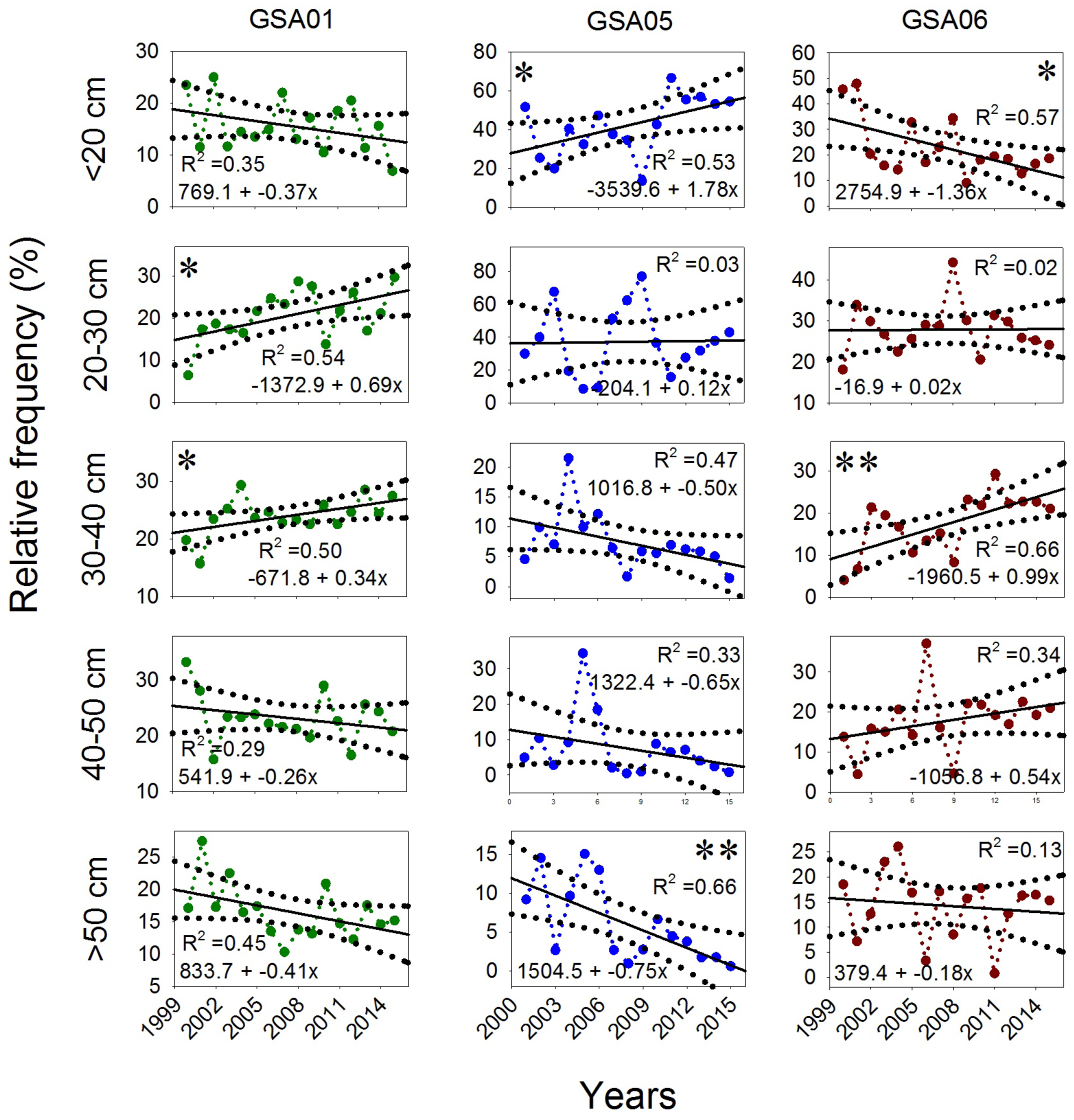


Figure 4.4.10. Linear regression models of length frequency composition for *Galeus melastomus* by geographic sub-areas (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) during the periods 2000-2015 in GSA01 and GSA06, and 2001-2015 in GSA05. Dashed lines show 95% confidence intervals for regression lines, and asterisks indicate statistical significance: (*) $p$<0.05; (**) $p$<0.01; (***) $p$<0.001.

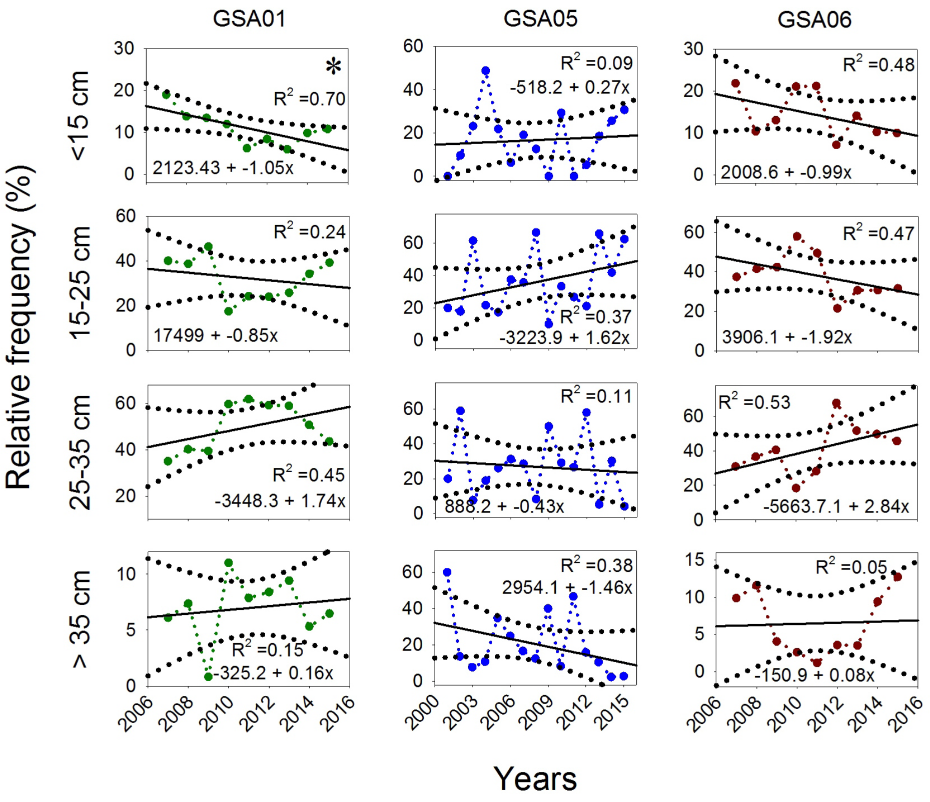


Figure 4.4.11. Linear regression models of length frequency composition for *Etmopterus spinax* by geographic sub-areas (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) during the periods 2007-2015 in GSA01 and GSA06 and 2001-2015 in GSA05. Dashed lines show 95% confidence intervals for regression lines, and asterisks indicate statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

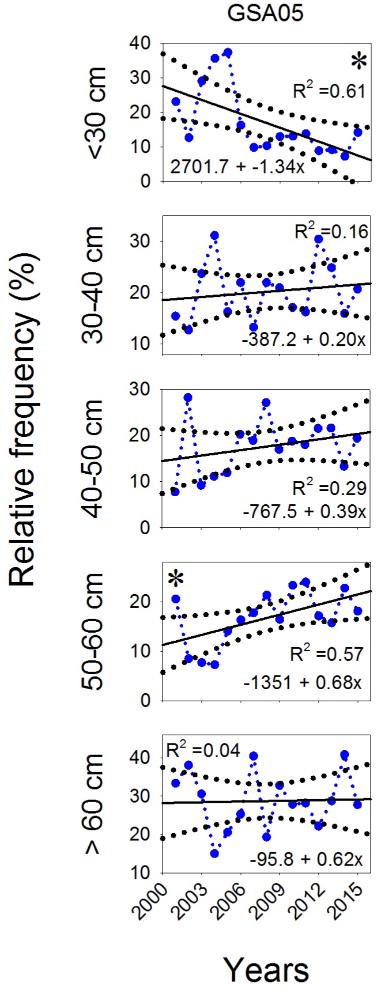


Figure 4.4.12. Linear regression models of length frequency composition for *Raja clavata* in the GSA05 (Balearic Islands) during the period 2001-2015. Dashed lines show 95% confidence intervals for regression lines, and asterisks indicate statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

### *Maturity*

Length at maturity was estimated for two sharks *S. canicula* and *G. melastomus* in all GSAs, and one batoid species, *R. clavata*, but only in GSA05. Maturity parameters estimated per year for each species are given in Tables S4.4.8-S4.4.10.

Linear regression models showed a significant decrease in length at first maturity of *S. canicula* for both sexes and in all GSAs (Figure 4.4.13). In females, the value of this parameter was reduced around 4.4 and 3.5 cm TL over 16 years in GSA01 and GSA06 and around 1.7 cm TL over 9 years in GSA05. For males this reduction was smaller, around 2 cm across the same period. For *G. melastomus* a significant downward trend was also detected in length at first maturity for females in GSA06 and males in GSA01, with a reduction of around 2 cm over 16 years in both sexes. *R. clavata* did not show any trends in length at first maturity in both sexes over the 9 years analysed. Linear regressions analyses of the trend of the size of the smallest mature female yielded the same results than the analysis of the trend of length at first maturity for all three species in each area (Figure 4.4.13).

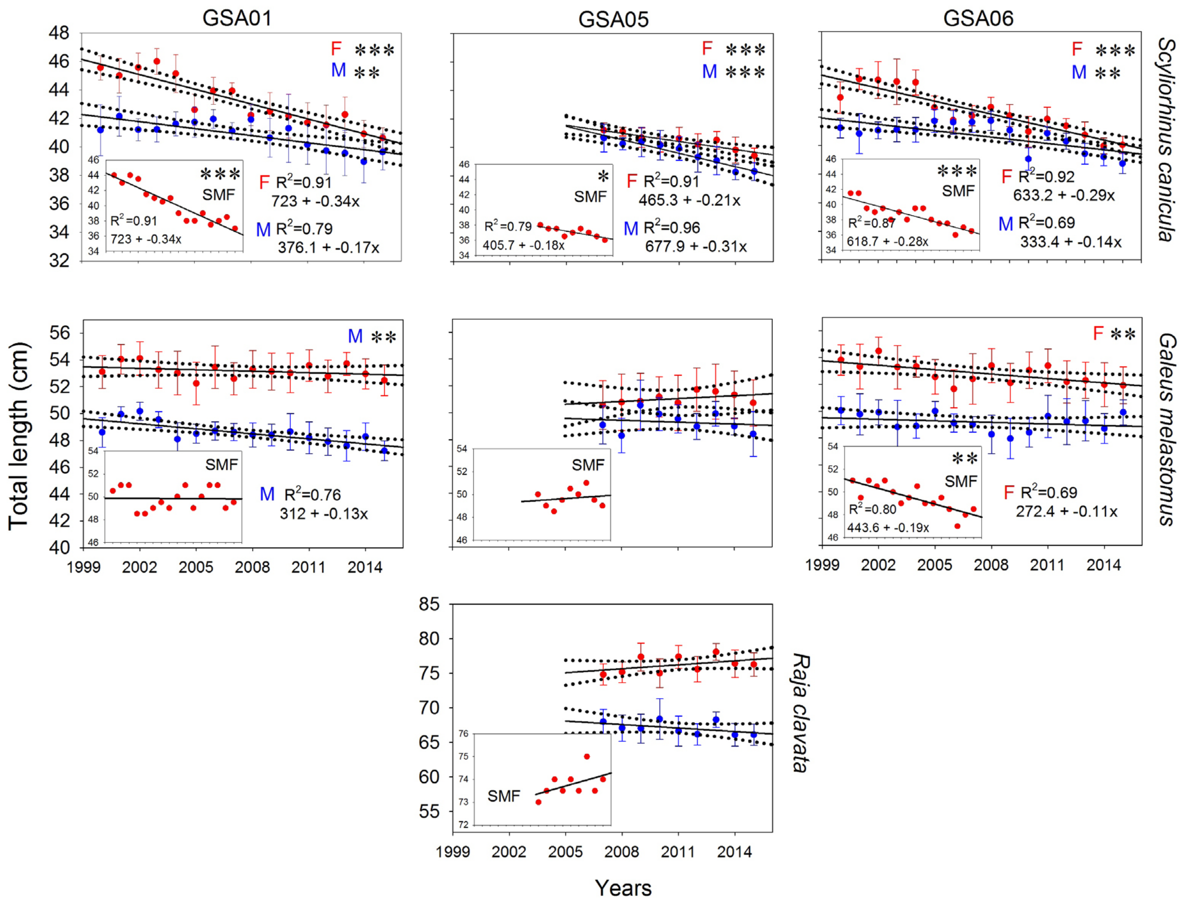


Figure 4.4.13. Linear regression models of length at first maturity ($L_{50}$; standard deviation values are included) for females (in red) and males (in blue) of *Scyliorhinus canicula* and *Galeus melastomus* for the three geographic sub-areas (GSAs) of the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain), and *Raja clavata* in GSA05. The SMF plot indicates the linear regression of smallest maturity females. Dashed lines show 95% confidence intervals for regression lines, and asterisks indicate statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

## 4.4.4 Discussion

Chondrichthyans are especially vulnerable fishes to the impact of fishing because of their particular life-history traits, including slow growth, late attainment of sexual maturity, long life spans and low fecundity (Stevens *et al*., 2000). Their decline has broad implications for marine ecosystems, because of their high position in trophic food webs (Scheffer *et al*., 2005; Baum and Worm, 2009). For these reasons, chondrichthyans are considered among the priority species for fisheries management and, in general, for the conservation of the marine environment. In this sense, in 1999 the Food and Agriculture Organization (FAO) developed the International Plan of action for the Conservation and Management of Sharks, and in 2003 the United Nations Enviroment Programme (UNEP) developed an action plan for cartilaginous fish in the Mediterranean, an area considered a key hotspot of extinction risk for these species (Dulvy *et al*., 2014).

The lack of accurate landings data on by-catch species, with chondrichthyans among them, and the scarcity of biological data has been a drawback for the assessment of their populations (Ellis *et al*., 2005). Nonetheless, assessment of by-catch species is crucial if the aim is moving from single species fisheries based management to an ecosystem based fisheries management and assessment (Browmand and Stergiou, 2004), particularly when these species can show comparable or even higher levels of overexplotation than the target ones (Ordines *et al*., 2014). Fishery-independent surveys provide valuable information of species exploited by fisheries (e.g. abundance and biomass, sex and maturity, size composition), which is particularly important for by-catch ones not studied in periodical samplings carried out throughout the year such as those ussually implemented for the most important target species undergoing periodical assessment of their exploitation state.

Here, we have assessed, at a community and population level, the spatio-temporal trends of demersal chondrichthyans in the western Mediterranean, where these species are an important by-catch of bottom trawl fishery (Carbonell *et al*., 2003). In contrast with the decreasing trends in diversity and abundance described for chondrichthyans throughout the Mediterranean, such as the Gulf of Lions (Aldebert, 1997), Lingurian Sea (Ligas *et al*., 2013), Adriatic Sea (Ferreti *et al*., 2013; Barausse *et al*., 2014) and Aegean Sea (Maravelias *et al*., 2012), our results suggest a generally stable situation and even sings of recovery for some species and groups over the last two decades. The bottom trawl fleet has gone through three phases during its recent temporal evolution (Quetglas *et al*., 2013): i) from mid 1960s to mid 1970s it increased by a factor of 2.5; ii) from mid 1970s to mid 1990s it continued to grow but at a slower rate; and iii) since mid 1990s it has gradually decreased. According to these authors, the sharp increase in the fishing effort caused that most demersal species populations became overfished during the 1980s. Chondrichthyans were not an exception and in some areas such as the Balearic Islands an abrupt decline in their landings was detected as early as mid decade of the 1960s (Guijarro *et al*., 2012), in coincidence with the increasing in effort. This early decline provoked that some of the most vulnerable chondrichthyan species such as *Galeorhinus galeus*, *Oxynotus centrina*, *Scyliorhinus stellaris*, *Squalus acanthias*, *Squatina* spp. and *Rhinobathos* spp., which appeared among the common ones in early description of fish communities of the trawl fishing grounds off the Balearic Islands (Ferrer, 1930; de Buen, 1935; Oliver, 1944; Maurin, 1968), are no longer present in the scientific surveys for the assessment of demersal resources and even have almost disappeared from commercial landings (Aldebert, 1997).These sharks have been catalogued by the International Union of the Conservation of Nature (IUCN) as near threatened, vulnerable, threatened or critically endangered species in the Mediterranean (Cavanagh and Gibson, 2007; Dulvy *et al*., 2016).

However, our results show, in general, recent increasing trends in abundance and biomass for both sharks and batoids on the continental shelf in the three GSAs, and more different species-dependent trends for deep water species with some increasing such as *Galeus melastomus*, and others decreasing such as Etmopterus spinax and *Dipturus oxyrinchus*. During last few decades, the technological improvement of fishing gears and vessels has allowed a worldwide expansion of fisheries to new areas, such as open ocean and deep sea bottoms, (Worm *et al*., 2013). It, has also occurred in the western Mediterranean, where trawl fishery moved from the continental shelf to the slope to capture the decapod crustacean red shrimp (*Aristeus antennatus*) and Norway lobster (*Nephrops norvegicus*) of higher economic value (Moranta *et al*., 2008; Hidalgo *et al*., 2009). This effort displacement involved a consequent reduction of fishing effort on the continental shelf and the above mentioned reduction of the trawl fleet since 1990s could explain the signs of recovery shown by sharks and batoids on the continental shelf. A recovery that has also been detected in the commercial landings of elasmobranchs from the continental shelf in the Balearic Islands (Guijarro *et al*., 2012). The implementation of marine protected areas, which in our study area are mostly located in shallow waters, could also have strengthened the recovery of these vulnerable species. In this sense, the Balearic Islands, one of the areas of the western Mediterranean with the greatest diversity and density (abundance and biomass) of these species (Massutí and Moranta, 2003; Ramírez-Amaro *et al*., 2015), have many protected areas, with up to seven marine reserves and one national park, covering 18% of the waters around the Archipelago (Coll *et al*., 2012).

Similar circumstances have been reported by serveral authors in different areas. In the Mediterranean, Marongiu *et al*. (2017) described stable and increasing trends in chondrichthyan populations off Sardinia, explained also by a decreasing number of trawlers and displacement of fishing effort. Increasing trends of *T. marmorata*, *R. miraletus* and *R. clavata* have also been observed in the Strait of Sicily (Garofalo *et al*., 2003), Tyrrhenian Sea (Ferretti *et al*., 2005), and Adriatic Sea (Ferretti *et al*., 2013), which could be explained by the decrease in fishing effort, particularly observed in the Strait of Sicily. In Atlantic waters off south-eastern United States, coastal sharks have increased their relative abundance, suggesting an initial recovery from past exploitation (Peterson *et al*., 2017). These authors also conclude that the distinct patterns of increment between species have been attributed to their different life histories and to the various management measures applied.

Both, *S. canicula* and *G. melastomus*, the two most abundant demersal sharks in the western Mediterranean, showed increasing trends despite their different bathymetric distribution (shelf and shelf-breack, and upper and middle slope, respectively; Ordines *et al*., 2011; Ramírez-Amaro *et al*., 2015). A similar increment in the population of *S. canicula* has been recorded in the Cantabrian Sea (north-eastern Atlantic; Arroyo *et al*., 2017). The particular life-history traits of these species (early maturity, shorter generation time, fast growth, continuous reproductive cycles; Camhi *et al*., 1998; Rey *et al*., 2004; Capapé *et al*., 2007), and even for *S. canicula* its high survival rate as a discarded catch of trawl fishery (Rodríguez-Cabello *et al*., 2001), may generates a higher resilience among elasmobranchs. According to Mendoza *et al*. (2014), these two species are the least vulnerable species in comparison to the rest of chondrichthyans species caught in the western Mediterranean. In addition, both species have also been considered opportunistic scavengers that may modify their natural diet to benefit from disturbed sediments and organic matter generated by fisheries discards (Olaso *et al*., 2005). A similar situation has been argued for some skates dwelling on the continental shelf (Abella and Serena, 2002); and for *R. clavata*, for which a high survival rate has also been observed in the Bristol Channel after discarding (Enever *et al*., 2009). In the case of *G. melastomus*, a very important additional factor must be considered on the basis of the increment in its populations. Furthermore, in the case of *G. melastomus*, it must be considered that the deep water trawling in the western Mediterranean has not reached more than 800-900 m depth, due to yields of the main target species in these bottoms, *Aristeus antennatus* decrease below these depths (Sardà *et al*., 2004). Moreover, the General Fisheries Commission for the Mediterranean in 2006 decided to ban this fishery beyond 1000 m. Taking into account the fact that *G. melastomus* is abundant down to depths of 1400 m, with the largest individuals dominating the populations inhabiting beyond 800 m depth (Moranta *et al*., 2004), an important fraction of its spawning stock remains unexploited. However, *G. melastomus* is an exception, because the other deep water chondrichthyans such as *D. oxyrinchus* and *E. spinax* exhibited decreasing trends. This decrease in *E. spinax* population in the Mediterranean has already been reported by other authors (Ordines *et al*., 2011; Cartes *et al*., 2013) and it has been explained by the fact that this shark is extremely vulnerable to fishing mortality, due to its late maturation, long reproductive cycle and low fecundity (Coelho and Erzini, 2005, 2008). In fact, deep water chodrichthyans, with longer turnover times (e.g. slower growth, older age at maturity and greater longevity) than the species dwelling on the continental shelf, are considered to be especially sensitive to fishery effect (Garcia *et al*., 2008; Simpfendorfer and Kyne, 2009). Given the depth of catch, the survival rates of discarded deep-water chondrichthyans are low, which make it dificult to cushion the effect of fishing (Dagit *et al*., 2007; Coelho *et al*., 2009).

Regarding the other population parameters of the most abundant species, analysed in addition to abundance and biomass, the results are different. While no clear trends were observed in size composition, a decreasing trend in length at first maturity ($L_{50}$) and the size of the smallest mature female of *S. canicula* was detected during the study period in all GSAs. The potential of fishing to drive changes in life-history traits (e.g. fecundity, maturity, growth rate) of exploited fish populations has long been recognized in at least three ways (see Sharpe *et al*., 2012 and references therein): i) often highly selective; ii) frequently inflict mortality much greater than natural mortality; and iii) many life history traits potentially changed under fisheries selection are heritable. Strong reductions in species abundances are often accompanied by changes in their life histories, as an adaption of the population to the new conditions (Darimont *et al*., 2009; Audzijonyte and Kuparinen, 2016). They are ecological and evolutionary responses due to the phenotypic plasticity of life strategies and the potential genetic changes in their populations, respectively. Over the last few decades, these changes have been documented in many commercial fish stock around the world (e.g., Jørgensen, 1990; Saborido-Rey and Junquera, 1998; Cardinale and Modin, 1999; Hutchings, 2005). The main drivers are likely to involve to fisheries-induced evolution and global environmental change, affecting both phenotypic and genetic components (Hendry *et al*., 2008; Sharpe and Hendry, 2009; Baudron *et al*., 2014).

Detecting changes in life-history traits of chondrichthyan populations is a difficult task since they are typical k-strategists in comparison to teleosts (Caillet *et al*., 1990; Stevens *et al*., 2000). However, in general, there is a tendency for small-sized coastal species, which mature earlier and have shorter life spans, and a higher intrinsic rate to increase than larger species, tend to have a high rebound potential (Smith *et al*., 1998; Au *et al*., 2015). In this sense, changes in life-history traits have only been detected in some small-medium sized sharks such as *Squalus acanthias* (Saunders and McFarlane, 1993) and *Rhizoprionodon terraenovae* (Carlson and Beremore, 2002). According to this, the decreasing trend of length at first maturity observed in *S. canicula*, which is also a small-sized shark, could be an indicator that its population has been under stress due to the above mentioned high levels of trawl fishery exploitation since the 1980s in the study area. The adaptive response of early maturation increases the likelihood of offspring reaching maturity, (Smith *et al*., 1998), which likely promoted the present recovery of *S. canicula* populations.

Our findings for $L_{50}$ can be explained by two possible mechanisms, an evolutionary response to selection for smaller size at maturity and/or by reducing intra-specific competition with the consequent increase in available food (Holden, 1973; Sharpe *et al*., 2012). Environment is another potential mechanism that seems to influence life-history changes in teleosts (e.g. Sharpe *et al*., 2012; Baudron *et al*., 2014), and which should be considered, especially in a semi-enclosed sea as the Mediterranean, where De Leiva Moreno *et al*. (2000) have drawn attention to an increase in productivity over last few decades. In teleost species it has been observed that environmental factors might determine differences in age and size at maturity, where favourable conditions (e.g., intensive upwelling, high productivity), produce higher quality offspring that grow at faster rates, resulting in early maturation (e.g., Domínguez-Petit *et al*., 2008; Jonsson *et al*., 2013). According to this, and considering that relative somatic condition of fish has been exposed as an important factor affecting growth rate (Rätz and Lloret, 2003; Ordines *et al*., 2009; Jonsson *et al*., 2013), potential changes in somatic condition of *S. canicula* could be explored, through the annual value of this parameter by sex over the last nine years (2007-2015), but considering only juvenile individuals (<35 cm TL), in order to avoid possible variation due to maturation process (statistical analysis is explained in Supplementary material: Text). No significant trends were found, with only the exception of females in GSA01 (Figure 4.4.14), which suggests that there have been no temporal changes in the growth rate of *S. canicula* populations. It is commonly cited that early maturation increases population growth rates; however, this correlation has been discussed by several authors (Partridge and Coyne, 1997; Angilletta and Dunham, 2003). The observed differences between both biological parameters may be because temperature and food quality affect energy deposits and survival rates differently (Smith *et al*., 1998; Jonsson *et al*., 2013). Before changes in growth, some of the resources are allocated to the running costs of the fish, mainly their basal metabolism (e.g., maintenance, immune defence, and cognition), digestion or routine activity (e.g., migration to breeding grounds, courting, competition for mates and breeding sites, copulation and parental care) (Jonsson and Jonsson, 2003; Enberg *et al*., 2012). In fact, according to Kuparinen and Hutchings (2012) during a period of recovery, fisheries-induced evolution might not have a substantive effect on the rate of population growth as previously hypothesized (Hutchings, 2005; Kuparinen *et al*., 2014).

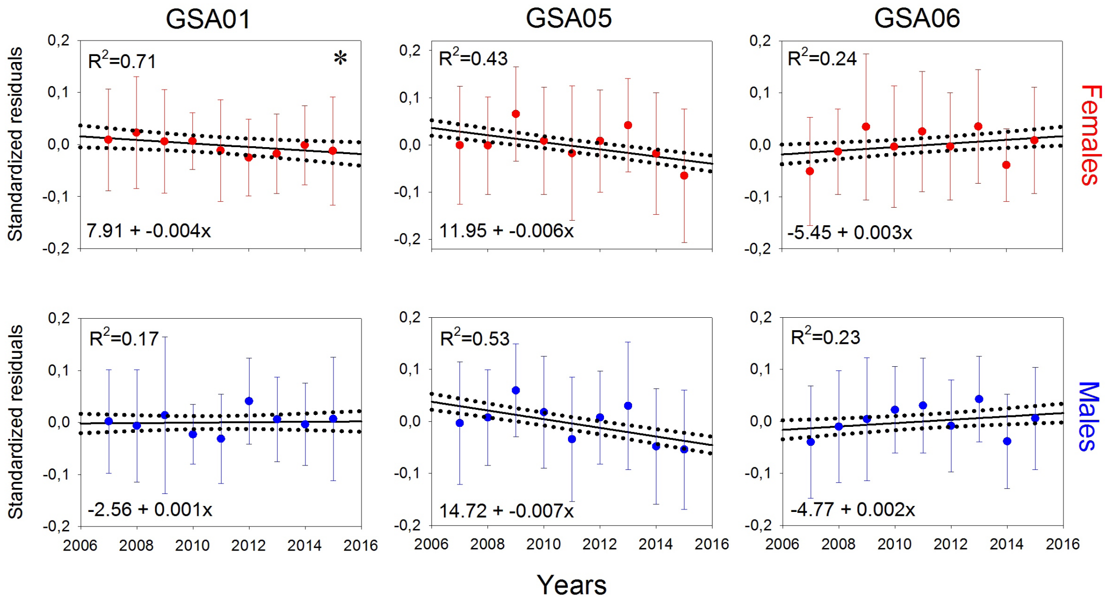


Figure 4.4.14. Linear regression models of somatic condition (mean and standard values) for females (in red) and males (in blue) of *Scyliorhinus canicula* for the three geographic sub-areas (GSAs) of the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) during the period 2007-2015. Dashed lines show 95% confidence intervals for regression lines and asterisks indicate statistical significance: (*) $p$<0.05; (**) $p$<0.01; (***) $p$<0.001.

In summary, this study shows the feasibility of fishery-independent scientific surveys, which are currently being developed in the Mediterranean, to assess the population status of by-catch vulnerable species such as demersal chondrichthyans. An analysis of the largest dataset from bottom trawl surveys in the study area (1994-2015), reveals fairly stable populations with an increment of species dwelling on the continental shelf, such as *S. canicula*, *R. clavata*, and *T. marmorata*, and even for the most abundant deep water species (*G. melastomus*). Thus, these species resist the impact of fishing on the western Mediterranean over the last two decades. By contrast, decreasing trends were detected for some deep-water species such as *E. spinax* and *D. oxyrinchus*. This can be explained by the reduction of the trawling effort at shallow depths over the last few decades, due to the decrease in the trawling fleet and to its displacement towards deep waters, jointly with the high resilience displayed by some of these species, like *S. canicula* which also showed a reduction of its length at first maturity, probably as an evolutionary response to the general overfishing of the demersal population exploited by trawl fishery during the 1980s. These results can be useful for implementing management measures that strengthen or initiate the recovery in the Mediterranean of these vulnerable species, whose populations play an important role in marine ecosystems, and to develop a true adaptive management in the area, making the sustainability of trawl fishery compatible with the conservation of marine ecosystems.

# Supplementary material: Tables

Table S4.4.1. Number of bottom trawl samples analysed by year, depth strata (D) considered in the MEDITS surveys (B: 50-100 m; C: 101-200 m; D: 201-500 m; E: 501-800 m) and geographic sub-areas (GSA) considered by the General Fisheries Commission for the Mediterranean (GFCM) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain).

| GSA | D | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GSA01 | B | 5 | 5 | 5 | 6 | 6 | 9 | 6 | 6 | 8 | 12 | 8 | 8 | 7 | 8 | 7 | 7 | 6 | 6 | 8 | 11 | 13 | 14 |
|  | C | 3 | 3 | 3 | 5 | 5 | 5 | 5 | 5 | 8 | 6 | 5 | 6 | 5 | 7 | 6 | 6 | 4 | 4 | 4 | 5 | 7 | 9 |
|  | D | 6 | 9 | 11 | 10 | 7 | 11 | 12 | 10 | 11 | 11 | 13 | 11 | 11 | 13 | 11 | 11 | 6 | 8 | 9 | 10 | 14 | 19 |
|  | E | 6 | 9 | 12 | 10 | 12 | 12 | 9 | 13 | 13 | 14 | 13 | 11 | 15 | 13 | 7 | 5 | 6 | 7 | 10 | 10 | 14 | 15 |
| GSA05 | B |  |  |  |  |  |  |  | 12 | 23 | 20 | 27 | 23 | 29 | 20 | 20 | 20 | 20 | 21 | 20 | 22 | 22 | 22 |
|  | C |  |  |  |  |  |  |  | 12 | 15 | 15 | 18 | 17 | 16 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
|  | D |  |  |  |  |  |  |  | 8 | 10 | 11 | 12 | 9 | 10 | 8 | 8 | 8 | 8 | 9 | 8 | 9 | 11 | 8 |
|  | E |  |  |  |  |  |  |  | 9 | 11 | 10 | 12 | 10 | 9 | 8 | 8 | 8 | 8 | 9 | 8 | 8 | 12 | 12 |
| GSA06 | B | 19 | 25 | 26 | 25 | 27 | 26 | 29 | 29 | 34 | 36 | 30 | 31 | 33 | 26 | 28 | 28 | 19 | 28 | 34 | 38 | 37 | 30 |
|  | C | 10 | 17 | 15 | 15 | 12 | 17 | 17 | 19 | 19 | 20 | 17 | 17 | 18 | 15 | 21 | 20 | 12 | 21 | 22 | 24 | 26 | 28 |
|  | D | 9 | 15 | 13 | 11 | 8 | 13 | 11 | 20 | 18 | 18 | 17 | 16 | 22 | 13 | 14 | 14 | 10 | 18 | 16 | 19 | 16 | 20 |
|  | E | 8 | 13 | 11 | 8 | 6 | 12 | 7 | 10 | 9 | 10 | 13 | 10 | 11 | 10 | 11 | 12 | 7 | 8 | 8 | 6 | 9 | 12 |

Table S4.4.2. Species composition, as percentage in standardized abundance (ABU) and biomass (BIO) indices, of sharks captured in the MEDITS surveys throughout the western Mediterranean, by geographic sub-area (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) and depth strata (B: 50-100 m; C: 101-200 m; D: 201-500 m; E: 501-800 m) over the period 1994-2015 for GSA01 and GSA06, and 2001-2015 for GSA05.

| **GSA** | **Species** | **Stratum B** | | **Species** | **Stratum C** | | **Species** | **Stratum D** | | **Species** | **Stratum E** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **ABU** | **BIO** | | **ABU** | **BIO** | | **ABU** | **BIO** | | **ABU** | **BIO** |
| GSA01 | *Scyliorhinus canicula* | 99.5 | 92.7 | *Scyliorhinus canicula* | 99.8 | 99.6 | *Galeus melastomus* | 77.1 | 76.1 | *Galeus melastomus* | 87.3 | 87.6 |
| | Other | <0.5 | <7.3 | Other | <0.2 | <0.4 | *Scyliorhinus canicula* | 10.4 | 9.9 | *Etmopterus spinax* | 8.1 | 4.5 |
| | | | | | | | *Etmopterus spinax* | 7.2 | 3.8 | *Galeus atlanticus* | 4.1 | 3.5 |
| | | | | | | | *Galeus atlanticus* | 4.8 | 3.9 | Other | <0.5 | <0.4 |
| | | | | | | | Other | <0.5 | <6.3 | | | |
| GSA05 | *Scyliorhinus canicula* | 99.5 | 96.1 | *Scyliorhinus canicula* | 94.2 | 74.9 | *Galeus melastomus* | 63.2 | 51.6 | *Galeus melastomus* | 93.3 | 95.2 |
| | *Mustelus mustelus* | 0.3 | 3.8 | *Squalus blainville* | 5.72 | 24.9 | *Scyliorhinus canicula* | 36.3 | 45.1 | *Etmopterus spinax* | 6.3 | 4.12 |
| | Other | <0.1 | <0.1 | Other | <0.1 | <0.2 | Other | <0.5 | <3.1 | Other | <0.4 | <0.7 |
| GSA05 | *Scyliorhinus canicula* | 100 | 100 | *Scyliorhinus canicula* | 97.9 | 90.3 | *Scyliorhinus canicula* | 54.6 | 81.8 | *Galeus melastomus* | 88 | 91.8 |
| | | | | *Squalus acanthias* | 1.7 | 8.9 | *Galeus melastomus* | 44.7 | 17.8 | *Etmopterus spinax* | 7.7 | 3.5 |
| | | | | Other | <0.4 | <0.8 | Other | <0.7 | <0.4 | *Scyliorhinus canicula* | 4 | 4.1 |
| | | | | | | | | | | Other | <0.3 | <0.6 |

Table S4.4.3. Species composition, as percentage in standardized abundance (ABU) and biomass (BIO) indices of batoids captured in the MEDITS surveys throughout the western Mediterranean, by geographic sub-area (GSAs) in the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain) and depth strata (B: 50-100 m; C: 101-200 m; D: 201-500 m) over the period 1994-2015 for GSA01 and GSA06, and 2001-2015 for GSA05.

| **GSA** | **Species** | **Stratum B** | | **Species** | **Stratum C** | | **Species** | **Stratum D** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **ABU** | **BIO** | | **ABU** | **BIO** | | **ABU** | **BIO** |
| GSA01 | *Torpedo marmorata* | 45.1 | 14.3 | *Leucoraja naevus* | 71.7 | 82.1 | *Leucoraja naevus* | 86.1 | 72.9 |
| | *Raja asterias* | 43.3 | 54.9 | *Torpedo marmorata* | 23.9 | 11.6 | *Torpedo nobiliana* | 7.6 | 11.9 |
| | *Leucoraja naevus* | 3.8 | 2.9 | *Raja asterias* | 2 | 4.1 | *Torpedo marmorata* | 4.3 | 4.4 |
| | Other | <7.8 | <28 | Other | <2.4 | <2.2 | Other | <2 | <10.8 |
| GSA05 | *Raja miraletus* | 24.6 | 5.4 | *Raja clavata* | 55.9 | 77.7 | *Raja clavata* | 58.7 | 61.8 |
| | *Raja radula* | 18.9 | 12.6 | *Raja montagui* | 18.3 | 3.9 | *Dipturus oxyrinchus* | 28.7 | 27.7 |
| | *Raja clavata* | 18.9 | 34.5 | *Leucoraja naevus* | 16.7 | 12.1 | *Leucoraja naevus* | 7.2 | 5.1 |
| | *Dasyatis pastinaca* | 4.9 | 9.5 | *Raja miraletus* | 3.9 | 0.8 | *Raja polystigma* | 2.9 | 0.8 |
| | *Raja brachyura* | 4.9 | 6.2 | Other | <0.6 | <0.6 | Other | <2.5 | <4.5 |
| | *Leucoraja naevus* | 3.1 | 1.6 | | | | | | |
| | Other | <24 | <40 | | | | | | |
| GSA06 | *Raja clavata* | 28.2 | 42.7 | *Raja clavata* | 30.2 | 39.6 | *Raja clavata* | 36.2 | 54.8 |
| | *Raja asterias* | 21.9 | 22.7 | *Raja asterias* | 11.6 | 9.2 | *Raja asterias* | 32.9 | 15.1 |
| | *Torpedo marmorata* | 20.2 | 9.8 | *Torpedo marmorata* | 9.8 | 4.3 | *Leucoraja naevus* | 12.5 | 11.6 |
| | *Leucoraja naevus* | 10 | 6.8 | *Leucoraja naevus* | 8.7 | 3.4 | *Torpedo nobiliana* | 3.8 | 2.5 |
| | *Raja montagui* | 5.3 | 3.9 | *Raja miraletus* | 7.7 | 3.2 | Other | <14.6 | <16 |
| | Other | <14.4 | <14.1 | *Raja montagui* | 5.2 | 3.5 | | | |
| | | | | Other | <26.8 | <36.8 | | | |

Table S4.4.4. Results from dynamic factor analysis (DFA) model fitting applied to standardized abundance (AB) and biomass (BI) indices for sharks by depth strata (B: 50-100 m; C: 101-200 m; D: 201-500 m; E: 501-800 m) over the period 1994-2015 in GSA01 (Northern Alboran Sea) and GSA06 (Northern Spain) and 2001-2015 in GSA05 (Balearic Islands). For each model, the covariance matrix structure, the number of common trends (m), and the corrected Akaike Information Criterion (AICc) are given. Italicized models represent the models for each depth stratum that were selected as the models that produced the most favorable covariance matrix structure (with the lowest AICc values) and number of common trends.

| **Strata** | **Covariance matrix structure** | | **m** | | **Log-likelihood** | | **AICc** | |
|---|---|---|---|---|---|---|---|---|
| | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** |
| B | *Diagonal and equal* | *Diagonal and equal* | *1* | *1* | *-76.17* | *-77.00* | *161.08* | *162.74* |
| | Diagonal and unequal | Diagonal and unequal | 1 | 1 | -74.43 | -75.00 | 162.48 | 163.63 |
| | Equalvarcov | Equalvarcov | 1 | 1 | -75.72 | -76.76 | 162.58 | 164.67 |
| | Diagonal and equal | Diagonal and equal | 2 | 2 | -76.16 | -76.98 | 165.95 | 167.60 |
| | Equalvarcov | Diagonal and unequal | 2 | 2 | -75.72 | -75.00 | 167.64 | 168.88 |
| | Diagonal and unequal | Equalvarcov | 2 | 2 | -74.43 | -76.72 | 167.74 | 169.64 |
| | Diagonal and equal | Diagonal and equal | 3 | 3 | -76.16 | -76.97 | 168.53 | 170.18 |
| | Unconstrained | Unconstrained | 1 | 1 | -73.81 | -74.51 | 169.31 | 170.70 |
| | Equalvarcov | Diagonal and unequal | 3 | 3 | -75.72 | -75.00 | 170.33 | 171.68 |
| | Diagonal and unequal | Equalvarcov | 3 | 3 | -74.43 | -76.72 | 170.53 | 172.33 |
| | Unconstrained | Unconstrained | 2 | 2 | -73.80 | -74.46 | 175.22 | 176.54 |
| | Unconstrained | Unconstrained | 3 | 3 | -73.80 | -74.46 | 178.39 | 179.71 |
| C | *Diagonal and equal* | *Diagonal and equal* | *1* | *1* | *-80.80* | *-81.55* | *170.34* | *171.84* |
| | Equalvarcov | Equalvarcov | 1 | 1 | -80.35 | -80.39 | 171.83 | 171.91 |
| | Diagonal and unequal | Diagonal and unequal | 1 | 1 | -80.70 | -81.47 | 175.03 | 176.56 |
| | Diagonal and equal | Diagonal and equal | 2 | 2 | -80.79 | -81.54 | 175.21 | 176.72 |
| | Equalvarcov | Equalvarcov | 2 | 2 | -80.35 | -80.39 | 176.90 | 176.98 |
| | Diagonal and equal | Diagonal and equal | 3 | 3 | -80.79 | -81.54 | 177.80 | 179.30 |
| | Equalvarcov | Equalvarcov | 3 | 3 | -80.35 | -80.39 | 179.58 | 179.66 |
| | Diagonal and unequal | Unconstrained | 2 | 1 | -80.70 | -79.80 | 180.29 | 181.27 |
| | Unconstrained | Diagonal and unequal | 1 | 2 | -79.85 | -81.47 | 181.37 | 181.83 |
| | Diagonal and unequal | Diagonal and unequal | 3 | 3 | -80.70 | -81.47 | 183.09 | 184.62 |
| | Unconstrained | Unconstrained | 2 | 2 | -79.85 | -79.80 | 187.31 | 187.22 |
| | Unconstrained | Unconstrained | 3 | 3 | -79.84 | -79.79 | 190.48 | 190.38 |
| D | *Equalvarcov* | *Diagonal and equal* | *1* | *1* | *-81.60* | *-78.20* | *169.45* | *165.14* |
| | Diagonal and equal | Equalvarcov | 1 | 1 | -79.15 | -77.94 | 171.94 | 167.03 |
| | Unconstrained | Diagonal and unequal | 1 | 1 | -76.39 | -77.53 | 174.46 | 168.67 |
| | Equalvarcov | Diagonal and equal | 2 | 2 | -79.15 | -78.02 | 174.51 | 169.67 |
| | Diagonal and unequal | Equalvarcov | 1 | 2 | -81.07 | -77.97 | 175.65 | 172.15 |
| | Diagonal and equal | Diagonal and equal | 2 | 3 | -81.59 | -78.02 | 176.81 | 172.25 |
| | Equalvarcov | Unconstrained | 3 | 1 | -79.15 | -75.56 | 177.19 | 172.79 |
| | Diagonal and equal | Diagonal and unequal | 3 | 2 | -80.60 | -77.53 | 179.39 | 173.94 |
| | Unconstrained | Equalvarcov | 2 | 3 | -76.41 | -77.97 | 180.45 | 174.83 |
| | Diagonal and unequal | Diagonal and unequal | 3 | 3 | -81.01 | -77.53 | 183.70 | 176.73 |
| | Unconstrained | Unconstrained | 3 | 2 | -76.52 | -75.56 | 183.82 | 178.74 |
| | Diagonal and unequal | Unconstrained | 2 | 3 | -80.04 | -75.56 | 187.68 | 181.90 |
| E | *Equalvarcov* | *Equalvarcov* | *1* | *1* | *-77.12* | *-81.23* | *165.37* | *169.88* |
| | Unconstrained | Equalvarcov | 1 | 2 | -73.29 | -79.37 | 168.26 | 172.44 |
| | Equalvarcov | Unconstrained | 2 | 1 | -76.90 | -78.12 | 170.00 | 173.41 |
| | Equalvarcov | Equalvarcov | 3 | 3 | -76.90 | -75.87 | 172.68 | 175.12 |
| | Diagonal and unequal | Diagonal and equal | 1 | 1 | -74.08 | -78.12 | 173.78 | 175.20 |
| | Diagonal and equal | Diagonal and unequal | 1 | 1 | -74.07 | -80.23 | 174.80 | 176.67 |
| | Unconstrained | Unconstrained | 2 | 2 | -74.39 | -75.74 | 176.41 | 179.11 |
| | Diagonal and unequal | Diagonal and equal | 2 | 2 | -75.71 | -81.02 | 178.31 | 179.95 |
| | Unconstrained | Diagonal and unequal | 3 | 2 | -74.45 | -80.58 | 179.69 | 182.05 |
| | Diagonal and equal | Unconstrained | 2 | 3 | -73.33 | -75.74 | 180.28 | 182.26 |
| | Diagonal and equal | Diagonal and equal | 3 | 3 | -76.80 | -81.03 | 181.90 | 182.96 |
| | Diagonal and unequal | Diagonal and unequal | 3 | 3 | -76.60 | -80.97 | 182.74 | 185.62 |

Table S4.4.5. Results from dynamic factor analysis (DFA) model fitting applied to standardized abundance (AB) and biomass (BI) indices for most abundant sharks, *Scyliorhinus canicula* (SC), *Galeus melastomus* (GM) and Etmopterus spinax (ES) over the period 1994-2015 in GSA01 (Northern Alboran Sea) and GSA06 (Northern Spain) and 2001-2015 in GSA05 (Balearic Islands). For each model, the covariance matrix structure, the number of common trends (m), and the corrected Akaike Information Criterion (AICc) are given. Italicized models represent the models for each depth stratum that were selected as the models that produced the most favorable covariance matrix structure (with the lowest AICc values) and number of common trends.

| **Species** | **Covariance matrix structure** | | **m** | | **Log-likelihood** | | **AICc** | |
|---|---|---|---|---|---|---|---|---|
| | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** |
| SC | *Equalvarcov* | *Equalvarcov* | *1* | *1* | *-79.95* | *-80.29* | *166.77* | *168.81* |
| | Diagonal and equal | Diagonal and equal | 1 | 1 | -77.82 | -78.83 | 168.64 | 169.32 |
| | Equalvarcov | Equalvarcov | 2 | 2 | -77.82 | -78.81 | 171.84 | 173.82 |
| | Diagonal and unequal | Diagonal and unequal | 1 | 1 | -79.77 | -80.20 | 173.16 | 174.03 |
| | Diagonal and equal | Diagonal and equal | 2 | 2 | -79.95 | -80.28 | 173.52 | 174.19 |
| | Equalvarcov | Equalvarcov | 3 | 3 | -77.82 | -78.81 | 174.52 | 176.50 |
| | Diagonal and equal | Diagonal and equal | 3 | 3 | -79.95 | -80.28 | 176.10 | 176.77 |
| | Unconstrained | Unconstrained | 1 | 1 | -77.47 | -78.07 | 176.62 | 177.83 |
| | Diagonal and unequal | Diagonal and unequal | 2 | 2 | -79.77 | -80.20 | 178.42 | 179.29 |
| | Diagonal and unequal | Diagonal and unequal | 3 | 3 | -79.77 | -80.20 | 181.21 | 182.08 |
| | Unconstrained | Unconstrained | 2 | 2 | -77.47 | -78.13 | 182.57 | 183.88 |
| | Unconstrained | Unconstrained | 3 | 3 | -77.47 | -78.13 | 185.73 | 187.05 |
| GM | *Diagonal and equal* | *Equalvarcov* | *1* | *1* | *-71.90* | *-83.17* | *152.64* | *170.11* |
| | Equalvarcov | Diagonal and equal | 1 | 1 | -71.01 | -79.49 | 153.31 | 173.09 |
| | Diagonal and unequal | Equalvarcov | 1 | 2 | -71.52 | -82.17 | 156.87 | 175.20 |
| | Diagonal and equal | Unconstrained | 2 | 1 | -71.90 | -79.50 | 157.63 | 177.15 |
| | Equalvarcov | Diagonal and unequal | 2 | 1 | -71.01 | -77.74 | 158.52 | 177.79 |
| | Equalvarcov | Equalvarcov | 3 | 3 | -71.01 | -82.08 | 161.30 | 177.85 |
| | Diagonal and unequal | Diagonal and equal | 2 | 2 | -71.52 | -79.48 | 161.31 | 177.97 |
| | Unconstrained | Diagonal and equal | 1 | 3 | -70.18 | -82.17 | 162.55 | 180.55 |
| | Diagonal and equal | Unconstrained | 3 | 2 | -70.52 | -82.17 | 164.55 | 183.18 |
| | Diagonal and unequal | Diagonal and unequal | 3 | 2 | -71.52 | -77.78 | 165.23 | 185.22 |
| | Unconstrained | Unconstrained | 2 | 3 | -69.81 | -83.17 | 168.07 | 186.26 |
| | Unconstrained | Diagonal and unequal | 3 | 3 | -71.72 | -77.74 | 176.36 | 188.20 |
| ES | *Diagonal and equal* | *Equalvarcov* | *2* | *1* | *-76.96* | *-74.62* | *161.78* | *160.39* |
| | Unconstrained | Diagonal and equal | 1 | 1 | -70.23 | -75.85 | 162.15 | 160.44 |
| | Diagonal and unequal | Unconstrained | 1 | 1 | -74.41 | -69.40 | 162.44 | 160.48 |
| | Diagonal and equal | Diagonal and unequal | 1 | 1 | -76.90 | -74.59 | 162.66 | 162.80 |
| | Equalvarcov | Equalvarcov | 2 | 2 | -73.85 | -74.62 | 163.90 | 165.45 |
| | Diagonal and equal | Diagonal and equal | 3 | 2 | -74.08 | -73.01 | 164.35 | 165.63 |
| | Diagonal and unequal | Unconstrained | 2 | 2 | -72.87 | -69.60 | 164.63 | 166.82 |
| | Equalvarcov | Diagonal and equal | 1 | 3 | -76.80 | -75.93 | 164.75 | 168.06 |
| | Equalvarcov | Equalvarcov | 3 | 3 | -73.85 | -74.62 | 166.58 | 168.13 |
| | Diagonal and unequal | Unconstrained | 3 | 3 | -72.87 | -69.60 | 167.41 | 169.99 |
| | Unconstrained | Diagonal and unequal | 2 | 2 | -70.23 | -75.79 | 168.09 | 170.46 |
| | Unconstrained | Diagonal and unequal | 3 | 3 | -70.23 | -74.59 | 171.25 | 170.85 |

Table S4.4.6. Results from dynamic factor analysis (DFA) model fitting applied to standardized abundance (AB) and biomass (BI) indices for batoids by depth strata (B: 50-100 m; C: 101-200 m; D: 201-500 m) over the period 1994-2015 in GSA01(Northern Alboran Sea) and GSA06 (Northern Spain), and 2001-2015 in GSA05 (Balearic Islands). For each model, the covariance matrix structure, the number of common trends (m), and the corrected Akaike Information Criterion (AICc) are given. Italicized models represent the models for each depth stratum that were selected as the models that produced the most favorable covariance matrix structure (with the lowest AICc values) and number of common trends.

| **Strata** | **Covariance matrix structure** | | **m** | | **Log-likelihood** | | **AICc** | |
|---|---|---|---|---|---|---|---|---|
| | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** |
| B | *Equalvarcov* | *Equalvarcov* | *1* | *1* | *-79.78* | *-79.61* | *168.21* | *164.48* |
| | Diagonal and equal | Diagonal and equal | 1 | 1 | -78.54 | -76.67 | 168.30 | 166.90 |
| | Diagonal and unequal | Equalvarcov | 1 | 2 | -79.73 | -76.61 | 173.10 | 169.42 |
| | Diagonal and equal | Diagonal and unequal | 2 | 1 | -79.74 | -79.01 | 173.17 | 171.64 |
| | Equalvarcov | Diagonal and equal | 2 | 2 | -79.77 | -79.08 | 173.27 | 171.77 |
| | Diagonal and equal | Equalvarcov | 3 | 3 | -78.54 | -76.61 | 173.75 | 172.10 |
| | Equalvarcov | Unconstrained | 3 | 1 | -79.77 | -75.61 | 175.75 | 172.91 |
| | Unconstrained | Diagonal and equal | 1 | 3 | -77.50 | -79.60 | 176.68 | 174.35 |
| | Diagonal and unequal | Diagonal and unequal | 2 | 2 | -79.74 | -79.00 | 178.37 | 176.89 |
| | Diagonal and unequal | Unconstrained | 3 | 2 | -79.74 | -75.55 | 181.16 | 178.72 |
| | Unconstrained | Diagonal and unequal | 2 | 3 | -77.40 | -79.00 | 182.42 | 179.69 |
| | Unconstrained | Unconstrained | 3 | 3 | -77.40 | -75.55 | 185.58 | 181.88 |
| C | *Diagonal and equal* | *Diagonal and equal* | *1* | *1* | *-80.49* | *-81.42* | *169.73* | *171.59* |
| | Diagonal and unequal | Equalvarcov | 1 | 1 | -78.58 | -81.40 | 170.79 | 173.95 |
| | Equalvarcov | Diagonal and unequal | 1 | 1 | -80.46 | -80.77 | 172.06 | 175.17 |
| | Unconstrained | Diagonal and equal | 1 | 2 | -75.66 | -81.36 | 173.00 | 176.74 |
| | Diagonal and equal | Unconstrained | 2 | 1 | -80.57 | -78.18 | 174.75 | 178.05 |
| | Diagonal and unequal | Equalvarcov | 2 | 2 | -78.59 | -81.40 | 176.06 | 179.01 |
| | Diagonal and equal | Diagonal and equal | 3 | 3 | -80.48 | -81.41 | 177.18 | 179.04 |
| | Equalvarcov | Diagonal and unequal | 2 | 2 | -80.51 | -80.83 | 177.22 | 180.54 |
| | Diagonal and unequal | Equalvarcov | 3 | 3 | -78.59 | -81.40 | 178.85 | 181.69 |
| | Unconstrained | Diagonal and unequal | 2 | 3 | -75.69 | -80.77 | 179.01 | 183.22 |
| | Equalvarcov | Unconstrained | 3 | 2 | -80.46 | -78.29 | 179.80 | 184.20 |
| | Unconstrained | Unconstrained | 3 | 3 | -75.69 | -78.18 | 182.17 | 187.16 |
| D | *Diagonal and equal* | *Diagonal and equal* | *1* | *1* | *-81.65* | *-82.49* | *172.04* | *173.73* |
| | Equalvarcov | Equalvarcov | 1 | 1 | -81.21 | -82.16 | 173.55 | 175.45 |
| | Diagonal and unequal | Diagonal and unequal | 1 | 1 | -80.66 | -82.11 | 174.94 | 177.84 |
| | Unconstrained | Diagonal and equal | 1 | 2 | -77.86 | -82.48 | 177.40 | 178.60 |
| | Diagonal and equal | Equalvarcov | 2 | 2 | -81.93 | -82.16 | 177.48 | 180.53 |
| | Equalvarcov | Diagonal and equal | 2 | 3 | -81.29 | -82.49 | 178.78 | 181.18 |
| | Diagonal and equal | Diagonal and unequal | 3 | 2 | -81.64 | -82.11 | 179.49 | 183.10 |
| | Diagonal and unequal | Unconstrained | 2 | 1 | -80.97 | -80.73 | 180.82 | 183.14 |
| | Equalvarcov | Equalvarcov | 3 | 3 | -81.21 | -82.16 | 181.30 | 183.20 |
| | Unconstrained | Diagonal and unequal | 2 | 3 | -78.27 | -82.09 | 184.16 | 185.86 |
| | Diagonal and unequal | Unconstrained | 3 | 2 | -81.67 | -80.75 | 185.03 | 189.12 |
| | Unconstrained | Unconstrained | 3 | 3 | -78.23 | -80.75 | 187.25 | 192.29 |

Table S4.4.7. Results from dynamic factor analysis (DFA) model fitting applied to standardized abundance (AB) and biomass (BI) indices for *Torpedo marmorata* over the period 1994-2015 in GSA01(-Northern Alboran Sea) and GSA06 (Northern Spain). For each model, the covariance matrix structure, the number of common trends (m), and the corrected Akaike Information Criterion (AICc) are given. Italicized models represent the models for each depth stratum that were selected as the models that produced the most favorable covariance matrix structure (with the lowest AICc values) and number of common trends.

| **Species** | **Covariance matrix structure** | | **m** | | **Log-likelihood** | | **AICc** | |
|---|---|---|---|---|---|---|---|---|
| | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** | **AB** | **BI** |
| *Torpedo marmorata* | *Equalvarcov* | *Diagonal and equal* | *1* | *1* | *-60.59* | *-60.98* | *126.21* | *128.56* |
| | Diagonal and equal | Diagonal and unequal | 1 | 1 | -58.59 | -60.81 | 127.79 | 130.66 |
| | Unconstrained | Equalvarcov | 1 | 1 | -58.36 | -60.87 | 128.31 | 130.78 |
| | Equalvarcov | Diagonal and equal | 2 | 2 | -58.59 | -60.97 | 128.76 | 130.99 |
| | Diagonal and unequal | Unconstrained | 1 | 1 | -60.39 | -60.74 | 129.81 | 133.06 |
| | Diagonal and equal | Diagonal and unequal | 2 | 2 | -60.59 | -60.81 | 130.22 | 133.21 |
| | Unconstrained | Equalvarcov | 2 | 2 | -58.36 | -60.87 | 131.00 | 133.33 |

Table S4.4.8. Logistic regression coefficients (α and β) of maturity ogive and length at first maturity ($L_{50}$; or length in cm TL at which 50% of specimens had become mature) for *Scyliorhinus canicula* per year and sex (F: female; M: males), and the smallest mature female (SMF; cm TL) in geographic sub-areas GSA01 (Northern Alboran Sea), GSA05 (Balearic Islands), and GSA06 (Northern Spain). n: number of samples analysed. Statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

| **GSAs** | **Years** | **n** | | **$L_{50}$** | | **α** | | **β** | | **SMF** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | |
| GSA01 | 2000 | 56 | 52 | 45.54 | 41.17 | -51.81** | -15.09** | 0.11** | 0.03*** | 44 |
| | 2001 | 64 | 62 | 44.99 | 42.14 | -38.46** | -25.52* | 0.08** | 0.06* | 43 |
| | 2002 | 205 | 332 | 45.62 | 41.20 | -27.18** | -22.22*** | 0.06** | 0.05*** | 44 |
| | 2003 | 163 | 202 | 45.99 | 41.22 | -33.45*** | -25.30*** | 0.07*** | 0.06*** | 43.5 |
| | 2004 | 93 | 104 | 45.13 | 41.60 | -22.04*** | -13.03*** | 0.04*** | 0.03*** | 41.5 |
| | 2005 | 114 | 148 | 42.59 | 41.72 | -25.12*** | -19.65*** | 0.05*** | 0.04*** | 41 |
| | 2006 | 164 | 254 | 43.92 | 41.94 | -26.85*** | -45.46*** | 0.06*** | 0.10*** | 40.5 |
| | 2007 | 240 | 292 | 43.92 | 41.11 | -30.96*** | -34.20*** | 0.07*** | 0.08*** | 41 |
| | 2008 | 125 | 151 | 42.14 | 41.90 | -25.54*** | -26.78*** | 0.06*** | 0.06*** | 39 |
| | 2009 | 104 | 146 | 42.47 | 40.63 | -23.03*** | -20.01*** | 0.05*** | 0.04*** | 38 |
| | 2010 | 51 | 60 | 42.15 | 41.28 | -27.30* | -14.87** | 0.06* | 0.03** | 38 |
| | 2011 | 63 | 61 | 42.70 | 40.13 | -31.26** | -37.24** | 0.07** | 0.09** | 39 |
| | 2012 | 87 | 101 | 41.52 | 39.72 | -25.04** | -16.20*** | 0.06** | 0.04*** | 37.5 |
| | 2013 | 149 | 138 | 42.20 | 39.30 | -22.50** | -25.10** | 0.06** | 0.06** | 38 |
| | 2014 | 98 | 97 | 40.90 | 38.95 | -34.31*** | -24.42*** | 0.08*** | 0.06*** | 38.5 |
| | 2015 | 299 | 316 | 40.61 | 39.69 | -26.92*** | -22.74*** | 0.06*** | 0.06*** | 37 |
| GSA05 | 2007 | 670 | 917 | 41.19 | 40.98 | -23.72*** | -20.54*** | 0.06*** | 0.05*** | 38 |
| | 2008 | 698 | 869 | 41.44 | 40.25 | -21.42*** | -22.52*** | 0.05*** | 0.05*** | 37.5 |
| | 2009 | 722 | 890 | 40.84 | 40.05 | -25.20*** | -43.43*** | 0.06*** | 0.10*** | 37.5 |
| | 2010 | 785 | 925 | 40.75 | 40.01 | -24.26*** | -50.30*** | 0.06*** | 0.12*** | 36.5 |
| | 2011 | 626 | 854 | 40.64 | 39.87 | -17.18*** | -19.78*** | 0.04*** | 0.02*** | 37 |
| | 2012 | 246 | 362 | 39.98 | 39.38 | -12.44*** | -11.75*** | 0.03*** | 0.03*** | 37.5 |
| | 2013 | 282 | 388 | 40.60 | 39.20 | -23.40** | -24.10** | 0.05** | 0.06** | 37 |
| | 2014 | 568 | 743 | 39.96 | 38.26 | -22.20*** | -16.87*** | 0.05*** | 0.04*** | 36.5 |
| | 2015 | 729 | 827 | 39.45 | 38.30 | -26.84*** | -13.22*** | 0.06*** | 0.03*** | 36 |
| GSA06 | 2000 | 247 | 307 | 43.37 | 41.24 | -19.85*** | -51.32*** | 0.04*** | 0.12*** | 41.5 |
| | 2001 | 402 | 296 | 44.66 | 40.83 | -29.12*** | -20.42*** | 0.06*** | 0.05*** | 41.5 |
| | 2002 | 372 | 509 | 44.59 | 41.06 | -19.30*** | -25.97*** | 0.04*** | 0.06*** | 39.5 |
| | 2003 | 363 | 370 | 44.48 | 41.12 | -15.51*** | -18.96*** | 0.03*** | 0.04*** | 39 |
| | 2004 | 254 | 366 | 43.83 | 41.22 | -19.61*** | -15.67*** | 0.04*** | 0.03*** | 39.5 |
| | 2005 | 555 | 632 | 42.66 | 41.72 | -21.03*** | -14.23*** | 0.05*** | 0.03*** | 38 |
| | 2006 | 295 | 421 | 41.77 | 41.63 | -19.97*** | -14.17** | 0.04*** | 0.03** | 39 |
| | 2007 | 412 | 463 | 42.08 | 41.64 | -22.10*** | -20.29*** | 0.05*** | 0.04*** | 38 |
| | 2008 | 267 | 346 | 42.68 | 41.73 | -29.37*** | -21.03*** | 0.06*** | 0.05*** | 39.5 |
| | 2009 | 282 | 351 | 42.10 | 41.07 | -30.84** | -20.67*** | 0.07** | 0.05*** | 39.5 |
| | 2010 | 235 | 259 | 40.98 | 39.05 | -17.81*** | -30.49*** | 0.04*** | 0.07*** | 38 |
| | 2011 | 318 | 386 | 41.86 | 40.86 | -18.28*** | -38.04*** | 0.04*** | 0.09*** | 37.5 |
| | 2012 | 442 | 476 | 41.37 | 40.30 | -34.11*** | -29.65** | 0.08*** | 0.07** | 37.5 |
| | 2013 | 393 | 433 | 40.90 | 39.60 | -26.00** | -17.90** | 0.06** | 0.04** | 36 |
| | 2014 | 330 | 382 | 39.97 | 39.19 | -28.17*** | -26.61*** | 0.07*** | 0.06*** | 37 |
| | 2015 | 534 | 539 | 40.04 | 38.74 | -21.08*** | -18.14*** | 0.05*** | 0.04*** | 36.5 |

Table S4.4.9. Logistic regression coefficients (α and β) of maturity ogive and length at first maturity ($L_{50}$; or length in cm TL at which 50% of specimens had become mature) for *Galeus melastomus* per year and sex (F: female; M: males), and the smallest mature female (SMF; cm TL) in geographic sub-areas GSA01 (Northern Alboran Sea), GSA05 (Balearic Islands) and GSA06 (Northern Spain). n: number of samples analysed. Statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

| **GSAs** | **Years** | **n** | | **$L_{50}$** | | **α** | | **β** | | **SMF** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **F** | **M** | **F** | **M** | **F** | **M** | **F** | **M** | |
| GSA01 | 2000 | 168 | 241 | 53.21 | 48.59 | -23.30*** | -18.54*** | 0.04*** | 0.03*** | 50.5 |
| | 2001 | 264 | 295 | 54.04 | 49.94 | -29.20*** | -44.63*** | 0.05*** | 0.08*** | 51 |
| | 2002 | 247 | 348 | 54.11 | 50.17 | -50.26** | -40.57*** | 0.09** | 0.08*** | 51 |
| | 2003 | 270 | 384 | 53.26 | 49.55 | -37.78*** | -44.20*** | 0.07*** | 0.08*** | 48.5 |
| | 2004 | 182 | 185 | 53.02 | 48.09 | -22.50** | -29.30*** | 0.04** | 0.06*** | 48.5 |
| | 2005 | 196 | 348 | 52.90 | 48.49 | -22.01*** | -37.20*** | 0.04*** | 0.07*** | 49 |
| | 2006 | 401 | 567 | 53.45 | 48.70 | -31.52*** | -27.45*** | 0.05*** | 0.05*** | 49.5 |
| | 2007 | 260 | 329 | 52.93 | 48.62 | -31.91*** | -46.90*** | 0.06*** | 0.09*** | 49 |
| | 2008 | 225 | 302 | 53.29 | 48.41 | -39.64*** | -27.51*** | 0.07*** | 0.05*** | 50 |
| | 2009 | 270 | 323 | 53.46 | 48.39 | -36.46*** | -42.85*** | 0.06*** | 0.08*** | 51 |
| | 2010 | 124 | 129 | 53.11 | 48.64 | -27.72*** | -27.31*** | 0.05*** | 0.05*** | 49 |
| | 2011 | 121 | 150 | 53.57 | 48.18 | -29.21** | -28.86*** | 0.05** | 0.05*** | 50 |
| | 2012 | 248 | 326 | 53.54 | 47.90 | -27.63*** | -27.19*** | 0.05** | 0.05*** | 51 |
| | 2013 | 446 | 481 | 54.10 | 47.20 | -25.90* | -35.80* | 0.05* | 0.07* | 51 |
| | 2014 | 219 | 201 | 53.38 | 48.28 | -20.29*** | -36.12*** | 0.03*** | 0.07*** | 49 |
| | 2015 | 389 | 482 | 52.94 | 47.21 | -22.91*** | -37.24*** | 0.04*** | 0.07*** | 49.5 |
| GSA05 | 2007 | 359 | 438 | 50.58 | 49.08 | -19.69*** | -29.49*** | 0.03*** | 0.06*** | 50 |
| | 2008 | 319 | 323 | 50.31 | 48.39 | -17.28** | -14.27*** | 0.03** | 0.02*** | 49 |
| | 2009 | 215 | 254 | 51.86 | 50.22 | -14.66*** | -25.57** | 0.02*** | 0.05** | 48.5 |
| | 2010 | 264 | 317 | 51.51 | 49.89 | -34.90** | -33.31*** | 0.06** | 0.06*** | 49.5 |
| | 2011 | 147 | 270 | 51.07 | 49.53 | -23.02*** | -24.21*** | 0.04*** | 0.04*** | 50.5 |
| | 2012 | 191 | 317 | 52.22 | 48.97 | -32.63* | -24.97*** | 0.06* | 0.05*** | 50 |
| | 2013 | 136 | 143 | 52.10 | 49.90 | -50.90* | -31.30* | 0.09* | 0.06* | 51 |
| | 2014 | 266 | 301 | 51.33 | 48.99 | -20.33* | -17.22** | 0.03* | 0.03** | 49.5 |
| | 2015 | 854 | 448 | 50.89 | 48.74 | -16.52*** | -23.00*** | 0.03*** | 0.04*** | 49 |
| GSA06 | 2000 | 195 | 216 | 53.81 | 50.04 | -49.54** | -34.54*** | 0.09** | 0.06*** | 51 |
| | 2001 | 394 | 413 | 53.66 | 49.77 | -19.76** | -23.37* | 0.03** | 0.04* | 49.5 |
| | 2002 | 321 | 431 | 54.14 | 49.91 | -43.09** | -37.81*** | 0.07** | 0.07*** | 51 |
| | 2003 | 145 | 145 | 53.94 | 48.81 | -22.83* | -25.55*** | 0.04* | 0.05*** | 50.5 |
| | 2004 | 262 | 271 | 53.32 | 48.89 | -45.55*** | -30.13*** | 0.08*** | 0.06*** | 51 |
| | 2005 | 319 | 286 | 52.55 | 50.00 | -29.05*** | -47.18*** | 0.05*** | 0.09*** | 50 |
| | 2006 | 346 | 431 | 51.66 | 49.12 | -23.23*** | -51.22*** | 0.04*** | 0.10*** | 49 |
| | 2007 | 424 | 463 | 52.73 | 49.00 | -30.39*** | -46.21*** | 0.05*** | 0.09*** | 49.5 |
| | 2008 | 112 | 91 | 53.05 | 48.06 | -36.84* | -23.16** | 0.06* | 0.04** | 50.5 |
| | 2009 | 141 | 150 | 52.77 | 47.95 | -29.72** | -36.20** | 0.05** | 0.07** | 49 |
| | 2010 | 86 | 115 | 53.04 | 48.41 | -22.15** | -46.46*** | 0.04** | 0.09*** | 49 |
| | 2011 | 197 | 198 | 53.37 | 49.61 | -42.21*** | -30.07*** | 0.07*** | 0.06*** | 49.5 |
| | 2012 | 366 | 385 | 52.18 | 49.22 | -37.99** | -28.34*** | 0.07** | 0.05*** | 48.5 |
| | 2013 | 210 | 214 | 52.80 | 49.30 | -34.50* | -39.70* | 0.06* | 0.08* | 47 |
| | 2014 | 192 | 213 | 51.97 | 48.70 | -32.79*** | -42.44*** | 0.06*** | 0.08*** | 48 |
| | 2015 | 229 | 263 | 51.92 | 49.91 | -31.12*** | -37.81*** | 0.05*** | 0.07*** | 48.5 |

Table S4.4.10. Logistic regression coefficients (α and β) of maturity ogive and length at first maturity ($L_{50}$; or length in cm TL at which 50% of specimens had become mature) for *Raja clavata* per year and sex (F: female; M: males), and the smallest mature female (SMF; cm TL) in the GSA05 (Balearic Islands). n: number of samples analysed. Statistical significance: (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$.

| Years | n | | $L_{50}$ | | α | | β | | SMF |
|---|---|---|---|---|---|---|---|---|---|
| | F | M | F | M | F | M | F | M | |
| 2007 | 107 | 158 | 75.15 | 68.00 | -62.63* | -21.98*** | 0.08* | 0.03*** | 73 |
| 2008 | 69 | 86 | 75.48 | 67.71 | -15.44** | -21.22** | 0.02** | 0.03** | 73.5 |
| 2009 | 94 | 83 | 77.02 | 66.99 | -39.03* | -42.05** | 0.05* | 0.06** | 74 |
| 2010 | 93 | 100 | 75.67 | 69.16 | -18.47*** | -18.46*** | 0.02*** | 0.02*** | 73.5 |
| 2011 | 93 | 74 | 77.70 | 66.29 | -30.13 | -16.27*** | 0.03** | 0.02*** | 74 |
| 2012 | 88 | 70 | 75.90 | 66.41 | -27.84* | -17.61** | 0.03* | 0.02** | 73.5 |
| 2013 | 71 | 79 | 78.10 | 68.60 | -21.30* | -13.10* | -0.03* | 0.02* | 75 |
| 2014 | 113 | 120 | 76.88 | 66.52 | -53.30* | -17.34*** | 0.06* | 0.02*** | 73.5 |
| 2015 | 82 | 73 | 76.09 | 66.32 | -24.59** | -24.08*** | 0.03** | 0.03*** | 74 |

## Supplementary material: Text

The individual somatic condition for females and males of *Scyliorhinus canicula* was estimated. This analysis used data on total weight (TW) and total length (TL) collected during the MEDITS surveys over the period 2007-2015 for the three geographic sub-areas (GSAs) of the study area (GSA01: Northern Alboran Sea; GSA05: Balearic Islands; GSA06: Northern Spain). Moreover, since somatic growth slows upon maturation, and in order to prevent possible variation, we used small-sized individuals (< 35 cm TL) for both sexes, taking into account the length at first maturity estimated in the western Mediterranean (Ramírez-Amaro et al., 2015). To do so, first, the individual TW and TL were log-transformed and then, the linear relationship between log (TW) and log (TL) was established by year. Residuals of the differences between the observed and predicted log (TW) were calculated and standardized, dividing each one by the standard deviation of their predicted values. An individual that was a lighter for its corresponding length, as predicted from the regression equation, would have a negative residual, and would be considered in poorer condition than one heavier for its corresponding length predicted from the regression equation, which would have a positive residual (Hayes and Shonkwiler, 2001). Lastly, linear regression analysis was applied to detect temporal trends during the study period for each GSA.

# 5. GENERAL DISCUSSION

Most Mediterranean demersal chondrichthyans are caught incidentally, mainly from bottom trawling, and a large portion is usually discarded (FAO, 2000; Tsagarakis *et al*., 2017). For this reason, they are rarely incorporated into national or international fishing statistics, and it is difficult to gather information about them in order to know the impact of fishing on their populations. In this sense, the present Thesis provides knowledge of population dynamics regarding western Mediterranean demersal chondrichthyans, using datasets and samples from scientific surveys, which could assist in their assessment and management. These results could be a reference point for the present status of demersal chondrichthyans in the Northern Alboran Sea (GSA01), Alboran Island (GSA02), Balearic Islands (GSA05) and Northern Spain (GSA06) – four GSAs established by the General Commission for the Mediterranean (GFCM) – and highlight the effects of depth, bottom trawl and benthic habitats on their populations in different ways: species composition, assemblages, diversity, species richness, biomass, abundance, size structure, length at first maturity, optimal depth, genetic diversity and phylogeny. While a detailed discussion of each topic can be found within each previous chapter, this general discussion aims to integrate the knowledge obtained in each chapter in four different ways, i) genetic and biological results, ii) phylogeny and ecological traits, iii) phylogeny and taxonomy, and iv) global and local richness patterns.

The comparative analysis of biological parameters, population structure, connectivity, demographic history and temporal trends of the two most abundant demersal chondrichthyans with a distinct bathymetric distribution in the study area, *Scyliorhinus canicula* and *Galeus melastomus*, reveal mismatches in two senses. On the one hand, there are discrepancies between biological parameters (Chapter I) and genetic results (Chapter II). Biological parameters play a continued role in identifying a stock structure (McBride, 2014). These parameters are particularly promising for identifying stocks because they may be directly linked to genetic process, specifically when they are subject to natural selection. The estimation of biological parameters for *S. canicula* and *G. melastomus* suggest the existence of a single population in the western Mediterranean, which is supported by genetic results, but only in the case of *G. melastomus*. The relationship between reproductive isolation, genetic structure and phenotypic diversity is not always clear, as in the case of *S. canicula*. Fishing mortality can itself select genotypes or otherwise alter vital rates in ecological time in a way that can confound the appearance of stock structure (Ames, 2004). In this way, in some study cases there are mismatches between biological parameters and genetic data that require an experimental approach to resolve them (Lankford *et al*., 2001). Even if variation in a heritable life-history trait exists, and this could confer adaptive value, measurable genetic structure may be difficult to demonstrate, because of adults staying, recent colonization events, low frequency of mixture of juvenile or larval stages, or limits to sampling methods (Thorpe, 1994; Ward, 2000). In fact, in spite of the genetic population structure detected in *S. canicula*, occasional migration events could be suggested between its populations (Chapter II). According to Rodríguez-Cabello *et al*. (2004) *S. canicula* does not have wide migration, but rather its individual movement can be up to 286 km. This migration behavior can make it difficult to define its population limits when using only biological parameters. The most successful way of focusing the problem of defining the limits of stocks is the application of a holistic approach (Begg and Waldman, 1999). This approach involves a broad spectrum of complementary techniques, such as morphometry, parasitology, genetics and biological parameters (Abaunza *et al*., 2008).

On the other hand, there are differences between the geographic sub-areas (GSAs) currently considered by the GFCM for assessment or management of fisheries, and genetic population structure for both species (Chapter II) were observed. Ignoring the congruence of spatial scales between stocks (populations) and GSAs can result in a local reduction of exploited populations (Reusch *et al*., 2005; Worm *et al*., 2006). However, integration of genetic information into fisheries models is a difficult task (Waples *et al*., 2008). An important benefit of the genetic approach is that it measures average levels of population connectivity (Ovenden *et al*., 2015). Genetic structure broadly measure the detection of genetic differences between spatially or temporally separated samples, which imply the existence of some level of demographic independence and the presence of separate stocks (Waples, 1998; Waples and Gaggiotti, 2006). Accurately identifying stock boundaries is critical to successful fisheries assessment (DeCelles and Zemeckis, 2014). In this sense, new clustering methods (e.g. Bayesian Phylogeographic and Ecological Clustering; Chapter II) have been developed that do not rely on predefined stock boundaries to frame the analysis. These flexible approaches work by grouping individuals in such a way that the most genetically cohesive groupings are identified (Ovenden *et al*., 2015; Galarza *et al*., 2009). In fact, these methods lend themselves well to combining genetic information with geographical, oceanographic, and other environmental information in order to increase the explanatory power of the analysis (Galarza *et al*., 2009).

*S. canicula* and *G. melastomus* have represented more than 85% of the total abundance and biomass of demersal chondrichthyans in the western Mediterranean (Chapters I and IV). Likewise, increasing trends in terms of abundance and biomass in both sharks has been observed in the same study area (Chapter IV). These results support the great effective population size for *S. canicula* and *G. melastomus* suggested by the high genetic diversity estimated in both species (Chapter II). Genetic diversity describes the set of genetic variants retained by a group of organisms, most commonly by species or population (Ovenden *et al*., 2015). This is required for a population to evolve in response to environmental change, habitat loss, alteration to patterns of connectivity, and even fishing impact. Consequently, if there is low genetic diversity in a population, it is likely to become extinct in response to a major environmental change (Frankham *et al*., 2010). The conservation of genetic diversity within and among populations stems from the need to i) maintain adaptability of natural populations; and ii) minimize changes in life-history traits (e.g. age and size at maturity, growth) and behavior that influence dynamics of fish populations (Kenchington *et al*., 2003). Monitoring spatial and temporal variance in genetic diversity is as valid as monitoring biological parameters (Ovenden *et al.*, 2015). In this sense, tissue sample collections should be incorporated into scientific surveys such as the MEDITS programme, in order to assess and compare genetic diversity in the future.

In particular, microsatellite analysis (Chapter II) suggests that *S. canicula* has been subjected to bottleneck events in the past, due to environmental factors (Pleistocene glacial/interglacial period). Since bottlenecks lead to a loss of genetic variation that can lower levels of individual fitness, reduced resistance to diseases, and reduced ability to respond to environmental changes (Frankham *et al.*, 1999), it can be concluded that these effects on current genetic diversity (with high values) are not reflected, suggesting a rapid post-bottleneck recovery of genetic diversity. This recovery behavior can also be observed in the recovery of this species due to the effects of overfishing in the past (Chapter IV). In fact, earlier maturity, an evolutionary response which likely promoted the present recovery of *S. canicula* populations, could be an example of its high resilience to adverse scenarios.

The batoids phylogeny study (Chapter III) highlight the relevance of integrating genetic, ecological traits (bathymetric distribution and habitat preferences) and paleography history information so as to disentangle the possible drivers of speciation and evolutionary events in Mediterranean fish fauna. Evolutionary and phylogenetic knowledge has been recognized in conservation theory, but is rarely considered in conservation and policy planning (Davies and Buckley, 2011; Pollock *et al.*, 2015). Recent approaches to identifying conservation priority combine information on species status from the IUCN with information on the evolutionary history of the species from phylogenetic trees (e.g. Moores *et al.*, 2008; May-Collado and Agnarsson, 2011; May-Collado *et al.*, 2016). This new approach provides a value measure of biodiversity, which complements estimations of species richness (May-Collado *et al.*, 2016). One general agreement of this approach is to conserve groups of species that include as much "evolutionary history" as possible (Krajewski, 1991; Linder, 1995). From this perspective, and according to the batoid phylogenetic results (Chapter III), the ancient, and monotypic (with few or no sister taxa) lineages were *Rostroraja alba*, *Raja miraletus*, *Pteromylaeus bovines*, *Myliobatis aquila* and *Torpedo marmorata*. These species provide a relatively large contribution to phylogenetic diversity of batoids. Thus, these species should be of high priority for the implementation of management measures within the framework conservation strategy.

Differences in terms of abundance, biomass, bathymetric distribution, benthic habitats and feeding behaviour are shown for *Raja* species (Chapters III and IV). These findings suggest important functional diversity within the genus *Raja*. For example, populations of two closely related species in the Balearic Islands, *Raja clavata* and *Raja radula*, show fairly stable trends in terms of abundance and biomass, with even an increment in *R. clavata* (Chapter IV). Both species have a different bathymetric distribution and benthic habitat preferences compared to other *Raja* species in the Balearic Islands. *R. radula* is closely related to shallow shelf bottoms, particularly those with a presence of *Peyssonnelia* beds, and *R. clavata* is related to deep shelf bottoms, with a presence of cronoid beds. These differences could cause low interspecific competition, and may be related to the population stability of both species. However, human impact such as habitat degradation and overfishing could increase interspecific competition and alter the evolutionary and speciation processes of batoids, resulting in loss of phylogenetic diversity. Due to the lack of available biological data, ecological trait analyses for other closely related species, such as *Raja montagui* vs. *R. polystigma* and *Leucoraja naevus* vs. *L. circularis*, were not possible to estimate in this Thesis. However, similar studies could be carried out in the future in order to know their differences in ecological parameters.

Geographical patterns of species and ecosystem level diversity are often considered in conservation assessment, but, rarely have the underlying processes per se been examined in this framework (Myers *et al*., 2000; Cowling *et al*., 2003). Spatial components of the evolutionary processes that generate and maintain biodiversity must be identified, mapped and presented in an easily understood framework prior to their incorporation into conservation plans (Vázquez and Gittleman, 1998; Crowling and Pressey, 2001). A report made for the European Commission on Sensitive and Essential Fish Habitat in the Mediterranean Sea (European Commission, 2016) established that there is a need for the identification and mapping of marine habitats crucial for the conservation of fish population in the Mediterranean. This is even more important with the implementation of the European Marine Strategy Framework Directive, which mandates incorporating potential environmental drivers and any other ecosystem knowledge into fisheries assessment and scientific advice. The results obtained in the present Thesis make it possible to determine which benthic areas could be considered to be more important in terms of evolutionary and ecological processes of Mediterranean batoids. Identification of these benthic habitats could be considered as the first step in regional conservation measures to protect and preserve Mediterranean batoid populations, where the Balearic Islands should be prioritized as a special area for conservation.

Taxonomic misidentification and the presence of cryptic species can compromise the veracity of fisheries data, ecological parameters and conservation related to research and management (Coulson *et al*., 2011; Cerutti-Pereyra *et al*., 2012). Phylogenetic results (Chapter III) in combination with species delimitation analysis evidence a mismatch between taxonomic and molecular approaches (16 *vs*. 18 species, respectively), and confirm *Dasyatis* cf. *tortonesei* as a valid species and also the presence of *Dipturus nidarosiensis* in the Mediterranean. These findings, together with the misidentifications between morphologically similar skates (mainly juvenile stages of *Raja brachyura*, *R. montagui* and *R. polystigma*), suggest that an accurate taxonomic revision is necessary for these species. Even when adequate taxonomic keys and field guides are available, it is often difficult to identify batoids in the field with confidence, due to the high plasticity of the characters or local adaptation in response to varying environmental conditions (e.g. polychromatism in *R. clavata*; Mnasri *et al*., 2009), as well as sexual dimorphism. These taxonomic issues mean that accurate identification might require examination of microanatomy or measurements of a complex combination of morphometric attributes (Cerutti-Pereyra *et al*., 2012). According to genetic results, DNA sequencing for batoid identification (Chapter III) is proved to be an excellent tool. Currently, species identification using DNA is a burgeoning scientific field and the most rapidly growing areas are where genetic tools are being taken for fisheries management (Ovenden *et al*., 2015). The next step would be the integration of the molecular approach and taxonomic description in order to obtain accurate identification.

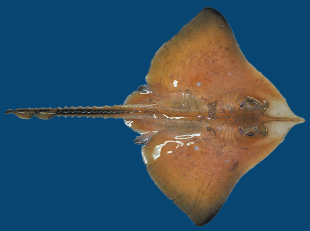
Broad-scale models have improved understanding of the global distribution of diversity, but some inconsistencies may occur on a regional scale (Witman *et al*., 2004). A problem in comparing predictions across taxa and geographic areas is the “ecologic fallacy” (Steel and Holt, 1996), whereby data that are aggregated are analysed and then the results are assumed falsely to extend to either the level of an individual or alternative aggregation (Costello *et al*., 2012). Broad-scale spatial richness patterns in Mediterranean chondrichthyans, based on general information on distribution of species, “local” checklists, and the probability threshold of species occurrence, have shown a general decrease from northwestern to southeastern regions (e.g. Coll *et al*., 2010; Dulvy *et al*., 2016; Meléndez *et al*., 2017). In particular, these richness models showed the same richness patterns throughout the Mediterranean Iberian Peninsula (GSA01 and GSA06) and the Balearic Islands (GSA05). Nonetheless, the results of this Thesis have reveal a clear difference in demersal chondrichthyan richness between the three geographic subareas (Chapter I and IV). The greaest richness was detected on the continental shelf of GSA05 and the slope of GSA01, while GSA06 showed the lowest values both for the continental shelf and slope. In this sense, biological interactions, productivity, habitat complexity, disturbance and environmental stress interact to produce variability in local species richness (Gaston, 2000; Witman *et al*., 2004). Thus, these factors must be taken into account for future global richness models. Likewise, broad-scale spatial richness patterns could be estimated using scientific surveys dataset, such as the MEDITS programme whose data are based on a standardized sampling method, which could give more realistic results.

Lastly, the results of this Thesis can be useful for implementing management measures of exploited western Mediterranean chondrichthyan populations, and for detecting future changes in their vulnerable community structure. Likewise, it highlights the usefulness of a scientific survey database (MEDITS), making it necessary to follow these monitoring programmes since can provide a critical feedback loop for learning about ecosystem dynamics, which is fundamental to adaptive management.

# 6. Conclusions

## Conclusiones

## Conclusions



## Conclusions

1. A total of 33 species of demersal chondrichthyans were identified from 3158 bottom trawl samples, collected on circalittoral and bathyal soft bottoms along the western Mediterranean, during the MEDITS scientific surveys, developed annually since 1994, throughout the four geographic sub-areas (GSAs) established by the General Fisheries Commission for the Mediterranean (GFCM): Northern Alboran Sea (GSA01) and Alboran Island (GSA02) considered jointly as GSA01, Balearic Islands (GSA05) and Northern Spain (GSA06).

2. Depth was confirmed as the main driver determining chondrichthyan communities. Two different assemblages were identified in each GSA, which correspond to the continental shelf (41-252 m) and the slope (284-813 m). The highest values of diversity, abundance and biomass were recorded on the continental shelf of GSA05 and the slope of GSA01, while GSA06 showed the lowest values of these parameters at both depth strata. This is explained by the different trawl fishing effort exerted in these areas, with the highest and lowest levels in GSA06 and GSA05, respectively, and because deep-water trawl is not well developed in GSA01. The dominance of biogenic sand and gravel bottoms in the Balearic shelf can also explain the greater abundance of rays and skates than along the Iberian Peninsula, where muddy bottoms predominate.

3. The sharks *Scyliorhinus canicula* and *Galeus melastomus* were the most abundant species on the continental shelf and slope, respectively, both representing more than 85% of total abundance and biomass. From data obtained in 2013, their length at first maturity ($L_{50}$) does not reveal any differences between GSAs, estimated at 41 cm total length (TL) for females and 39 cm for males in *S. canicula*, and 53 and 48 cm, respectively, in *G. melastomus*. By contrast, geographic differences were detected in their bathymetric distribution with similar optimum depths in GSA01 and GSA06 (250 and 242 m, respectively) for *S. canicula*, but shallower in GSA05 (163 m). For *G. melastomus* the optimum depth was greater in GSA01 (686 m), followed by GSA06 (549 m) and GSA05 (483 m). Other relatively abundant species in which it was possible to estimate $L_{50}$ were (36 cm TL for females and 29 cm for males in GSA01), and *Raja clavata* (78 cm TL for females and 69 cm for males in GSA05).

4. Contrasting patterns in population genetic structure and connectivity of *S. canicula* and *G. melastomus* were detected. *S. canicula* displayed genetic differences and low connectivity between Alboran Sea (GSA01 and GSA02) and Balearic Sea (GSA05 and GSA06), while *G. melastomus* showed high connectivity with no geographic differences in its population structure. This can be attributed to their distinct dispersal ability and bathymetric distribution, as well as their philopatric behaviour. These results suggest a mismatch between populations of these species and the GSAs established by the GFCM in the western Mediterranean, and highlight the need for better integration of genetics into the assessment and management of the fisheries developed.

5. Differences were also detected in the demographic history of both species. While *G. melastomus* showed recent population expansion, *S. canicula* showed stability at present but with signs of past bottleneck events, which could be due to climate changes. This result should be taken into account for its conservation, because they can lead to inbreeding and the appearance of mildly deleterious alleles, thereby compromising the adaptive evolutionary potential of *S. canicula*.

6. Estimation of divergence times from phylogenetic relationships between batoid species showed a parallel evolutionary history of these species and the history of the Mediterranean. Since the Cretaceous, two important speciation events were detected: a first one during the

Eocene-Oligocene transition, when almost all genera of batoids were originated; and a second one during the Miocene, when almost all current species of batoids appeared. This diversification corresponds to crucial paleo-climatic and paleo-geographical events that took place in the Mediterranean. The main drivers of speciation events of *Raja* species are their bathymetric distribution and the benthic habitats where they dwell, both ecological factors being of equal importance to physical distance and geographical barriers.

7. Molecular analysis showed the presence of 18 batoid species in the western Mediterranean and revealed morphological misidentifications based on morphological characters. *Dasyatis* cf. *tortonesei* was confirmed as a valid species, as well as the presence of *Dipturus nidarosiensis* in the Mediterranean, where its distribution area has been extended from the central Mediterranean to its western basin.

8. The feasibility of the fishery-independent scientific surveys to assess the population status of vulnerable species as chondrichthyans has been demonstrated. Although these are important by-catch species in the Mediterranean bottom trawl fishery, the lack of accurate landings data in the area have restricted their assessment.

9. The analysis of the largest database from bottom trawl surveys existing in the study area (1994-2015) showed a fairly stable demersal chondrichthyan population in terms of species richness and density (abundance and biomass) levels. Even an increment in the abundance and biomass of species dwelling on the continental shelf was observed, especially *S. canicula*, *R. clavata*, and *Torpedo marmorata*, and even for the most abundant deep water species (*G. melastomus*). In contrast, decreasing trends in some deep-water species (*E. spinax* and *Dipturus oxyrinchus*) were showed. These findings can be explained by the reduction of the trawling effort on the continental shelf over the last few decades, due to the decrease of the trawling fleet and to its displacement towards deep waters, jointly with the high resilience displayed by some of these species.

10. No trends were detected in other biological parameters (size structure and $L_{50}$) of the most abundant demersal chondrichthyans during the study period, except in *S. canicula* which showed a decreasing trend in $L_{50}$ for the three GSAs, estimated at 46-41, 41-39, and 45-40 cm TL for females, and 42-39, 41-38, and 42-39 cm TL for males, in GSA01, GSA05, and GSA06, respectively. These decreases can be explained as an evolutionary response to the general overfishing of the demersal populations exploited by the trawl fishery during the 1980s.

11. The results of the present Thesis are useful for improving the assessment of chondrichthyans in the Mediterranean, and to implement management measures that contribute to the recovery and conservation of these vulnerable species whose populations play an important role in marine ecosystems.

## Conclusiones

1. El análisis de 3158 muestras recolectadas con arte de arrastre de fondo durante la campañas MEDITS, que se realizan anualmente desde 1994, han permitido identificar un total de 33 especies de condrictios demersales en fondos circalitorales y batiales blandos del Mediterráneo Occidental, a lo largo de las cuatro sub-áreas geográficas (GSAs) establecidas por la Comisión General de Pesca para el Mediterráneo (CGPM): Norte del Mar de Alborán (GSA01) e Isla de Alborán (GSA02), considerados conjuntamente como GSA01, Islas Baleares (GSA05) y Norte de España (GSA06).

2. Se ha confirmado que la profundidad es el principal factor que determina la estructura de las comunidades de condrictios. Se han identificado dos comunidades diferentes en cada GSA, que se corresponden con la plataforma continental (31-252 m) y el talud (284-813 m). Los valores más elevados de diversidad, abundancia y biomasa se han obtenido en la plataforma continental de la GSA05 y en el talud de la GSA01, mientras que la GSA06 ha mostrado los valores más bajos de estos parámetros en ambos estratos batimétricos. Esto se explica por el esfuerzo de la pesquería de arrastre, cuyos niveles más altos y más bajos se dan en la GSA06 y GSA05, respectivamente, y por el hecho de que en la GSA01 esta pesquería no está totalmente desarrollada en aguas profundas. El predominio de fondos biogénicos de arena y grava en la plataforma balear puede explicar también la mayor abundancia de rayas, en comparación con la Península Ibérica, donde predominan los fondos fangosos.

3. Los tiburones *Scyliorhinus canicula* y *Galeus melastomus* han sido las especies más abundantes en la plataforma continental y el talud, respectivamente, con más del 85% de la abundancia y biomasa total. La talla de primera madurez ($L_{50}$) de estas especies, estimada con datos obtenidos en 2013, no ha mostrado diferencias entre GSAs, con valores de 41 cm de longitud total (LT) en hembras y 39 cm en machos de *S. canicula*, y 53 y 48 cm LT en hembras y machos de *G. melastomus*, respectivamente. Sin embargo, se han detectado diferencias geográficas en la distribución batimétrica de ambas especies. En *S. canicula* se han estimado profundidades optimas similares en la GSA01 y GSA06 (250 y 242 m, respectivamente), mientras que en la GSA05 esta profundidad ha sido menor (163 m). Para *G. melastomus*, la profundidad óptima ha sido mayor en la GSA01 (686 m), seguida de la GSA06 (549 m) y la GSA05 (483 m). Otras especies relativamente abundantes, en las que se ha podido estimar $L_{50}$ fueron *Etmopterus spinax* (36 cm LT para hembras y 29 cm para machos en la GSA01) y *Raja clavata* (78 cm LT para hembras y 69 cm para machos en la GSA05).

4. Se han detectado patrones diferentes en la estructura genética poblacional y conectividad de *S. canicula* y *G. melastomus*. La primera especie ha mostrado diferencias genéticas y baja conectividad entre el Mar de Alborán (GSA01 y GSA02) y el Mar Balear (GSA05 y GSA06), mientras que en la segunda se ha detectado una conectividad elevada, sin diferencias geográficas en su estructura poblacional. Esto se ha atribuido a capacidades de dispersión y distribución batimétrica diferentes entre ambas especies y a un comportamiento filopátrico distinto. Estos resultados sugieren una discordancia entre las poblaciones de *S. canicula* y *G. melastomus* y las GSAs establecidas por la CGPM en el Mediterráneo Occidental, y sugieren la necesidad de integrar información genética en la evaluación y gestión de pesquerías.

5. También se han detectado diferencias en la historia demográfica de estas especies. Mientras que *G. melastomus* ha mostrado una expansión demográfica reciente, la población actual de *S. canicula* es estable, pero en el pasado sufrío eventos de cuello de botella, posiblemente debido a cambios climáticos. Ello se debería tener en cuenta para la conservación de la es-

pecie, ya que estos eventos pueden provocar endogamia y el desarrollo de alelos deletéreos, comprometiendo así el potencial evolutivo adaptativo de *S. canicula*.

6. Los tiempos de divergencia estimados en las relaciones filogenéticas entre batoideos han evidenciado que estas especies tienen una historia evolutiva paralela a la del Mediterráneo. Desde el Cretácico, se han detectado dos eventos importantes de especiación: el primero durante la transición Eoceno-Oligoceno, que originó la mayoría de géneros; y el segundo durante el Mioceno, cuando aparecieron casi todas las especies actuales. Esta diversificación se corresponde con eventos paleo-climáticos y paleo-geográficos cruciales, que tuvieron lugar en el Mediterráneo. Los factores más determinantes en los procesos de especiación en el género *Raja* han sido la profundidad y los hábitats bentónicos donde se distribuyen las especies, siendo ambos factores ecológicos de igual importancia que la distancia física o las barreras geográficas.

7. El análisis molecular ha demostrado la presencia de 18 especies de batoideos en el Mediterráneo Occidental y ha evidenciado identificaciones erróneas, basadas sólo en caracteres morfológicos. Se ha confirmado *Dasyatis* cf. *tortonesei* como una especie válida, así como la presencia de *Dipturus nidarosiensis* en el Mediterráneo, donde su área de distribución se ha extendido desde la cuenta central hasta la occidental.

8. Se ha mostrado que las campañas científicas, desarrolladas independientemente de la actividad pesquera, son de gran utilidad para evaluar la situación poblacional de especies vulnerables como los condrictios. La falta de datos precisos sobre estas especies en los desembarcos pesqueros ha limitado su evaluación, a pesar de ser una parte importante de la captura accesoria de la pesca de arrastre del Mediterráneo.

9. El análisis de la base de datos más extensa, disponible a partir de campañas científicas realizadas en el área de estudio (1994-2015), ha evidenciado una población de condrictios bastante estable en lo referente a la riqueza de especies y densidad (abundancia y biomasa). Incluso se ha observado un incremento en la abundancia y biomasa de las especies que se distribuyen en la plataforma continental (*S. canicula*, *R. clavata* y *Torpedo marmorata*) y también de la especie más abundante en el talud (*G. melastomus*). Por el contrario, se han detectado tendencias decrecientes en otras especies de aguas profundas (*E. spinax* y *Dipturus oxyrinchus*). Estos resultados se explican por la reducción del esfuerzo de la pesca de arrastre en la plataforma continental durante las últimas décadas, consecuencia de la disminución del número de unidades de la flota y su desplazamiento hacia aguas profundas y por la capacidad de resiliencia elevada que muestran algunas de estas especies.

10. No se han detectado tendencias en otros parámetros biológicos (estructura de tallas y $L_{50}$) de los condrictios demersales más abundantes, excepto en *S. canicula* que ha mostrado una tendencia decreciente en su $L_{50}$, con intervalos de 46-41, 41-39 y 45-40 cm LT para las hembras y 42-39, 41-38 y 42-39 cm para los machos de la GSA01, GSA05 y GSA06, respectivamente. Esta disminución puede explicarse como una respuesta evolutiva a la sobrepesca, que desde los años ochenta ha afectado las poblaciones demersales explotadas por la pesquería de arrastre en el Mediterráneo Occidental.

11. Los resultados de esta Tesis pueden ser de utilidad para mejorar la evaluación de los condrictios del Mediterráneo y aplicar medidas de gestión que contribuyan a la recuperación y conservación de estas especies vulnerables, cuyas poblaciones juegan un papel clave en los ecosistemas marinos.

## Conclusions

1. L'anàlisi de 3158 mostres recol·lectades amb art de ròssec de fondària durant les campanyes MEDITS, que es realitzen anualment des de 1994, ha permès identificar un total de 33 espècies de condrictis demersals en fons circalitorals i batials tous de la Mediterrània Occidental, al llarg de les quatre subàrees geogràfiques (GSAs) establertes per la Comissió General de Pesca per a la Mediterrània (CGPM): Nord del Mar d'Alborán (GSA01) i Illa d'Alborán (GSA02), considerats conjuntament com GSA01, Illes Balears (GSA05) i Nord d'Espanya (GSA06).

2. S'ha confirmat que la profunditat és el principal factor que determina l'estructura de les comunitats de condrictis. S'han identificat dues comunitats diferents a cada GSA, que es corresponen amb la plataforma continental (31-252 m) i el talús (284-813 m). Els valors més elevats de diversitat, abundància i biomassa s'han obtingut a la plataforma continental de la GSA05 i en el talús de la GSA01, mentre que la GSA06 ha mostrat els valors més baixos d'aquests paràmetres en ambdós estrats batimètrics. Això s'explica per l'esforç de la pesqueria de ròssec, on els nivells més alts i més baixos es donen en la GSA06 i GSA05, respectivament, i pel fet que en la GSA01 aquesta pesqueria no està totalment desenvolupada en aigües profundes. El predomini de fons biogènics d'arena i grava a la plataforma balear, pot explicar també la major abundància de rajades, en comparació amb la Península Ibèrica, on predominen els fons fangosos.

3. Els taurons *Scyliorhinus canicula* i *Galeus melastomus* han estat les espècies més abundants en la plataforma continental i el talús, respectivament, amb més del 85% de l'abundància i biomassa total. La talla de primera maduresa ($L_{50}$) d'aquestes espècies, estimada amb dades obtingudes el 2013, no ha mostrat diferències entre GSAs, amb valors de 41 cm de longitud total (LT) en femelles i 39 cm en mascles a *S. canicula*, i 53 i 48 cm LT en femelles i mascles de *G. melastomus*, respectivament. No obstant això, s'han detectat diferències geogràfiques en la distribució batimètrica de les dues espècies. *S. canicula* ha mostrat profunditats òptimes similars a la GSA01 i GSA06 (250 i 242 m, respectivament), mentre que a la GSA05 aquesta profunditat ha estat menor (163 m). Per *G. melastomus*, la profunditat òptima ha estat més gran a la GSA01 (686 m), seguida de la GSA06 (549 m) i la GSA05 (483 m). Altres espècies relativament abundants, a les quals s'ha pogut estimar $L_{50}$ varen ser *Etmopterus spinax* (36 cm LT per a femelles i 29 cm per a mascles a la GSA01) i *Raja clavata* (78 cm LT per a femelles i 69 cm per a mascles a la GSA05).

4. S'han detectat patrons diferents en l'estructura genètica poblacional i connectivitat de *S. canicula* i *G. melastomus*. La primera espècie ha mostrat diferències genètiques i baixa connectivitat entre la Mar d'Alborán (GSA01 i GSA02) i la Mar Balear (GSA05 i GSA06), mentre en la segona s'han detectat una connectivitat elevada, sense diferències geogràfiques en la seva estructura poblacional. Aquest fet s'ha atribuït a capacitats de dispersió i distribució batimètrica diferents entre ambdues espècies i a un comportament filopàtric distint. Aquests resultats suggereixen una discordança entre les poblacions de *S. canicula* i *G. melastomus* i les GSAs establertes per la CGPM a la Mediterrània Occidental, i suggereixen la necessitat d'integrar informació genètica en l'avaluació i gestió de les pesqueries.

5. També s'han detectat diferències en la història demogràfica d'aquestes espècies. Mentre que *G. melastomus* ha mostrat una expansió demogràfica recent, la població actual de *S. canicula* és estable, però en el passat va sofrir esdeveniments de coll de botella, possiblement deguts a canvis climàtics. Aquest fet s'hauria de tenir en compte per a la seva conservació, ja que aquests esdeveniments poden provocar endogàmia i el desenvolupament d'al·lels deleteris, comprometent així el potencial evolutiu de *S. canicula*.

6. Els temps de divergència estimats en les relacions filogenètiques entre batoïdeus han evidenciat que aquestes espècies tenen una història evolutiva paral·lela a la de la Mediterrània. Des del Cretaci, s'han detectat dos esdeveniments importants d'especiació: i) el primer durant la transició Eocè-Oligocè, que va originar la majoria de gèneres; i ii) el segon durant el Miocè, moment en el qual varen aparèixer gairebé totes les espècies actuals. Aquesta diversificació es correspon amb esdeveniments paleo-climàtics i paleo-geogràfics crucials, que van tenir lloc a la Mediterrània. Els factors més determinants en els processos d'especiació en el gènere *Raja* han estat la profunditat i els hàbitats bentònics on es distribueixen les espècies, essent ambdós factors ecològics d'igual importància que la distància física o les barreres geogràfiques.

7. L'anàlisi molecular ha demostrat la presència de 18 espècies de batoïdeus a la Mediterrània Occidental i ha evidenciat identificacions errònies, basades només en caràcters morfològics. S'ha confirmat *Dasyatis* cf. com una espècie vàlida, així com la presència de *Dipturus nidarosiensis* a la Mediterrània, on la seva àrea de distribució s'ha estès des de la conca central fins l'occidental.

8. S'ha mostrat que les campanyes científiques, desenvolupades independentment de l'activitat pesquera, són de gran utilitat per avaluar la situació poblacional d'espècies vulnerables com els condrictis. La manca de dades precises sobre aquestes espècies en els desembarcaments pesquers ha limitat la seva avaluació, tot i ser una part important de la captura accessòria de la pesca de ròssec de la Mediterrània.

9. L'anàlisi de la base de dades més extensa, disponible a partir de les campanyes científiques realitzades en l'àrea d'estudi (1994-2015), ha evidenciat una població de condrictis força estable pel que fa a la riquesa d'espècies i densitat (abundància i biomassa). Fins i tot, s'ha observat un increment en l'abundància i biomassa de les espècies que es distribueixen a la plataforma continental (*S. canicula*, *R. clavata* i *Torpedo marmorata*) i també de l'espècie més abundant en el talús (*G. melastomus*). Per contra, s'han detectat tendències decreixents en altres espècies d'aigües profundes (*E. spinax* i *Dipturus oxyrinchus*). Aquests resultats poden explicar-se per la reducció de l'esforç de la pesca de ròssec en la plataforma continental durant les últimes dècades, conseqüència de la disminució del nombre d'unitats de la flota i del seu desplaçament cap a aigües profundes, a més de l'elevada capacitat de resiliència que mostren algunes d'aquestes espècies.

10. No s'han detectat tendències en altres paràmetres biològics (estructura de talles i $L_{50}$) dels condrictis demersals més abundants, excepte en *S. canicula* que ha mostrat una tendència decreixent en el seu $L_{50}$, amb intervals de 46-41, 41-39 i 45-40 cm LT per a les femelles i 42-39, 41-38 i 42-39 cm per als mascles de la GSA01, GSA05 i GSA06, respectivament. Aquesta disminució pot explicar-se com una resposta evolutiva a la sobrepesca que des dels anys vuitanta ha afectat les poblacions demersals explotades per la pesca de ròssec a la Mediterrània Occidental.

11. Els resultats d'aquesta Tesi poden ser d'utilitat per millorar l'avaluació dels condrictis de la Mediterrània i aplicar mesures de gestió que contribueixin a la recuperació i conservació d'aquestes espècies vulnerables, les poblacions de les quals juguen un paper clau en els ecosistemes marins.

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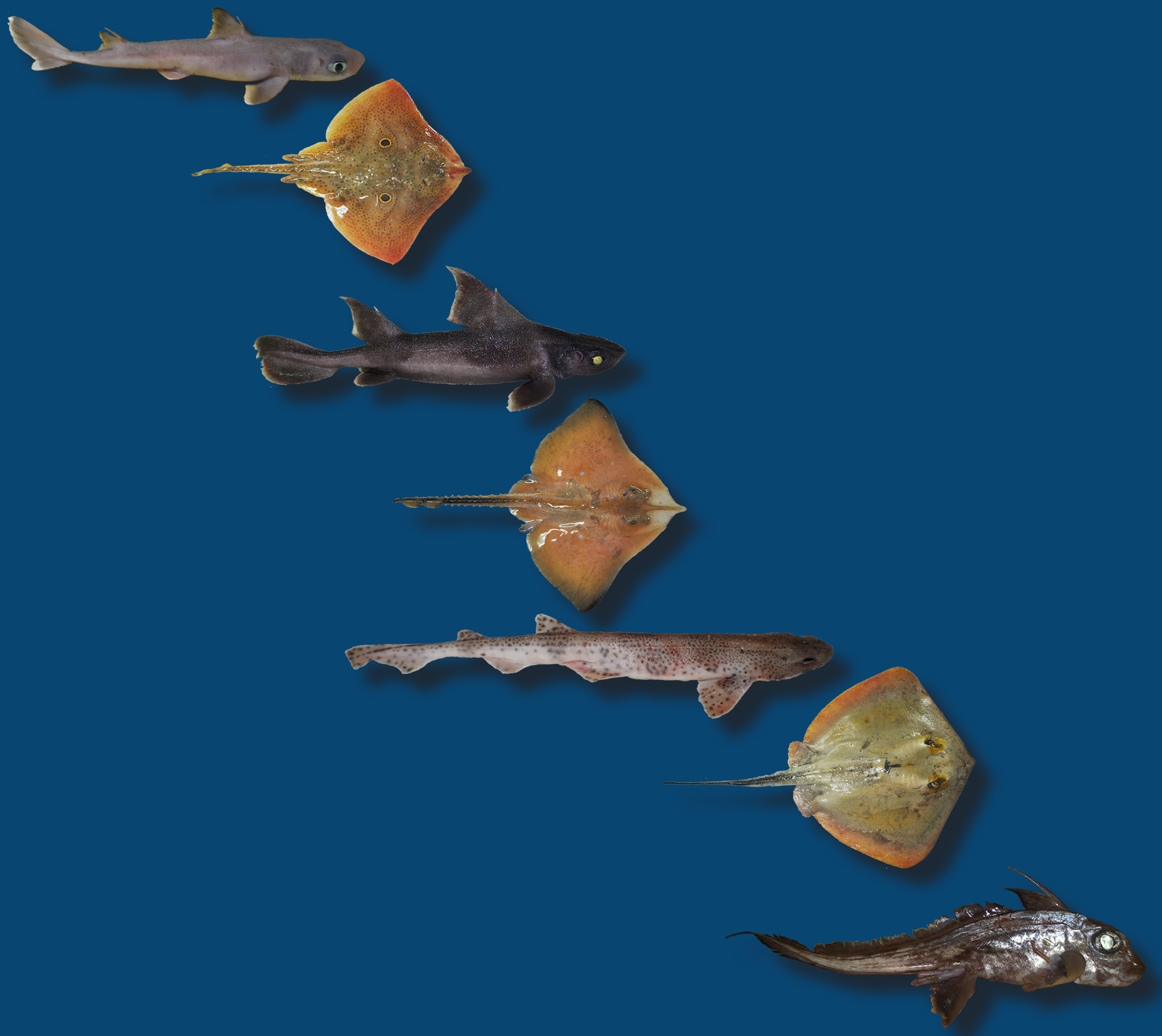

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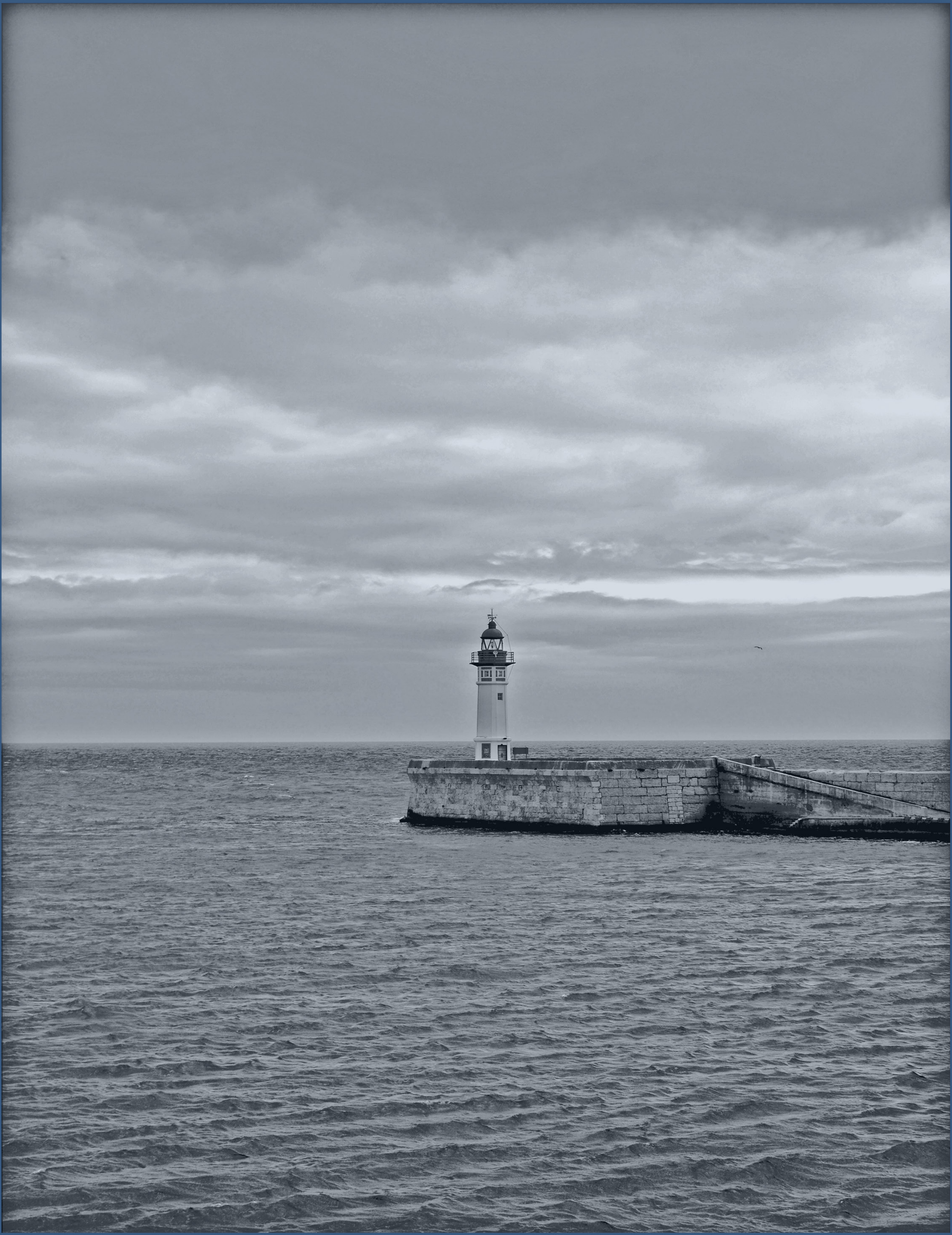

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