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Tesis Doctoral

Twenty thousand parasites under the sea: a multidisciplinary approach to parasite communities of deep-dwelling fishes from the slopes of the Balearic Sea (NW Mediterranean)

Tesis doctoral presentada por Sara Maria Dallarés Villar para optar al título de Doctora en Acuicultura bajo la dirección de la Dra. Maite Carrassón López de Letona, del Dr. Francesc Padrós Bover y de la Dra. Montserrat Solé Rovira.

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Los directores

Maite Carrassón López de Letona
Universitat Autònoma de Barcelona

Francesc Padrós Bover
Universitat Autònoma de Barcelona

Montserrat Solé Rovira
Institut de Ciències del Mar (CSIC)

La tutora

Maite Carrassón López de Letona
Universitat Autònoma de Barcelona

La doctoranda

Sara Maria Dallarés Villar

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Cover: Drawings by the author of the fish species addressed in the present thesis.

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ABSTRACT

The Mediterranean deep-sea remains mostly unexplored and, specifically, the parasite communities of Mediterranean deep-dwelling fishes are largely unknown.

Parasites are known to be effective bioindicators. Because many of them are trophically-transmitted and show complex life cycles involving more than one host, parasites can reflect host feeding habits, trophic interactions and species composition of their ecosystems. Parasites can also reveal environmental changes of anthropogenic or natural origin, and respond to environmental conditions that may influence their own biology or their hosts' population dynamics. Furthermore, the differentiation of parasite communities across geographical gradients allows their use as discrimination tags of host populations.

Besides these applications, parasites are considered stressors to their hosts and, as such, would be expected to have an impact on fish general health condition, to alter stress markers (such as some enzymatic activities) or to induce histological alterations (such as variations of splenic or hepatic melano-macrophages) and pathological conditions.

Considering the poor existing knowledge on parasite assemblages and their dynamics in the Mediterranean deep-sea, the central purpose of the present thesis is to characterize the parasite communities infecting the following important deep-dwelling ichthyc species in the Balearic Sea: *Mora moro* (Risso, 1810), *Phycis blennoides* (Brünnich, 1768), *Galeus melastomus* Rafinesque, 1810, *Scyliorhinus canicula* (Linnaeus, 1758), *Etmopterus spinax* (Linnaeus, 1758) and *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864. Another main objective is to assess the responsiveness of these parasite communities to environmental gradients and variables, to host diet and trophic ecology and their possible impact on host health condition.

In the third chapter of the present thesis, the parasite community of the common mora *M. moro* (Gadiformes: Moridae) is described in two seasons and three localities of the Balearic Sea. This teleost shows a rich and abundant parasite fauna, with 18 different endoparasite taxa found, of which 17 constituted new host records. Significant differences were detected among groups resulting from the combination of the localities and seasons sampled, in turn related to different environmental variables, for Anisakidae gen. sp., *Anisakis* Type II and Tetraphyllidea fam. gen. sp.; therefore, these parasites were proposed as potentially useful biological tags for geographical discrimination of *M. moro* in the NW Mediterranean Sea. Detailed relationships were found between parasite taxa and prey ingested (e.g. Anisakidae gen. sp. associated to meso-bathypelagic crustaceans, *Anisakis* Type I to benthopelagic squids). Most parasites were linked to samples with highest levels of near-bottom O_2 concentration, which is consistent with direct relationships found between near-bottom O_2 and zooplankton biomass in the Balearic Basin. Total parasite abundance and the abundance of Tetraphyllidea fam. gen. sp. showed significant positive relationships with acetylcholinesterase (AChE) activity, and the abundance of *Anisakis* Type II with lactate dehydrogenase (LDH) activity. Acetylcholinesterase activity was further associated with fish hepatosomatic index (HSI) and condition factor (K), and LDH activity with fish gonadosomatic index (GSI), K and total length (TL). Lactate dehydrogenase activity displayed differences among sampling groups. Splenic melano-macrophage centres and hepatic granulomas were not associated to fish parasite load. Positive relationships were found between the area of melano-macrophage centres and fish TL and LDH activity.

In the fourth chapter of the present thesis, the complete parasite community of *P. blennoides* (Gadiformes: Phycidae), at present unknown, is described in two bathymetric strata, four seasons and four localities of the Balearic Sea. A total of 20 different parasites were recovered, of which 11 constituted new host records. The most important parasites were the monogenean *Diclidophora phycidis,* the digeneans *Bathycreadium brayi* and *Lepidapedon* spp., the nematodes *Capillaria gracilis*, *Collarinema collaris*, *Cucullanus* sp. and *Hysterothylacium aduncum*, and the copepod *Clavella alata*. Overall, the parasite community of *P. blennoides* is characterized by high abundance, richness and diversity. Significant differences in the structure of the parasite community were detected between samples from < 1,000 and > 1,000 m depth and between samples from off the mainland and insular slopes. Significant seasonal and/or geographical differences were found for some specific parasites. Abundance of the nematode *C. collaris* was associated to high levels of turbidity and O_2 concentrations near the bottom. Abundances of *H. aduncum*, *D. phycidis*, *B. brayi* and *Lepidapedon* spp. were linked to high near-bottom temperature and salinity. Dietary analyses evidenced the role as potential intermediate hosts in parasite transmission by some prey (e.g. the teleost *Gaidropsarus biscayensis* for the cestode *Grillotia* cf. *erinaceus* and the nematodes *Anisakis* spp. or the euphausiid *Meganyctiphanes norvegica* for the acanthocephalan *Echinorhynchus* sp.). While the abundance of *B. brayi*, *C. collaris*, *Cucullanus* sp. and *Echinorhynchus* sp. was negatively linked to

AChE activity suggesting an inhibition of this enzyme as by infection-related stress, the abundance of *Echinorhynchus* sp. and *H. aduncum* correlated positively with lipid peroxidation levels, maybe due to oxidative stress linked to parasitism. Cysts of unknown etiology in fish gills were detected at higher prevalence than in any other fish from the same area. Number and area of hepatic macrophage centres seemed not significantly influenced by parasite infection levels.

In the fifth chapter of the present thesis, the parasite communities of *S. canicula* and *G. melastomus* (Carcharhiniformes: Scyliorhinidae) are described from the mainland slope of the Balearic sea at four seasons and two localities. For *S. canicula*, five parasites were recovered, of which the nematode *Proleptus obtusus* was the most important parasite in terms of prevalence and abundance. For *G. melastomus*, 13 parasites were recovered, of which the cestodes *Ditrachybothridium macrocephalum* and *Grillotia* sp. were the most frequent and abundant parasites. Overall, the parasite communities of both sharks were characterized by low mean richness and diversity, and high dominance. However, infracommunity structure and composition was significantly different between both species probably due to the consumption of different prey associated to their different bathymetric distributions. For *G. melastomus*, parasite infracommunity structure differed across seasons, with winter and spring samples grouping apart from summer and autumn ones, and between localities, with higher parasite burden in samples from off Besós than off Vilanova. The latter pattern is attributed to the vicinity of Besós to the Besós submarine canyon. *Ditrachybothridium macrocephalum* was more abundant in juvenile specimens of *G. melastomus* as a result of an ontogenic diet shift and *Grillotia* sp. accumulated in adult hosts, displaying higher abundance than in juveniles. The abundance of *Proleptus obtusus* was significantly higher in *S. canicula* than in *G. melastomus*, likely due to the higher consumption of reptantian decapods by the former. Monogenean parasites were associated to high turbidity and temperature levels, which are known to enhance monogenean infection and reproductive success. Cestodes of *G. melastomus* were linked to high turbidity and O_2 levels, which increase zooplankton biomass and thus favour the transmission of heteroxenous parasites

In the sixth chapter of the present thesis, the parasite communities of *G. melastomus*, *E. spinax* (Squaliformes: Etmopteridae) and *C. coelolepis* (Squaliformes: Somniosidae) are described between 400–2,200 m depth at two seasons and three localities off the mainland and insular slopes of the Balearic Sea. Environmental and fish biological, parasitological, dietary, enzymatic and histological data were obtained for each specimen, and the relationships among them tested. For *G. melastomus*, *E. spinax* and *C. coelolepis* a total of 15, two and eight parasites were respectively recovered. The parasite community of *G. melastomus* is characterized by high abundance, richness and diversity, and the cestodes *D. macrocephalum* and *Grillotia adenoplusia* dominate the infracommunities of juvenile and adult specimens, respectively. A differentiation of parasite communities, linked to a diet shift, has been observed between ontogenic stages of this species. *Etmopterus spinax* displays a depauperate parasite community and that of *C. coelolepis*, described for the first time, shows moderate richness and diversity. Detailed parasite-prey relationships have been discussed and possible transmission pathways suggested for different parasites of the three hosts. Parasites were mostly related to high water turbidity and O_2 levels, which enhance zooplankton proliferation and could thus enhance parasite transmission. The nematodes *H. aduncum* and *P. obtusus* were linked to high salinity levels, as already reported by previous studies, which are associated to high biomass and diversity of benthic and benthopelagic crustaceans. A decrease of AChE activity and lower hepatosomatic index, possibly linked to infection-related stress, have been observed. Lesions associated to encapsulated larvae of *G. adenoplusia* have been observed in the muscle of *G. melastomus*, especially in the tail region, which can be indicative of the hunting strategy of its final host and may compromise the escape response of *G. melatomus* thus facilitating parasite transmission.

In the seventh chapter of the present thesis, new morphological, molecular and ecological data on the tapeworm *D. macrocephalum* based on specimens recovered from the blackmouth catshark *G. melastomus* in the western Mediterranean, are presented and discussed. A redescription of the plerocercus of this parasite, new data on juvenile and mature worms and the first description of the eggs, based on light microscopy and SEM observations, are presented. Molecular analyses based on 28S rDNA (domains D1–D3) sequences from plerocerci, immature and adult specimens revealed that they are conspecific with the specimens previously recovered from the North East Atlantic. Despite previous authors had considered that museum specimens identified as *D. macrocephalum* may represent more than one species, examination of type- and voucher material has yielded the conclusion that no relevant morphological differences exist among them or between them and the present material. Information on infection levels of *D. macrocephalum* in a large host sample (n = 170) is provided. This parasite was more abundant in juvenile than in adult *G. melastomus* and from the middle slope with respect to the upper slope, probably related to ontogenetic and bathymetric diet shifts of its definitive host.

In the eighth chapter of the present thesis, the family Sphyriocephalidae Pintner, 1913, which comprises the genera *Hepatoxylon* Bosc, 1811, *Sphyriocephalus* Pintner, 1913 and *Heterosphyriocephalus* Palm, 2004, is revised from newly-collected and museum material. *Heterosphyriocephalus encarnae* n. sp. is described from the pelagic thresher, *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae) collected from the Pacific Ocean off Boca del Alamo, Mexico. This species can be readily distinguished from the rest of sphyriocephalids by its small size, low number of proglottids and long velum with a characteristically irregular and folded border, among other features. The tentacles show a distinctive basal armature, and a heteroacanthous typical metabasal armature with heteromorphous hooks. Redescriptions are provided for S*phyriocephalus tergestinus* and *Sphyriocephalus viridis* based on novel morphological data. Furthermore, *S. tergestinus* is allocated into *Heterosphyriocephalus* as *H. tergestinus* n. comb. based on new molecular data. New generic diagnoses are provided for *Sphyriocephalus* and *Heterosphyriocephalus*, as well as keys for the determination of genera and of species. Although the morphology of the genus *Hepatoxylon* is not addressed in the present study, the available sequence of its type-species has been incorporated in the phylogenetic analysis and its relationship to the other two genera of the family is discussed.

RESÚMEN

El Mar Mediterráneo profundo permanece en gran parte inexplorado, y, específicamente, las comunidades parásitas de los peces mediterráneos de aguas profundas son mayormente desconocidas.

Es sabido que los parásitos son efectivos bioindicadores. Debido a que muchos de ellos se transmiten por vía trófica y tienen complejos ciclos vitales que involucran a más de un hospedador, los parásitos pueden reflejar los hábitos alimentarios de sus hospedadores, interacciones tróficas y la composición de especies de sus ecosistemas. Los parásitos pueden también poner de manifiesto cambios ambientales de origen antropogénico o natural, y responder a condiciones ambientales que influyan en su propia biología o en las dinámicas poblacionales de sus hospedadores. Asimismo, la diferenciación de las comunidades de parásitos a lo largo de gradientes geográficos posibilita su uso como marcadores de discriminación de las poblaciones de hospedadores.

Al margen de estas aplicaciones, los parásitos se consideran agentes estresantes para sus hospedadores y, como tales, sería esperable que ejerzan un impacto en el estado general de salud de los peces, que alteren marcadores de estrés (como por ejemplo las actividades de algunas enzimas), o que induzcan alteraciones histológicas (como variaciones de melano-macrófagos esplénicos o hepáticos) y condiciones patológicas.

Teniendo en cuenta el poco conocimiento que se tiene de las comunidades parásitas y de sus dinámicas en el Mediterráneo profundo, el objetivo central de la presente tesis es caracterizar las comunidades parasitarias de las importantes especies ícticas de aguas profundas siguientes: *Mora moro* (Risso, 1810), *Phycis blennoides* (Brünnich, 1768), *Galeus melastomus* Rafinesque, 1810, *Scyliorhinus canicula* (Linnaeus, 1758), *Etmopterus spinax* (Linnaeus, 1758) y *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864. Otro propósito importante es evaluar la respuesta de estas comunidades a gradientes y variables ambientales, a la dieta y a la ecología trófica de los hospedadores y su posible impacto en el estado de salud de los estos últimos.

En el tercer capítulo de la presente tesis, la comunidad parasitaria de la mora común *M. moro* (Gadiformes: Moridae) se describe en dos estaciones y tres localidades del Mar Balear. Este teleósteo muestra un parasitofauna rica y abundante, con 18 endoparásitos hallados distintos, de los cuales 17 constituyen nuevas citas para este hospedador. Se detectaron diferencias significativas entre los grupos resultantes de la combinación de

las localidades y las estaciones muestreadas, asociadas a su vez a distintas variables ambientales, para Anisakidae gen. sp., *Anisakis* Tipo II y Tetraphyllidea fam. gen. sp.; por lo tanto, estos parásitos son sugeridos como marcadores biológicos útiles para la discriminación geográfica de *M. moro* en el NO del Mar Mediterráneo. Se hallaron relaciones detalladas entre taxones parásitos y presas ingeridas (e.g. Anisakidae gen. sp. Asociado a crustáceos meso-batipelágicos, *Anisakis* Tipo I a calamares bentopelágicos). La mayor parte de los parásitos se asociaron a muestras con máximos niveles de concentración de O_2 cerca del fondo, lo que es coherente con las relaciones directas halladas entre el O_2 cerca del fondo y la biomasa de zooplancton en la Cuenca Balear. La abundancia total de parásitos y la de Tetraphyllidea fam. gen. sp. mostraron relaciones positivas significativas con la actividad de la acetilcolinesterasa (ACe), y la abundancia de *Anisakis* Tipo II con la de la lactato deshidrogenasa (LDH). La actividad de la ACe estaba además asociada con el índice hepatosomático (IHS) y con el factor de condición (FC) de los peces, y la actividad de la LDH con el índice gonadosomático, el FC y la longitud total (LT) de los peces. La actividad de la LDH mostró diferencias entre grupos muestrales. Los centros melano-macrofácigos esplénicos y los granulomas hepáticos no se encontraron asociados a la carga parasitaria. Se hallaron relaciones positivas entre el área de los centros melano-macrofágicos y la LT y la actividad de la LDH de los peces.

En el cuarto capítulo de la presente tesis, la comunidad parásita completa de *P. blennoides* (Gadiformes: Phycidae), desconocida por ahora, se describe en dos estratos batimétricos, cuatro estaciones y cuatro localidades del Mar Balear. Se halló un total de 20 parásitos distintos, de los que 11 constituían nuevas citas para este hospedador. Los parásitos más importantes eran el monogeneo *Diclidophora phycidis*, los digeneos *Bathycreadium brayi* y *Lepidapedon* spp., los nematodos *Capillaria gracilis*, *Collarinema collaris*, *Cucullanus* sp. e *Hysterothylacium aduncum* y el copépodo *Clavella alata*. En conjunto, la comunidad parásita de *P. blennoides* se caracteriza por alta abundancia, riqueza y diversidad. Se detectaron diferencias significativas en la estructura de la comunidad parásita entre muestras de < 1,000 y > 1,000 m de profundidad y entre muestras procedentes de las vertientes continental e insular. Se hallaron diferencias estacionales y/o geográficas significativas para algunos parásitos concretos. La abundancia del nematodo *C. collaris* se asoció a niveles altos de turbidez y de concentraciones de O_2 cerca del fondo. Las abundancias de *H. aduncum*, *D. phycidis*, *B. brayi* y *Lepidapedon* spp. se asociaron a alta temperatura y salinidad cerca del fondo. Los análisis de dieta evidenciaron el papel como hospedadores intermediarios en la transmisión de parásitos de algunas presas (e.g. el teleósteo *Gaidropsarus biscayense* para el cestodo *Grillotia* cf. *erinaceus* y los nematodos *Anisakis* spp. o el eufausiáceo *Meganyctiphanes norvegica* para el acantocéfalo *Echinorhynchus* sp.). Mientras que las abundancias de *B. brayi*, *C. collaris*, *Cucullanus* sp. y *Echinorhynchus* sp. se relacionaron negativamente con la actividad de la ACe sugiriendo la inhibición de esta enzima por el estrés asociado a las infecciones parásitos, las abundancias de *Echinorhynchus* sp. y *H. aduncum* se relacionaron positivamente con los niveles de peroxidación de lípidos, quizás debido a estrés oxidativo asociado a parasitismo. Se detectaron quistes de etiología desconocida en las branquias de los peces, a niveles de prevalencia superiores a los mostrados por cualquier otro pez de la misma área. El número y área de los centros macrofágicos hepáticos no parecieron significativamente afectados por los niveles de infección parasitaria.

En el quinto capítulo de la presente tesis, las comunidades parásitas de *S. canicula* y *G. melastomus* (Carcharhiniformes: Scyliorhinidae) se describen en la vertiente continental del Mar Balear en cuatro estaciones y dos localidades. Para *S. canicula*, se hallaron cinco parásitos, de los cuales el nematodo *Proleptus obtusus* era el parásito más abundante en términos de prevalencia y abundancia. Para *G. melastomus*, se hallaron 13 parásitos, de los cuales los cestodos *Ditrachybothridium macrocephalum* y *Grillotia* sp. eran los parásitos más frecuentes y abundantes. En conjunto, las comunidades parasitarias de ambos tiburones se caracterizaron por baja riqueza y diversidad medias, y por una alta dominancia. Sin embargo, la estructura y la composición de las infracomunidades eran significativamente distintas entre ambas especies debido al consumo de distintas presas asociadas a sus distintas distribuciones batimétricas. Para *G. melastomus*, la estructura de las infracomunidades parásitas difería entre estaciones, con las muestras de invierno y primavera agrupadas aparte de las de verano y otoño, y entre localidades, con mayor carga parasitaria en las muestras de Besós que en las de Vilanova. Este último patrón se atribuye a la vecindad de Besós al cañón submarino del mismo nombre. *Ditrachybothridium macrocephalum* era más abundante en juveniles de *G. melastomus* como resultado de un cambio ontogénico de dieta y *Grillotia* sp. se acumulaba en los hospedadores adultos, en los que mostró mayor abundancia que en juveniles. La abundancia de *P. obtusus* era significativamente mayor en *S. canicula* que en *G. melastomus*, probablemente debido al mayor consumo de decápodos reptantia por el primero. Los parásitos monogeneos se relacionaron con altos niveles de turbidez y temperatura, que se sabe que estimulan la infección por monogeneos y su éxito reproductivo. Los cestodos de *G. melastomus* se asociaron a altos niveles de turbidez y de O_2, que aumentan la biomasa de zooplancton y favorecen por tanto la transmisión de los parásitos heteroxenos.

En el sexto capítulo de la presente tesis, las comunidades parásitas de *G. melastomus*, *E. spinax* (Squaliformes: Etmopteridae) y *C. coelolepis* (Squaliformes: Somniosidae) se describen entre 400–2,200 m de profundidad en dos estaciones y tres localidades de las vertientes continental e insular del Mar Balear. Datos ambientales y biológicos, parasitológicos, dietarios, enzimáticos e histológicos de los peces fueron obtenidos para cada espécimen, y las relaciones entre ellos fueron testadas. Para *G. melastomus*, *E. spinax* y *C. coelolepis* se hallaron un total de 15, dos y ocho parásitos, respectivamente. La comunidad parásita de *G. melastomus* se caracteriza por alta abundancia, riqueza y diversidad, y los cestodos *D. macrocephalum* y *Grillotia adenoplusia* dominan las infracomunidades de especímenes juveniles y adultos, respectivamente. Una diferenciación de las comunidades parásitas, asociada a un cambio de dieta, se observó entre estadíos ontogénicos de esta especie. *Etmopterus spinax* muestra una comunidad parásita depauperada y la de *C. coelolepis*, descrita por primera vez, muestra una riqueza y diversidad moderadas. Se han discutido relaciones detalladas entre parásitos y presas, y se han sugerido posibles vías de transmisión para distintos parásitos de los tres hospedadores. Los parásitos se relacionaron mayormente con altos niveles de turbidez y de O_2, que estimulan la proliferación del zooplancton y pueden por lo tanto promover la transmisión parasitaria. Los nematodos *H. aduncum* y *P. obtusus* se asociaron a altos niveles de salinidad, como ya había sido reportado por estudios anteriores, que a su vez de asocian a alta biomasa y diversidad de crustáceos bentónicos y bentopelágicos. Se observaron un descenso de la actividad de la ACe y un menor índice hepatosomático, posiblemente ligados a estrés asociado a las infecciones parasitarias. Se detectaron lesiones asociadas a larvas encapsuladas de *G. adenoplusia* en la musculatura de *G. melastomus*, especialmente en la región caudal, lo que podría comprometer la respuesta de huida del tiburón frente a depredadores y por tanto promover la transmisión del parásito.

En el séptimo capítulo de la presente tesis, se presentan y discuten nuevos datos morfológicos, moleculares y ecológicos del cestodo *D. macrocephalum* a partir de especímenes extraídos del tiburón bocanegra *G. melastomus* en el oeste del Mediterráneo. Se aportan una redescripción del plerocerco de este parásito, nuevos datos de parásitos juveniles y adultos y la primera descripción de los huevos, en base a observaciones por microscopía óptica y SEM. Los análisis moleculares a partir de secuencias del ARNr del 28S (dominios D1−D3) de plerocercos y especímenes juveniles y adultos han revelado que éstos son conspecíficos con especímenes previamente encontrados en el noreste del Atlántico. A pesar de que autores previos habían considerado que los especímenes de museo identificados como *D. macrocephalum* podrían pertenecer a más de una especie, el análisis de material-tipo y de vouchers ha arrojado la conclusión de que no existen diferencias morfológicas relevantes entre los individuos de ese material, ni entre ellos y los del Mediterráneo. Se aporta información acerca de los niveles de infección de *D. macrocephalum* en una muestra amplia de hospedadores (n = 170). Este parásito era más abundante en hospedadores juveniles que en adultos y en el talud medio que en el talud superior, probablemente como consecuencia de cambios ontogénicos y batimétricos en la dieta de su hospedador definitivo.

En el octavo capítulo de la presente tesis, la familia Sphyriocephalidae Pintner, 1913, que comprende los géneros *Hepatoxylon* Bosc, 1811, *Sphyriocephalus* Pintner, 1913 y *Heterosphyriocephalus* Palm, 2004, se revisa a partir de material nuevo y de museo. Se describe la especie *Heterosphyriocephalus encarnae* n. sp. del tiburón zorro *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae), recolectada en el Océano Pacífico, en Boca del Alamo, México. Esta especie se puede distinguir fácilmente del resto de miembros de la familia por su pequeño tamaño, bajo número de proglótides y largo velo con un margen plegado y característicamente irregular, entre otras características. Los tentáculos muestran una armadura basal distintiva, y una armadura metabasal heteroacanta típica con ganchos heteromorfos. Se proporcionan redescripciones para *Sphyriocephalus tergestinus* y *Sphyriocephalus viridis* a partir de nuevos datos morfológicos. Además, S. tergestinus se asigna al género *Heterosphyriocephalus* como *H. tergestinus* n. comb. en base a nuevos datos moleculares. Se proporcionan nuevas diagnosis genéricas para *Sphyriocephalus* y *Heterosphyriocephalus*, así como claves para la determinación de géneros y especies. Aunque la morfología del género *Hepatoxylon* no se trata en el presente estudio, la secuencia disponible de su especie tipo se ha incorporado al análisis filogenético y su relación con los otros dos géneros de la familia se discute.

CHAPTER 1 - INTRODUCTION

INTRODUCTION

1.1. The Mediterranean deep-sea

The Mediterranean Sea is a semi-enclosed water body communicating with the Atlantic Ocean through the Strait of Gibraltar and with particular hydrographic, physical and biological features. It is a highly dynamic system and has an active overturning circulation, with one shallow and two deep circulation cells (the latter located in each of the two main basins) (Tanhua et al., 2013).

The Mediterranean mean depth is around 2,000 m and reaches its maximum value of 5102 m in the Matapan trench, in the Ionian Sea (eastern basin) (Türkay, 2004; Sardà et al., 2009). The deep-sea, usually considered to start at the break of the continental shelf, at around 200 m depth (Thistle, 2003; Sardà et al., 2004), comprises an approximate 83% of the total Mediterranean area (Türkay, 2004).

The Mediterranean Sea is a concentration basin with a negative hydrographic balance due to high evaporation rates, and consequently, salinity levels are high (about 38 psu in the western basin and 39 psu in the eastern one (Türkay, 2004)). Since the cold Atlantic deep waters cannot surpass the Gibraltar sill, Mediterranean deeper layers originate by the sinking of surface waters and, therefore, their temperature is also unusually high (*ca.* 13 ºC vs. 1-4ºC in oceanic waters) (Klein and Roether, 2004; Türkay, 2004). In consequence, many species adapted to the temperatures of Atlantic shallow waters have expanded their vertical distribution and colonized deeper habitats in the Mediterranean (Türkay, 2004), the so-called submergence effect (Ekman, 1953). Regarding to nutrient concentrations, the Mediterranean is an oligotrophic environment, mainly attributed to the antiestuarine water circulation at the Strait of Gibraltar, where the eastward flow of Atlantic nutrient-poor surface waters is compensated by a westward countercurrent of Mediterranean nutrient-rich deep waters (Huertas et al., 2012). Moreover, the high temperatures of Mediterranean waters enhance bacterial degradation of sinking organic particles, in such a way that most of them do not reach the seafloor (Stefanescu et al., 1993; Türkay, 2004). These factors are considered to account for the lesser richness and abundance of the deep-dwelling benthic fauna in the Mediterranean with respect to the Atlantic (Pérès, 1985; Stefanescu et al., 1992a; Türkay, 2004), as well as for the smaller body size and feeding rates often reported for Mediterranean deep-sea animals (Stefanescu et al., 1992b; Carrassón and Cartes, 2002; Carrassón and Matallanas, 2002).

The main habitats found in the Mediterranean deep-sea are the continental slopes and the abyssal plains (Türkay, 2004). Traditionally, the Mediterranean continental slope has been divided into three distinct regions: the upper slope (*ca.* between 200-800 m), the middle slope (*ca.* between 800-1,400 m) and the lower slope (*ca.* below 1,400 m) (Emig, 1997). Organic matter, mainly of terrestrial origin, is easily transported down the steep slopes (especially through submarine canyons) through cascading events of dense shelf waters (Foglini et al., 2016). As a result, benthic faunal communities are better sustained in the slope than in the abyssal depths: indeed, abundance, biomass and richness of deep-sea faunal assemblages generally decrease with depth, so that the richest communities are found in the upper and middle slopes while in the abyssal plains they remain comparatively sparse and depauperate (Cartes and Sardà, 1992; Stefanescu et al., 1993; Tecchio et al., 2011).

1.1.1. The study area: the Balearic Sea

The Balearic Sea is a subdivision of the Mediterranean Sea located on its western basin and delimited by the coastline of Spain and the Balearic Islands (IHO, 1953) (Fig. 1). The maximum depth in the Balearic Sea is of approximately 2400 m (Salat and Font, 1987) and its hydrographic characteristics are fairly homogeneous (Stefanescu et al., 1992a), with almost constant temperature and salinity values below 150 m (Stefanescu et al., 1993).

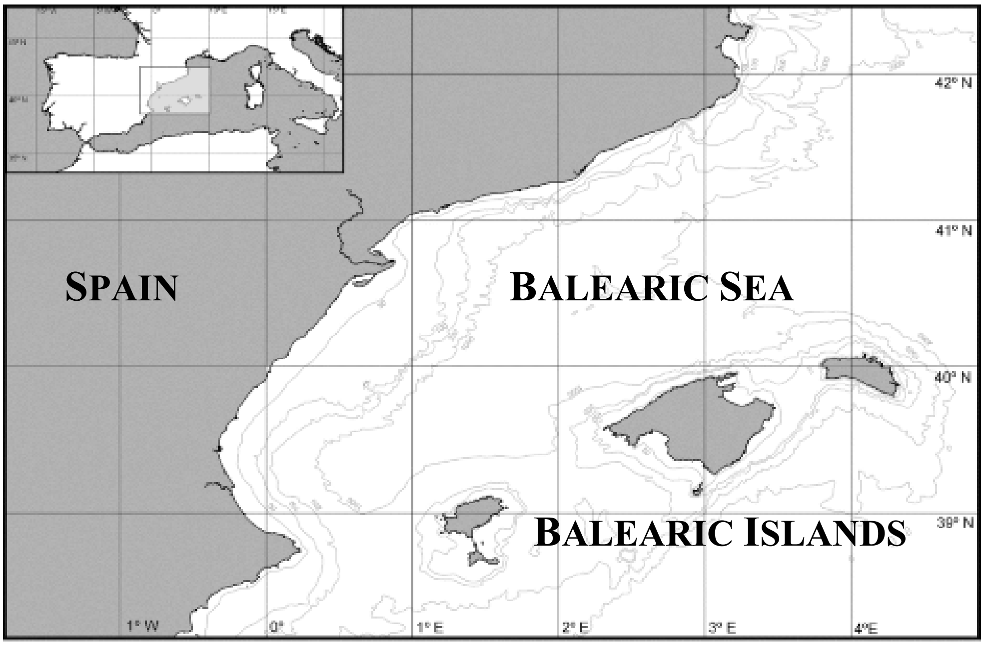

Figure 1. Map of the study area

However, different environmental and hydrographic conditions, in turn associated to different dynamics of trophic resources, are found between the mainland and the insular slopes. A complex system of submarine canyons extending from the Catalan coast crosses the mainland slope and transfers organic matter to the deep sea through cascading events (Sardá et al., 2004; Sanchez-Vidal et al., 2009; Duran et al., 2014). Water turbidity and O_2 concentration are known to favor the proliferation of invertebrate communities and are more elevated in the mainland slope (Cartes et al., 2013). As a result, enhanced biomass and faunal abundance and more complex trophic webs are found on the mainland with respect to the insular slope of the Balearic Sea (Cartes et al., 2009; Fanelli et al., 2013).

Inputs of organic material follow seasonal patterns, mainly associated to the stratification of the water column from spring to autumn, the late winter surface bloom of production and patterns of continental discharges through submarine canyons (Papiol et al., 2013, 2014; Rumolo et al., 2015). These are linked to the availability of food sources for the slope communities and determine their trophic, population and reproduction dynamics (Cartes et al., 2008; Papiol et al., 2014).

In addition, an heterogeneous depth-related nutrient distribution, alongside with different environmental conditions and fluxes of water masses, (e.g. the Levantine Intermediate water, characterized by low O_2 and high temperature and salinity levels (Aguzzi et al., 2013)), are found throughout the slopes (Puig and Palanques, 1998; Cartes et al., 2013). All these factors govern peaks of invertebrate populations (e.g. zooplankton) and are translated into important bathymetric changes regarding the composition and structure of deep-sea demersal faunal assemblages (Stefanescu et al., 1993; Cartes et al., 2004, 2009, 2013).

1.2. Importance of two large-sized gadiforms and four abundant chondrichthyans of the Mediterranean deep-sea

Among the deep-dwelling teleost species of the Balearic Sea, the common mora *Mora moro* (Risso, 1810) and the greater forkbeard *Phycis blennoides* (Brünnich, 1768) are species of large size that dominate, in terms of biomass, the ichthyofaunal assemblages found in the upper and middle slopes (Stefanescu et al., 1993; D'Onghia et al., 2004). Among the chondrichthyans inhabiting these same waters, the blackmouth catshark *Galeus melastomus* Rafinesque, 1810, the lesser spotted dogfish *Scyliorhynus canicula*

(Linnaeus, 1758), the velvet belly *Etmopterus spinax* (Linnaeus, 1758) and the Portuguese dogfish *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864 are the most important species, in terms of abundance, that can be found along the slopes (Massutí and Moranta, 2003).

1.2.1. The common mora, *Mora moro* (Risso, 1810)

Mora moro (Gadiformes: Moridae) (Fig. 2) is a worldwide-distributed species usually inhabiting depths below 1000 m in the Balearic sea, where it is one of the main contributors to biomass between 1000 and 1400 m (Stefanescu et al., 1992a; Froese and Pauly, 2016). While adults of *M. moro* live in close association with the sea-floor, juveniles have been suggested to have a pelagic existence (Stefanescu et al., 1992b). The diet of this species is mainly based on large epi- and suprabenthic prey, including crustaceans, cephalopods and fishes (Carrassón et al., 1997; Carrassón and Cartes, 2002). As suggested by its large size and high fecundity, it is a fish of high energy requirements (Gordon and Duncan, 1985), and its disappearance below 1400 m is likely a consequence of the scarcity of feeding resources in the lower slope (Carrassón et al., 1997). *Mora moro* is not a targeted species by commercial fisheries and its commercial importance is thus moderate (Froese and Pauly, 2016; IUCN, 2016).

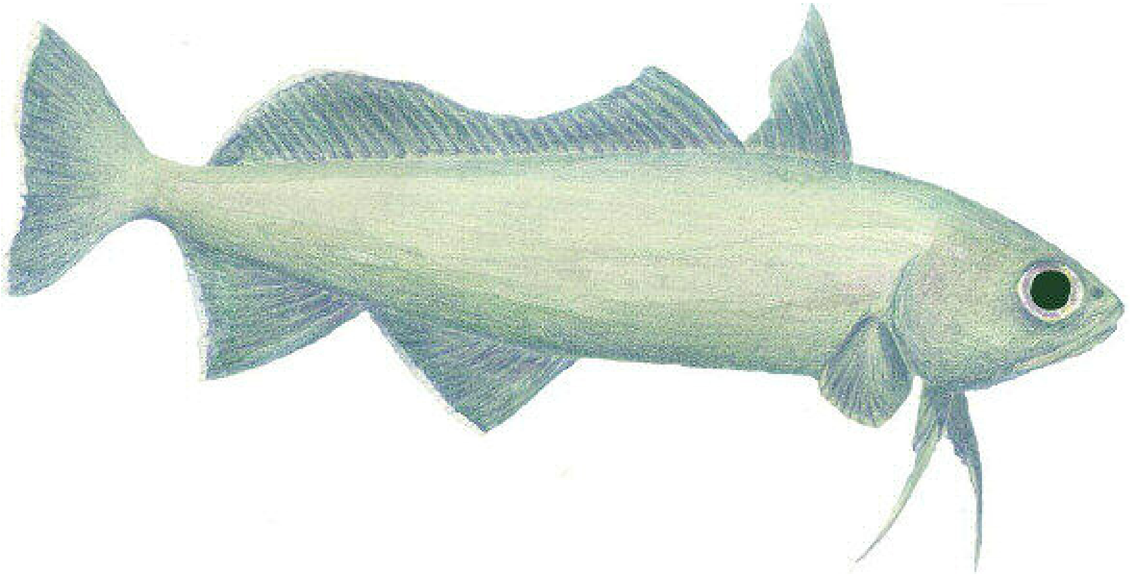

Figure 2. *Mora moro* (Risso, 1810)

1.2.2. The greater forkbeard, *Phycis blennoides* (Brünnich, 1768)

Phycis blennoides (Gadiformes: Phycidae) (Fig. 3) is a benthopelagic species distributed in the continental shelves and slopes of the northeastern Atlantic Ocean and the Mediterranean Sea (Froese and Pauly, 2016). In the Balearic Sea, it is a dominant species in the upper slope, although it has been found down to 1300 m depth (Stefanescu et al., 1992a; Cartes et al., 2004). Like *M. moro*, it is a large fish with high energetic demands (Carrassón and Cartes, 2002). Its diet is based on benthic prey, among which crustacean decapods are of major importance (MacPherson, 1978; Morte et al., 2002; Papiol et al., 2014). An ontogenic diet shift is known for this species, with young specimens feeding on small crustaceans (e.g. mysids, amphipods and isopods) and adult fishes consuming larger prey (e.g. natantia, reptantia, teleosts) (MacPherson, 1978; Morte et al., 2002). *Phycis blennoides* also shows an ontogenic migration, with juvenile specimens distributed up to 700 m depth and adults inhabiting deeper grounds (Massutí et al., 1996). This phenomenon is in all likelihood related to the different feeding habits of juvenile and adult specimens coupled with the bathymetric distribution of their respective prey (Massutí et al., 1996). It is a commercially exploited species in Atlantic and Mediterranean waters (Froese and Pauly, 2016).

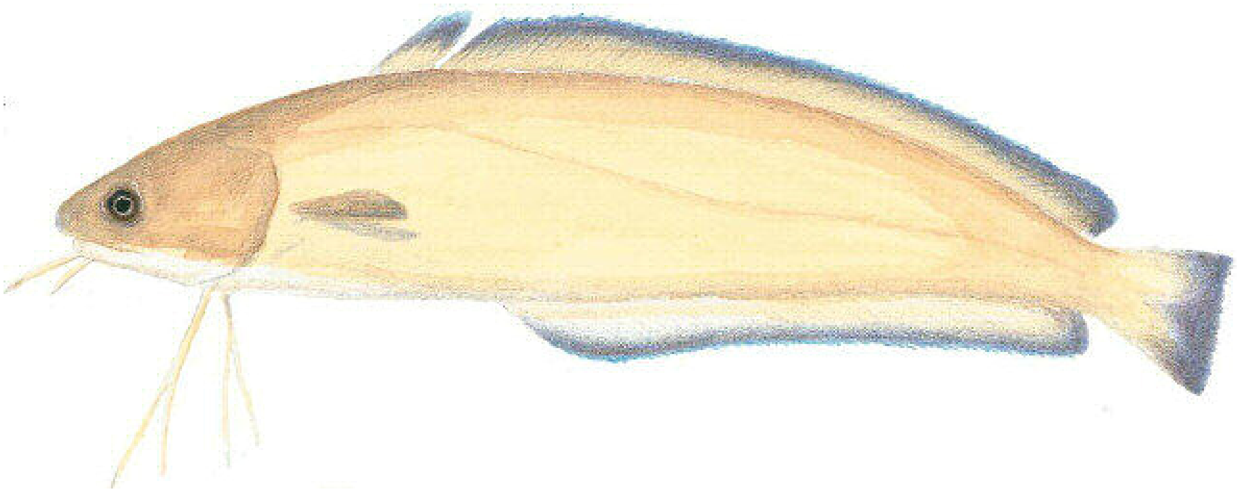

Figure 3. *Phycis blennoides* (Brünnich, 1768)

1.2.3. The blackmouth catshark, *Galeus melastomus* Rafinesque, 1810

Galeus melastomus (Carcharhiniformes: Scyliorhinidae) (Fig. 4) inhabits the outer continental shelves and the slopes of the northeastern Atlantic Ocean and the Mediterranean Sea (Froese and Pauly, 2016). It is one of the most abundant sharks in the Balearic Sea (Massutí and Moranta, 2003) and the most important one in the upper and middle slopes in terms of abundance and biomass (Carrassón et al., 1992; Massutí and Moranta, 2003; D'Onghia et al., 2004). This small demersal species consumes benthic and benthopelagic fauna, among which natantian decapods, fishes and cephalopods are the most common prey (Carrassón et al., 1992; Valls et al., 2011). A marked ontogenic diet shift has been reported, with adults selecting large-sized prey such as cephalopods, fishes and large crustaceans, and juveniles consuming mostly small crustaceans, with cephalopods and fishes being of low importance (Carrassón et al., 1992). *Galeus melastomus* also shows an ontogenic migration, with youngest specimens being most abundant above a depth of 700 m and adults inhabiting the greatest depths of its bathymetric range, at depths free of trawling activity (Carrassón et al., 1992; Massutí and Moranta, 2003; Papiol et al., 2014). It is an important by-catch in the Balearic Sea (Carbonell et al., 2003), and adult specimens are commonly traded and consumed in some areas (Erzini et al., 2001; Abella and Serena, 2005).

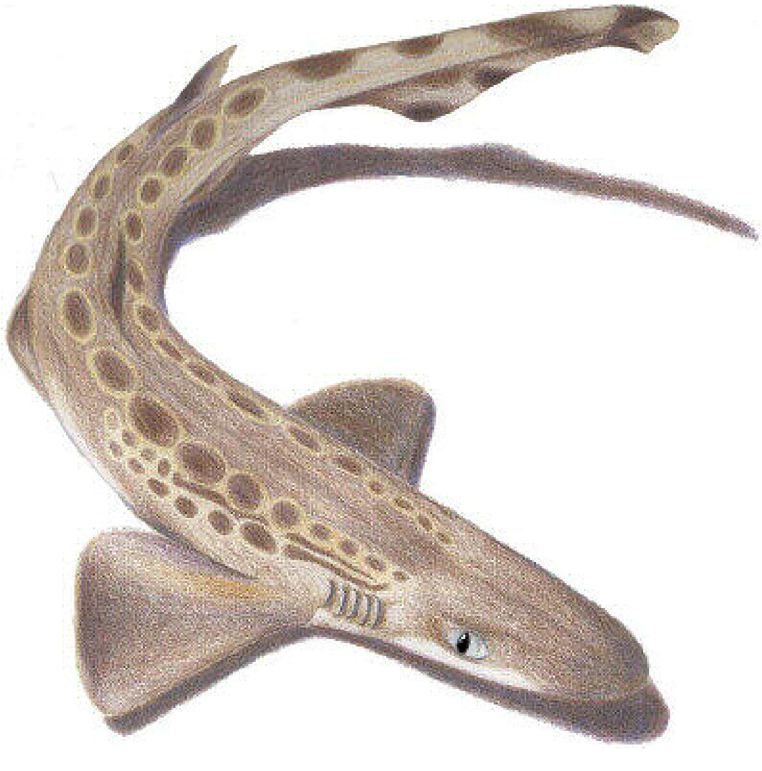

Figure 4. *Galeus melastomus* Rafinesque, 1810

1.2.4. The lesser spotted dogfish, *Scyliorhynus canicula* (Linnaeus, 1758)

Scyliorhynus canicula (Carcharhiniformes: Scyliorhinidae) (Fig. 5) is a small shark distributed in the northeastern Atlantic ocean and the Mediterranean Sea (Capapé, 1997; Froese and Pauly, 2016). Alongside with *G. melastomus*, it is one of the most common sharks in the Balearic Sea (Massutí and Moranta, 2003). This species dominates the elasmobranch fauna of the continental shelf of the Balearic Sea, showing its peak of abundance around 100 m depth, although it can be also found in the upper slope down to 500 m (Massutí and Moranta, 2003). In the northwestern Mediterranean, the diet of *S. canicula* is fairly generalist and based on crustaceans, polychaetes and teleosts (Valls et al., 2011). A more diverse diet based on larger prey as body size increases has been reported, with juveniles consuming mainly crustaceans and adults switching to a diet with a higher proportion of teleosts (Valls et al., 2011). This diet shift is coupled with a bathymetric segregation of maturity stages: while juvenile specimens inhabit deeper grounds, adults are located in shallower waters (Massutí and Moranta, 2003; Valls et al., 2011). *Scyliorhinus canicula* is a very common by-catch in the northwestern Mediterranean (Carbonell et al., 2003), although it has commercial value in some Mediterranean regions (Capapé et al., 2008).

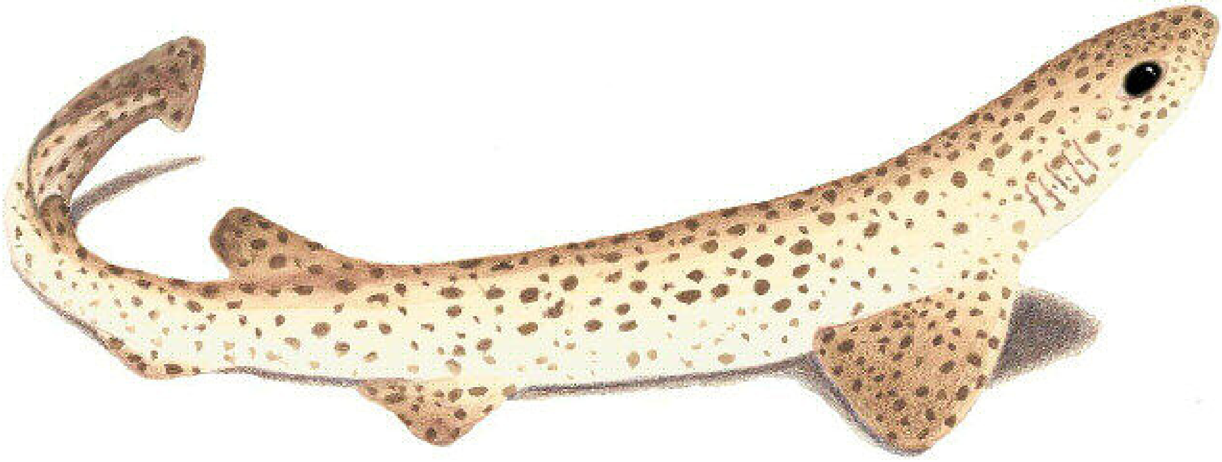

Figure 5. *Scyliorhinus canicula* (Linnaeus, 1758)

1.2.5. The velvet belly, *Etmopterus spinax* (Linnaeus, 1758)

Etmopterus spinax (Squaliformes: Etmopteridae) (Fig. 6) is distributed in the eastern Atlantic ocean and the Mediterranean Sea (Compagno et al., 2005). In the Mediterranean Sea, this small-sized shark shows its maximum peak of abundance between 800-1200 m (Stefanescu et al., 1992a; D'Onghia et al., 2004) and is one of the most abundant sharks below 1000 m (Carrassón et al., 1992). In the northwestern Mediterranean, *E. spinax* shows strongly pelagic habits and a poorly diversified diet essentially composed of cephalopods and teleosts, the former more abundant in adult specimens (Carrassón et al., 1992; Valls et al., 2011). It displays a differential bathymetric distribution according to size, with larger specimens located at deeper waters (Carrassón et al., 1992). Although it is captured as a by-catch by deep-water commercial fisheries, its commercial importance is very low (Coelho et al., 2015).

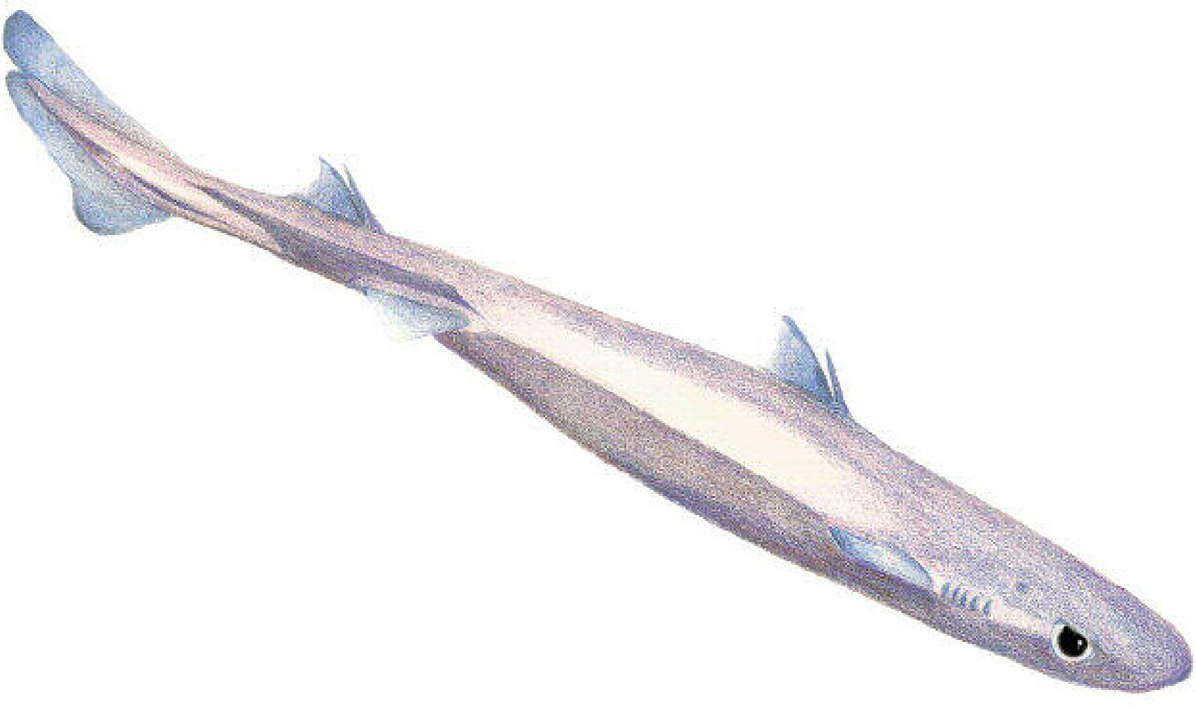

Figure 6. *Etmopterus spinax* (Linnaeus, 1758)

1.2.6. The portuguese dogfish, *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864

Centroscymnus coelolepis (Squaliformes: Somniosidae) (Fig. 7) is a bathydemersal shark inhabiting deep waters of the Atlantic, Indian and western Pacific oceans, as well as the Mediterranean Sea (Froese and Pauly, 2016). In the Mediterranean, this species inhabits deeper grounds than in the Atlantic or Pacific oceans (Carrassón et al., 1992; Bañón et al., 2006). Actually, in Mediterranean waters it is almost exclusively found on the lower slope (between *c.a.* 1500 and >2500 m depth), where it is the only abundant shark (Carrassón et al., 1992). *Centroscymnus coelolepis* has a diet characterized by poor diversity and based on nectobenthic prey (Carrassón et al., 1992; Carrassón and Cartes, 2002; Cartes et al., 2016), although moderate scavenging habits have also been reported (Carrassón et al., 1992; Cartes et al., 2016). Cephalopods are by far the preferred prey and, in a much lower proportion, decapod crustaceans and fishes are also consumed (Carrassón et al., 1992). This shark shows an important ontogenic diet shift: while cephalopods constitute practically the totality of prey in adult specimens, the diet of juveniles also includes natantian decapods in a good proportion (Carrassón et al., 1992). A possible trend in sexual and ontogenic segregation by depth has been suggested (Carrassón et al., 1992; Bañón et al., 2006), although available data are insufficient to confirm this hypothesis. Although its commercial value is generally low, in the northeast Atlantic (Portugal) it is moderately exploited and one of the most important commercial deep-dwelling sharks (Bañón et al., 2006; Froese and Pauly, 2016).

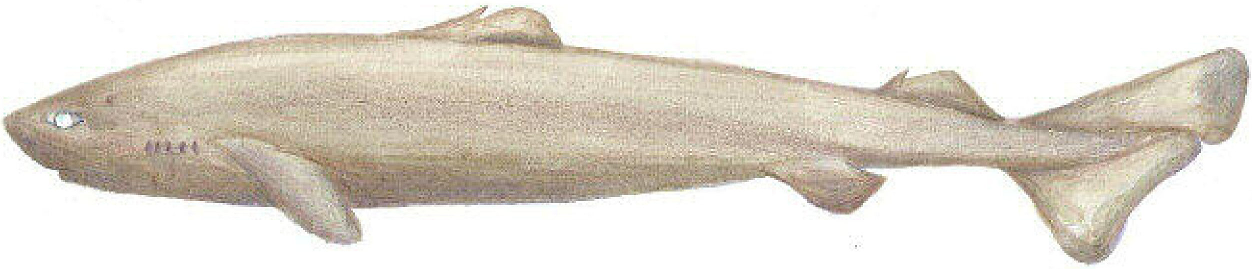

Figure 7. *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864

1.3. Parasites in the deep-sea environment

The XIX century naturalist H. N. Moseley, on returning from the Challenger expedition, considered the most important early contribution to deep-sea biology, stated that 'the unhappy deep-sea animals have not escaped their parasites in their cold and gloomy retreat' (Moseley, 1880). This observation highlighted the ubiquitous nature of parasitism in the marine environment, which at that time was not as obvious as it may seem today. Actually, parasitism is so successful that has evolved in almost every phylum of the animal kingdom, as well as in many groups of plants, and the number of parasitic species outnumbers by far that of non-parasitic ones (Roberts and Janovy, 2009).

Sampling the deep is difficult and costly; these facts coupled with the huge extension of the deep-sea environment yield a scenario in which the vast majority of it remains unexplored (Levinton, 2011). As a consequence, there is still a great lack of knowledge on the parasites infecting deep-sea hosts, for which life cycles, host relationships, distribution and zoogeography are largely unknown (Bray, 2005). The fact that fishes recovered from deep waters often evert their stomach as a result of pressure changes during their capture and transport to the surface, with the subsequent loss of gut contents (parasites among them) (Bray et al., 1999), does not help.

In spite of these difficulties, important efforts have been undertaken to sample deep-sea parasite fauna. Manter (1934) carried out the first deep-sea parasitological survey in the Atlantic, with a special focus on digeneans. Campbell et al. (1980), Houston and Haedrich (1986), Zubchenko (1981), Gartner and Zwerner (1989) also carried out extensive samplings in north-Atlantic waters. These authors outlined the general patterns followed by deep-sea parasite populations and communities for the first time, and set the stage for the more recent parasitological studies. Comprehensive parasitological studies in the southern hemisphere have been comparatively neglected. The first detailed study on deep-sea fish parasites from this area, off southeastern Australia, was provided by Heath (1989). In the Mediterranean Sea, although several early parasitological citations exist (e.g. see Brian (1912), Pintner (1913), Guiart (1935)), no extensive surveys on deep-sea parasite fauna have been performed, and, in general, parasites of deep-waters in this region have been barely addressed.

From the above mentioned and subsequent studies, some patterns followed by fish parasite diversity and abundance as encroaching into deep waters have been elucidated.

As a general rule, meso- and bathypelagic fish harbour less diversity and abundance of parasites than benthic species (Campbell et al., 1980; Klimpel et al., 2009). In turn, since abundance and diversity of the parasite fauna tends to decrease with depth and with distance from the continental slope, benthic fish inhabiting shallower waters show higher helminth diversity than those dwelling in deeper ones, (Campbell et al., 1980). Concerning deep-sea fish living on the continental slopes, species living in or close to submarine canyons accumulate more parasites and more diversity than those living at equivalent depths outside them (Campbell et al., 1980).

For heteroxenous parasites (those needing more than one host to complete their life cycle), these trends are mostly correlated with the abundance of benthic organisms acting as intermediate hosts along vertical and horizontal gradients (Campbell et al., 1980; Marcogliese, 2002). In the case of monoxenous parasites (those needing a single host to complete their life cycle), such as monogeneans, density of host populations is considered to be the most determinant factor (Campbell et al., 1980).

The Digenea is recognized as the largest group of metazoan parasites found in the deep-sea, with approximately an 11% of digenean families reported in this environment (Bray 2004, 2005). According to Klimpel et al. (2009), who carried out an extensive survey on deep-sea metazoan fish parasites reported from all around the globe, approximately 39% of deep-sea parasites belong to Digenea, 17% to Crustacea, 14% to Cestoda, 9% to Monogenea, 6% to Nematoda, 5% to Acanthocephala and 1% to Hirudinea, with an average of 1.5 metazoan parasites per individual host. These authors also reported low host-specificity in many deep-sea parasites, attributed to the generalist feeding habits of many deep-sea fish and the comparative scarcity of adequate intermediate hosts with respect to shallower waters (Klimpel et al., 2009).

1.4. Parasites as indicators of fish biology

Parasites are widely used as effective biological indicators of different aspects of fish biology. Hosts feeding habits and trophic relationships, phylogeny, ontogenic shifts, population segregation, migratory patterns, exposition to pollutants and even ecosystem alterations can all be inferred by the study of their parasite fauna (Williams et al., 1992). Many trophically-transmitted parasites have complex life cycles involving two or more hosts, and if at least part of these hosts is known, parasites allow elucidating trophic interactions among them (Marcogliese, 2005). Inferring dietary habits of a given host species by identifying individual parasites for which its previous hosts are known is a common approach (Bertrand et al., 2008). This is particularly useful when these components are unrecognizable or difficult to identify in gut contents (i.e. soft preys, such as gelatinous plankton). In a similar way, the identification of larval forms for which the subsequent or final hosts are known allows deducing which species prey onto the studied host (Valtonen et al., 2010). Host diet shifts are easily detected through parasitological studies, since the consumption of different intermediate hosts is corresponded with changes in the composition of the parasite assemblage (Münster et al., 2015). From a wider perspective, the structure of local food webs and the position that the studied host occupies within them can also be addressed (Marcogliese, 2005 and references therein; Valtonen et al., 2010; Culurgioni et al., 2015). In comparison with the analysis of stomach contents, which provides a snapshot of the recent diet composition, parasites can be used to make inferences about long-term feeding habits (Marcogliese, 2005; Knudsen et al., 2010). Actually, the combination of both approaches (short and long-term feeding habits inferred by stomach contents and parasites, respectively) is recommended to improve resolution in dietary and food web studies (Valtonen et al., 2010; Locke et al., 2013; Isbert et al., 2015)

The study of parasites can be also applied to reveal similarities among groups of hosts. For instance, host species phylogenetically closer tend to share more parasite species than unrelated species (Seifertová et al., 2008; Locke et al., 2013). Therefore, some light can be shed on dubious taxonomic relationships by the accurate determination of the parasites infecting the host species under consideration (Mateu et al., 2014). Ontogenic changes occurring throughout the lifespan of the hosts are also reflected by their parasite fauna in such a way that the latter changes as a function of host age (Timi et al., 2010a). As exposed above, ontogenic diet shifts, which modify the host exposure to parasites, are especially well reflected by parasite assemblages (Münster et al., 2015). Ecological convergence of groups of hosts (e.g. similar habitat use or same trophic level) seems to be an important predictor of parasite community resemblance as well (Poulin, 2010; Locke et al., 2013), in a similar way as geographical proximity (Locke et al., 2012). Conversely, similarity among parasite communities is expected to decay with geographical distance (Timi et al., 2010b). Actually, decreasing similarity among parasite assemblages should occur along any dimension that implies some sort of separation among them.

If baselines are set for the composition and structure of the parasite assemblage present in a given host, it is possible to detect alterations of such baselines as a consequence of environmental disturbances that have affected food chains and local food webs (Pérez-del-Olmo et al., 2007, 2009; Vidal-Martínez et al., 2010). Different studies have proved that effects of environmental impacts are aggravated for parasites with respect to their hosts, and that re-establishment of baseline levels takes longer for parasites too (Koch, 2004; Rohde, 2005). Therefore, parasite assemblages provide greater resolution than host assemblages when reflecting environmental conditions that may be negatively affecting the host populations.

At the individual level, parasites effectively serve as accumulation indicators (sentinels) of specific contaminants present in the environment (Nachev and Sures, 2016). Accumulation indicators are organisms able to concentrate in their tissues certain substances present in the environment, to significantly higher levels (Beeby, 2001). Some intestinal helminths, out of which acanthocephalans and tapeworms appear as the most promising ones, are able to concentrate metals in their tissues at concentrations several orders of magnitude higher that those present in the environment, and much more effectively than the tissues of their hosts (Sures and Reimann, 2003). This means that parasites easily allow the detection of some pollutants present in the environment in small concentrations, even below the detection limits for available techniques, which may not be revealed by analyzing the environment or host tissues. Parasites can even be more efficient in accumulating pollutants than established free-living sentinels, such as mussels (Sures et al., 1999; Sures and Reimann, 2003). In a similar way as described for environmental disturbances but on a different scale, parasites again magnify negative environmental conditions thus becoming more reliable as bioindicators than their own hosts.

One of the most important applications of establishing dissimilarities among parasite assemblages is discriminating distinct stocks of hosts. Different authors have discussed the criteria for determining whether a parasite can be considered a good tag of host populations (Kabata, 1963; Sindermann, 1983; MacKenzie, 1987; Williams et al., 1992; MacKenzie and Abaunza, 1998). The basic principle in which this procedure is based is that a fish becomes infected with a specific parasite when it enters the endemic region of that parasite (MacKenzie and Abaunza, 1998; MacKenzie, 2005). Therefore, fish populations living in different areas can be characterized on the basis of the parasite species that are infecting them, and that are associated to each area (Bertrand et al., 2008; Marcogliese and Jacobson, 2015; Mateu et al., 2014). Additionally, it can be inferred that a fish has been within the endemic area of a given parasite at some moment in the past when it is found infected by such parasite outside that endemic area, which is the base of studies on host migration patterns (MacKenzie, 2005; Mele et al., 2016).

In some of the applications described above, parasites are sometimes segregated into different functional groups that are analyzed separately since they provide different kinds of information. For instance, when using parasites as biological tags to investigate the population structure of their hosts, parasite taxa can be classified either as temporary or permanent, depending on their life spans in the studied host (MacKenzie, 2005 and references therein). The history of the contrasted host populations can be different depending on the time scale of the parasites considered: temporary parasites inform on the recent history, and permanent parasites inform on the long-term history of their respective hosts (Lester, 1990). In a similar way, in studies dealing with environmental impacts on parasite assemblages a separation is often established between monoxenous parasites (those whose life cycle involve a single host, generally exoparasites) and heteroxenous parasites (those whose life cycle involves two or more hosts, generally endoparasites). Exoparasities are more resistant due to their continuous exposition to external conditions and their levels are expected to increase in a stressed environment, meanwhile endoparasites show higher susceptibility given their dependence on different intermediate hosts and the vulnerability of their free-swimming larval stages (Diamant et al., 1999; MacKenzie, 1999; Pérez-del-Olmo et al., 2007).

1.5. The nature of host-parasite interactions

Because of the intrinsic nature of parasitism, in which the parasite lives at the expense of its host and in close association with it, harm to the host and benefit to the parasite are always implicit (Esch and Fernández, 1993; Roberts and Janovy, 2009). However, harm is not easy to quantify and it is a rather relative term (Esch and Fernández, 1993). While harm by the parasite is sometimes evident (e.g. reduced fecundity of trematode-infected snails), in many other cases damage is undetectable (Goater et al., 2014).

The relationships between parasites and their hosts are quite intimate and a certain degree of metabolic dependence of the parasite on the host always exists (Roberts and Janovy, 2009). Therefore, parasites use host resources to support their own development and reproduction and can thus reduce host fitness, either directly by stealing host food or energy reserves, or indirectly via the reallocation of limited host resources to defense and/or damage protection (Roberts and Janovy, 2009). The harm induced by the parasite can range from digging a hole in the skin of its host to stimulating an immune response, altering its behavior or stealing its food resources (Roberts and Janovy, 2009). Parasites are usually much smaller than their hosts and a single parasite may provoke a negligible impact. However, a host infected by a large number of individuals of this same parasite can undergo serious illness or even die. Similarly, a low parasite burden may have little impact on a healthy host, but the same burden infecting an unhealthy, starving host can have dramatic effects (Gunn and Pitt, 2012). Moreover, an enormous variation exists between species of parasites on the effects they may have on their hosts, since these effects depend not only on the overall parasite burden, but also on particularities of the parasite itself (e.g. specific kind of pathogenic action or site-specificity and related damage) and on host susceptibility (Goater et al., 2014).

The effects of parasite infection on the survival and fecundity of individual hosts are reflected at the population and the community levels. As long as parasites affect the survival and fecundity of individual hosts, they have the potential to reduce the size of their host populations (Goater et al., 2014). Anderson and May (1978) showed that this potential acts regulating hosts populations in a density-dependent way. As an example, the myxozoan *Myxobolus cerebralis* or the sea louse *Lepeophtheirus salmonis* can heavily reduce populations of infected salmonid fishes (Hoffman, 2011; Bateman et al., 2016).

At a higher scale, the effects of parasites on hosts' populations are translated into impacts on host communities and ecosystems. These impacts can be of crucial importance if the affected hosts are keystone species with vital ecological roles in the ecosystem. In such a case, parasites act as 'ecosystem engineers' due to their effects on biodiversity and their influence on the community and ecosystem structure (Goater et al., 2014).

CHAPTER 2 - OBJECTIVES

OBJECTIVES

Considering the poor existing knowledge on parasite assemblages and dynamics in the Mediterranean deep-sea, the central purpose of the present thesis is to characterize the parasite communities infecting important deep-dwelling ichthyc species of the Balearic Sea. Another main objective is to assess the responsiveness of these parasite communities to environmental gradients and variables, to host diet and trophic ecology and their possible impact on host health condition.

In order to fulfill these general goals, the following specific objectives are established:

1) To provide accurate descriptions of the parasite communities infecting the following deep-sea fishes in the Balearic Sea: *M. moro*, *P. blennoides*, *G. melastomus*, *S. canicula*, *E. spinax* and *C. coelolepis*.
2) To investigate whether variations in the structure and composition of such parasite communities and in the prevalence and abundance of individual parasites exist across temporal (i.e. different seasons), geographical (i.e. mainland vs. insular slopes and different sites within each) and bathymetric gradients.
3) To analyse the diet of the hosts addressed and elucidate how hosts feeding patterns influence the general structure and composition of parasite communities throughout the environmental gradients addressed and between host ontogenic stages.
4) To establish detailed parasite-prey relationships to reveal possible transmission pathways for individual parasites.
5) To test associations between individual parasite loads and environmental variables (i.e. temperature, salinity, O_2 concentration and turbidity levels of near-bottom water masses) to elucidate their influence on parasite abundance.
6) To determine activity levels of enzymes known to respond to different biological and stressing conditions (i.e. acetylcholinesterase, lactate dehydrogenase, citrate synthase) and lipid peroxidation levels in muscle of the hosts addressed and to relate them to parasite loads.
7) To analyse the relationship between general fish condition indices (i.e. condition factor, hepatosomatic and gonadosomatic indices) indicative of fish health status and their parasite burden.

8) To quantify the size and density of melano-macrophages found in spleen (in the case of teleosts) or liver (for elasmobranch species) sections, either isolated or forming centres, and to assess their variations as a function of parasite burden.
9) To carry out histopathological analyses of different organs (i.e. gills, liver, spleen, gonads and intestine) in order to detect microparasites, parasite-induced alterations and/or pathological conditions.
10) To address morphological, taxonomical or biological aspects of individual parasite species needing revision or additional studies that may arise during the study of the different parasite communities.

CHAPTER 3 - PARASITES OF THE DEEP-SEA FISH *MORA MORO* (RISSO, 1810) FROM THE NW MEDITERRANEAN SEA AND RELATIONSHIP WITH FISH DIET AND ENZYMATIC BIOMARKERS

CHAPTER 4 - THE PARASITE COMMUNITY OF *PHYCIS BLENNOIDES* (BRÜNNICH, 1768) FROM THE BALEARIC SEA IN RELATION TO DIET, BIOCHEMICAL MARKERS, HISTOPATHOLOGY AND ENVIRONMENTAL VARIABLES

CHAPTER 5 - PARASITE COMMUNITIES OF *SCYLIORHINUS CANICULA* (L., 1758) AND *GALEUS MELASTOMUS* RAFINESQUE, 1810 (ELASMOBRANCHII) FROM THE NW MEDITERRANEAN SEA, ASSESSING THE INFLUENCE OF SEASONALITY AND ENVIRONMENTAL VARIABLES

Parasite communities of *Scyliorhinus canicula* (L., 1758) and *Galeus melastomus* Rafinesque, 1810 (Elasmobranchii) from the NW Mediterranean Sea, assessing the influence of seasonality and environmental variables

Sara Dallarés[a], Ana Pérez-del-Olmo[a,b], Francisco E. Montero[b], Maite Carrassón[a*]

[a] Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Cerdanyola, 08193 Barcelona, Spain
[b] Unidad de Zoología Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Parc Científic, Universitat de València, PO Box 22085, Valencia 46071, Spain

*Corresponding author: E-mail address: Maite.Carrasson@uab.cat Tel.: +34 935814637

Sara Dallarés and Ana Pérez-del-Olmo contributed equally towards this study

Short running title: Parasite communities of *Scyliorhinus canicula* and *Galeus melastomus* from the NW Mediterranean Sea

SUMMARY

The parasite communities of *Scyliorhinus canicula* and *Galeus melastomus* are described for the first time in the NW Mediterranean. Their seasonal and geographical variations, as well as their relationship with environmental and fish biological data were tested. Overall, the parasite communities of both sharks were characterized by low mean richness and diversity, and high dominance. However, infracommunity structure and composition was significantly different between both species probably due to the consumption of different prey associated to their different bathymetric distributions. For *G. melastomus*, parasite infracommunity structure and the abundance of some parasites differed across seasons and/or localities as a result of different dynamics of the populations of intermediate hosts in turn linked to different environmental conditions. *Ditrachybothridium macrocephalum* was more abundant in juvenile specimens of *G. melastomus* as a result of ontogenic diet shifts and *Grillotia* sp. accumulated in adult hosts, displaying higher abundance than in juveniles. The abundance of *Proleptus obtusus* was significantly higher in *S. canicula* than in *G. melastomus*, likely due to the higher consumption of reptantian decapods by the former. Monogenean parasites of *S. canicula* both hosts were associated to high turbidity and temperature levels, which are known to enhance monogenean infection and reproductive success. Cestodes of *G. melastomus* were linked to high turbidity and O_2 levels, which increase zooplankton biomass and thus favour the transmission of heteroxenous parasites

Keywords: *Scyliorhinus canicula*, *Galeus melastomus*, Parasites, Communities, Seasonality, Mediterranean

INTRODUCTION

Elasmobranchs are of great relevance for the human being from an ecological, commercial, economic, conservationist and cultural point of view. Sharks and their relatives play a significant role as top predators in marine food webs, for which they are key components in their habitats, where they regulate trophic interactions and community composition (Stevens, 2000; Ruiz et al. 2016). Unfortunately, they are a main target of the fishing industry: the global market of shark products (which includes meat, fins, and other shark-derived commercial goods, such as liver oil, cartilage or skin) declares nearly 1 billion USD per year (Dent and Clarke, 2015). Such a high commercial importance has led to the overexploitation of these interesting animals, raising concerns about their vulnerability, conservation status and population decline (Stevens, 2000; Gross, 2014; Dent and Clarke, 2015). Of the approximately one thousand elasmobranch species included in the IUCN Red List of threatened species, nearly 20% hold one of the three endangered categories (IUCN, 2016), and approximately 44% are categorized as “data deficient”, which suggests that the real percentage of threatened species of this group can be actually higher. Among the factors that make elasmobranchs particularly vulnerable to population mining, their life-cycle strategy, characterized by slow growth, late sexual maturity and low fecundity, is of major relevance (Stevens, 2000). Sharks are, additionally, objects of great social interest and cultural significance, largely influenced by movies and the popular literature (Carrier et al. 2004).

However, in spite of their relevancy and justified research interest, there is still little knowledge on many aspects of sharks biology and ecology. A good example is the composition and dynamics of shark parasite communities, which have largely been neglected. Despite an increasing effort during the last years in describing and studying shark parasites, most publications either consist in taxonomic or phylogenetic studies (e.g. Caira et al. 2013, 2014; Dallarés et al. 2017) or deal with specific parasite groups, such as cestodes, or with the parasites found in a particular microhabitat, such as the spiral valve (e.g. Alarcos et al. 2006; Randhawa, 2012; Gračan et al. 2016).

While a large number of publications deal with teleost parasite communities, these have been barely addressed for shark species (the following list covers virtually all the existing literature on shark parasite communities: Henderson and Dunne, 1998; Moore, 2001; Henderson et al. 2002; Klimpel et al. 2003; Chambers, 2008; Isbert et al. 2015). In addition, these studies have been conducted in Northeastern Atlantic waters in almost all cases, and are completely absent from the Mediterranean Sea.

The spotted dogfish *Scyliorhinus canicula* (L., 1758) (Carcharhiniformes: Scyliorhinidae) and the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 (Carcharhiniformes: Scyliorhinidae) are the two most abundant sharks in the Balearic Sea (NW Mediterranean) (Massutí and Moranta, 2003). These small demersal sharks are an important by-catch in northwestern Mediterranean waters (Carbonell et al. 2003) and both have shown to be negatively affected by trawling activity (Carbonell et al. 2003; Dimech et al. 2012). Furthermore, *S. canicula* has been suggested as an indicator of fishing pressure in the surroundings of Majorca Island, in the Balearic Sea (Carbonell et al. 2003).

Galeus melastomus shows a very wide bathymetric range, being the most important shark in the upper and middle slopes (i.e. 400–800 m and 800–1,400 m, approximately) in terms of abundance and biomass (Carrassón et al. 1992; Massutí and Moranta, 2003; D'Onghia et al. 2004). In contrast, the spotted dogfish *Scyliorhinus canicula* (L., 1758) (Carcharhiniformes: Scyliorhinidae) reaches its maximum abundance around 100 m depth, dominating the elasmobranch fauna of the continental shelf, although it can be found in the upper slope down to 500 m (Massutí and Moranta, 2003).

The bathymetric distribution of juveniles and adults also differs for both species: while *G. melastomus* follows the deeper-bigger rule, with adults distributed in deeper waters than juveniles, *S. canicula* follows the opposite trend, with adults distributed above 100 m and juveniles below this depth (Carrassón et al. 1992; Massutí and Moranta, 2003).

Regarding their feeding biology, both species are generalistic predators and occasional scavengers that consume mainly benthic and benthopelagic prey (Carrassón et al. 1992;

Valls et al. 2011; Mnrasi et al. 2012). Although *G. melastomus* shows a more specialized diet than *S. canicula*, they can show a high diet overlap in the Balearic Sea, mainly in the upper slope (Valls et al. 2011). Furthermore, both sharks show similar ontogenic diet shifts, with adults consuming larger prey, such as cephalopods, teleosts and large crustaceans, than juveniles, for which smaller crustaceans like mysids, amphipods or euphausiids are the main target (Carrassón et al. 1992; Valls et al. 2011).

The metazoan parasite community of *S. canicula* was already described in the northeastern Atlantic Ocean by Henderson and Dunne (1998) and Moore (2001). In the case of *G. melastomus,* although several records of different parasites exist (Pintner, 1899, 1930; Sproston, 1946; Brinkmann, 1988; Euzet et al. 1993; Raibaut et al. 1998 Dallarés et al. 2015, among others), these come mainly from northern Atlantic waters.

Therefore, and given the high importance of *S. canicula* and *G. melastomus* in the NW Mediterranean marine ecosystems, the main aim of present study is to describe the parasite communities of both species in this area. Seasonal variability throughout the year (and geographical differentiation between two localities in the case of *G. melastomus*) is tested for parasite community descriptors and for the abundance of the most frequent parasites. The influence of environmental variables (namely temperature, salinity, turbidity, O_2 content of water masses and phytoplankton concentration) on the abundance patterns of individual parasites is further addressed. Finally, differences on the parasite community composition and structure between the two sharks addressed are assessed and discussed.

MATERIALS AND METHODS

Sampling area and specimen collection

A total of 41 specimens of *S. canicula* and 159 of *G. melastomus* was collected during 2007 at the continental slope of the Balearic Sea off the mouth of the River Besòs (Barcelona) (seasonally) and off Vilanova (only in summer). The samples were obtained using a semi-balloon otter trawl (OTSB) and a commercial fishing gear (BOU) at depths comprised between 53–68 m for *S. canicula* and between 549–809 m for *G. melastomus* (Table 1). On board, fish were freshly frozen at –20 ºC for further parasitological examination.

Simultaneously to sampling hauls, measures of temperature (T) in ºC, salinity (S) in psu, O_2 content of water masses in ml/l, turbidity (voltage) and phytoplankton pigment concentration (Chla) were obtained at 5 m above the bottom by deployment of a CTD profiler (Table 1).

Table 1. Sampling data for *Galeus melastomus* and *Scyliorhinus canicula*. n: number of individuals captured in each haul, Lat.: Latitude, Long.: Longitude, G. mel.: *G. melastomus*, S. can.: *S. canicula*. Environmental variables: TºC: temperature, S: salinity, O_2: oxygen concentration, Turb: turbidity, Chla: chlorophyll a concentration.

Locality-season group	**Haul**	**Date**	**Depth**	**Coordinates**		**n**	**N**	**Environmental variables**				
		(dd/mm/yy)	**(m)**	**Lat. (N)**	**Long. (E)**	**G. mel.**	**S. can.**	**TºC**	**S (psu)**	**O_2 (mL/L)**	**Turb (V)**	**Chla**
Besós-Winter	B1–BOU1	06/03/2007	651	41.24	2.46	25		13,29	38,53	3,93	0,15	0,05
	B1–BOU2	06/03/2007	784	41.15	2.40	28		13,19	38,51	4,12	1,10	0,05
	B1–BOU3	14/03/2007	53	41.41	2.29		1	13.64	38.22	4.88	0.35	1.02
	B1–BOU4	14/03/2007	63	41.42	2.38		6	13.64	38.22	4.88	0.35	1.02
Besós-Spring	B2–OTSB2	28/04/2007	650	41.17	2.38	5		13.29	38.53	5.76	0.27	0.02
	B2–OTSB3	28/04/2007	797	41.15	2.41	5		13.24	38.52	5.77	0.44	0.02
	B2–OTSB4	29/04/2007	809	41.15	2.40	3		13.24	38.52	5.77	0.44	0.02
	B2–OTSB5	29/04/2007	661	41.24	2.48	1		13.29	38.53	5.76	0.27	0.02
	B2–BOU1	09/05/2007	66	41.38	2.34		3	13.63	38.08	5.74	0.07	0.22
	B2–BOU2	09/05/2007	67	41.39	2.34		6	13.63	38.08	5.74	0.07	0.22
	B2–BOU3	11/05/2007	559	41.24	2.46	12		13.29	38.53	5.76	0.27	0.02
	B2–BOU4	11/05/2007	785	41.15	2.40	3		13.24	38.52	5.77	0.44	0.02
Besós-Summer	B3–OTSB5	01/07/2007	671	41.24	2.49	5		13.32	38.54	5.76	0.03	0.02
	B3–BOU3	18/07/2007	66	41.38	2.34		7	14.80	38.04	5.60	0.02	0.54
	B3–BOU4	18/07/2007	66	41.38	2.33		4	14.80	38.04	5.60	0.02	0.54
	B3–BOU5	18/07/2007	68	41.38	2.35		2	14.80	38.04	5.60	0.02	0.54
	B3–BOU6	19/07/2007	561	41.24	2.46	19		13.32	38.54	5.76	0.03	0.02
	B3–BOU7	19/07/2007	791	41.15	2.40	10		13.18	38.51	5.78	0.08	0.02
Besós-Autumn	B4–OTSB3	02/10/2007	811	41.14	2.36	2		13.18	38.51	8.25	0.18	0.02
	B4–OTSB6	03/10/2007	716	41.24	2.49	4		13.39	38.55	8.22	0.47	0.02
	B4–BOU1	13/11/2007	60	41.39	2.34		2	17.00	38.03	7.67	0.83	0.77
	B4–BOU2	13/11/2007	60	41.36	2.33		2	17.00	38.03	7.67	0.83	0.77
	B4–BOU3	13/11/2007	60	41.38	2.34		8	17.00	38.03	7.67	0.83	0.77
	B4–BOU4	28/12/2007	549	41.24	2.45	2		13.39	38.55	8.22	0.47	0.03
	B4–BOU5	28/12/2007	791	41.15	2.40	13		13.18	38.51	8.25	0.18	0.03
Vilanova-Summer	B3–OTSB6	05/07/2007	662	41.09	2.18	4		13.41	38.53	3.95	0.40	0.01
	B3–OTSB7	05/07/2007	803	41.07	2.21	2		13.17	38.50	5.78	0.09	0.02
	B3–BOU1	25/06/2007	780	41.07	2.20	16		13.17	38.50	5.78	0.09	0.02
Total number of specimens sampled						159	41					

Parasitological study

In the laboratory and prior to dissection, total length (TL) in mm and total weight (TW) in g were obtained for each fish. Subsequently, external surfaces and gills were examined for the presence of ectoparasites. All internal organs were dissected out and examined separately for endoparasites under stereomicrocope. In the case of *S. canicula*, body musculature was also examined under stereomicroscope by compression between two glass plates. All metazoan parasites collected were preserved in 70% ethanol. Monogeneans, digeneans and cestodes were stained with iron acetocarmine and examined as permanent mounts in Canada balsam. Nematode larvae and crustaceans were observed as temporary mounts in saline solution. All parasites were identified to the lowest possible taxonomic level.

Data analyses

For *G. melastomus*, two size-based groups of hosts were defined, corresponding to immature (TL<34 cm for males and TL<40cm for females) and mature specimens (TL≥34 cm for males and TL≥40 cm for females) (Capapé and Zaouali, 1977).

Ecological terms used for parasite populations and communities follow Bush et al. (1997): prevalence (P%) was calculated as the proportion of hosts in each sample infected by a given parasite and mean abundance (MA) as the total number of parasites found in a particular host species divided by the total number of hosts of such species. For both species, parasite species displaying a P%>10% in at least one seasonal/geographical group were considered not accidental and are henceforth called common (see indications in Table 2). Parasite infrapopulations and infracommunities (i.e., all parasites of a given species in an individual fish and all infrapopulations in an individual fish, respectively) were used as replicate samples in the analyses. Infracommunity richness, abundance, diversity and dominance were calculated, the two latter based on parasite abundance, using Brillouin's Index (PRIMER v6; Anderson et al. 2008) and Berger-Parker dominance index (calculated as the number of individuals of the most abundant parasite species in a given host divided by the total number of parasites found in such host), respectively. Fish condition was assessed by the condition factor (K, calculated as $TW \times 100 / TL^3$), and the hepatosomatic index (HSI, calculated as liver weight (g) × 100 / TW).

Spearman rank correlation (r_s) tests were applied in order to assess the association of parasite infracommunity parameters (i.e. richness, abundance, diversity and dominance) with host TL, and condition parameters (i.e. K and HSI). The same tests were used to test the relationship between the abundance of common parasites of both hosts and the same fish biological factors.

Kruskal-Wallis tests and generalized linear model (GZM) analyses were performed to test the differences on parasite infracommunity parameters and on the prevalence and abundance of common parasites (using fish TL as covariate), respectively, among seasons. Similarly, general linear models (GLM) followed by post hoc tests using Bonferroni correction were carried out to assess differences among seasons on fish TL and condition parameters for both species. The same analyses were repeated using matched seasonal samples (i.e. summer) of *G. melastomus* collected in Besós and Vilanova in order to test geographical variability in parasite infracommunity parameters, the prevalence and abundance of common parasites and in fish TL and condition parameters.

Permutational multivariate analyses of variance (PERMANOVA) were carried out using abundance data of parasite infracommunities to test differences in the parasite community structure among the four seasons sampled off Besós for the two species of sharks, and between matched seasonal samples from off Besós and Vilanova for *G. melastomus*. Such analyses were applied using PERMANOVA+ for PRIMERv6 (Anderson et al. 2008) on Bray-Curtis similarity matrices generated from the logarithmically transformed (log (X+1)) abundance data, and permutation p-values were obtained under unrestricted permutation of raw data (9999 perms).

Canonical correspondence analyses (CCA) were used to relate the abundance of the common parasites found in the two species of sharks with environmental variables (Ter Braak, 1986). In CCA plots, arrows represent explanatory variables and are proportional in length to their importance on the explained variable.

A non-parametric Multi-Dimensional Scaling (MDS) was applied on infracommunity data of *S. canicula* and *G. melastomus* in order to visualize the ordination of parasite infracommunities of the two distinct hosts. Then, using the samples collected from off Besós, T-Student tests (assuming non-equal variances) were applied to test differences on infracommunity richness, diversity and dominance between *S. canicula* and *G. melastomus*, and GZM were used to test differences on infracommunity abundance and on the overall abundance of the shared parasites between both hosts.

RESULTS

The parasite community of S. canicula

The parasite community of *S. canicula* included a total of five parasite species comprising three nematodes, one monogenean and one cestode (Table 2). *Hysterothylacium aduncum* (Rudolphi, 1802) constitutes a new host record for this species. The nematode *Proleptus obtusus* Dujardin, 1845 was, by far, the most important parasite in terms of prevalence and abundance. All sharks examined were infected by at least one parasite.

Table 2. Overall prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Galeus melastomus* and *Scyliorhinus canicula*. N: sample size for each host, [a] species considered common in *G. melastomus*, [b] species considered common in *S. canicula*. Dashes indicate absence of the parasite.

	Galeus melastomus		***Scyliorhinus canicula***	
N	**159**		**41**	
	P%	**MA ± SD**	**P%**	**MA ± SD**
Monogenea				
Erpocotyle sp.[a]	5.7	0.07 ± 0.30	–	–
Leptocotyle minor	1.3	0.01 ± 0.11	–	–
Hexabothrium appendiculatum[b]	–	–	17.1	0.46 ± 1.33
Digenea				
Otodistomum cestoides	1.9	0.02 ± 0.14	–	–
Accacoeliidae gen. sp. (met)	1.3	0.01 ± 0.11	–	–
Cestoda				
Ditrachybothridium macrocephalum[a]	17.1	0.53 ± 2.43	–	–
Grillotia sp.[a]	14.6	0.21 ± 0.60	–	–
Sphyriocephalus viridis	1.9	0.03 ± 0.26	–	–
Nybelinia lingualis[b]	–	–	2.4	0.02 ± 0.16
Nematoda				
Dychelyne (Cucullanellus) sp.	0.6	0.01 ± 0.08	–	–
Piscicapillaria baylisi	5.7	0.06 ± 0.23	–	–
Anisakis type II (sensu Berland, 1961)[b]	2.5	0.03 ± 0.16	7.3	0.10 ± 0.37
Hysterothylacium aduncum[b]	1.9	0.02 ± 0.14	7.3	0.07 ± 0.26
Proleptus obtusus[a,b]	5.1	0.06 ± 0.26	100	49.37 ± 37.76
Copepoda				
Eudactylina sp.	0.6	0.01 ± 0.08	–	–

No relationship was detected between any of the infracommunity parameters or the abundance of common parasites and fish TL, K or HSI ($p>0.05$ in all cases).

Kruskal-Wallis tests and GZMs revealed no seasonal differences for infracommunity parameters or for the abundance or prevalence of common parasites ($p>0.05$ in all cases) (Tables 3 and 4). Regarding fish biological factors, only K showed significant seasonal variations ($F_{(3, 37)}= 6.293$, $p= 0.001$) being higher in summer and autumn than in the other two seasons (Table 3).

The PERMANOVA applied on parasite infracommunities showed no seasonal effect on the structure of such communities ($p_{(perm)}>0.05$).

The CCA relating common parasites of *S. canicula* and environmental variables accumulated 99.8% of the total variance (Fig. 1). The abundance of the monogenean *Hexabothrium appendiculatum* (Kuhn, 1829) was strongly associated to high near-bottom turbidity and, to a lesser extent, temperature and O_2 concentration, while the nematode *H. aduncum* was associated to high salinity levels.

Table 3. Means and standar deviations of fish biological factors and parasite infracommunity descriptors across the seasons and localities sampled for *Scyliorhinus canicula* and *Galeus melastomus*. Different superscript letters and numbers show significant differences among seasons and between localities, respectively. S1: size 1, S2: size 2.

Host	***Scyliorhinus canicula***				***Galeus melastomus***				
Locality-season group	**Besós winter**	**Besós spring**	**Besós summer**	**Besós autumn**	**Besós winter**	**Besós spring**	**Besós summer**	**Besós autumn**	**Vilanova summer**
Total length	42.14 ± 2.73^{a}	42.88 ± 1.15^{a}	42.22 ± 4.78^{a}	43.62 ± 2.26^{a}	32.16 ± 9.54^{ab}	28.73 ± 14.50^{a}	36.95 ± 13.09^{b1}	46.37 ± 8.68^{c}	36.95 ± 11.94^{1}
Condition factor	0.26 ± 0.03^{a}	0.29 ± 0.06^{ab}	0.34 ± 0.06^{b}	0.34 ± 0.02^{b}	0.26 ± 0.07^{a}	0.27 ± 0.06^{a}	0.23 ± 0.03^{b1}	0.27 ± 0.04^{a}	0.21 ± 0.03^{2}
Hepatosomatic index	8.61 ± 3.36^{a}	7.76 ± 2.38^{a}	8.06 ± 2.87^{a}	5.74 ± 2.17^{a}	3.69 ± 1.00^{a}	4.27 ± 2.40^{a}	4.40 ± 1.96^{a1}	5.84 ± 1.43^{b}	4.86 ± 1.92^{1}
Total parasite richness	4	4	3	3	11	5	7	8	2
Infracommunity richness	1.43 ± 0.54^{a}	1.44 ± 1.01^{a}	1.38 ± 0.65^{a}	1.17 ± 0.39^{a}	0.70 ± 0.72^{a}	0.57 ± 0.69^{a}	0.59 ± 0.78^{a1}	0.90 ± 0.70^{a}	0.14 ± 0.35^{2}
Infracommunity abundance	29.14 ± 22.00^{a}	80.56 ± 59.93^{a}	48.00 ± 29.37^{a}	41.50 ± 18.53^{a}	0.87 ± 1.02	2.29 ± 5.41	0.76 ± 1.16^{1}	1.14 ± 1.06	0.18 ± 0.50^{2}
Infracommunity abndance – S1					0.71 ± 0.98^{a}	3.00 ± 6.12^{b}	0.58 ± 1.26^{a}	1.00 ± 0.82^{ab}	0.08 ± 0.28
Infracommunity abundance – S2					1.42 ± 1.00^{a}	0.14 ± 0.38^{a}	1.00 ± 1.00^{a}	1.18 ± 1.13^{a}	0.33 ± 0.71
Infracommunity diversity (Brillouin's index)	0.09 ± 0.12^{a}	0.03 ± 0.06^{a}	0.07 ± 0.11^{a}	0.04 ± 0.11^{a}	0.10 ± 0.16^{a}	0.08 ± 0.15^{a}	0.11 ± 0.20^{a1}	0.09 ± 0.16^{a}	0.00 ± 0.00^{1}
Infracommunity dominance (Berger-Parker's index)	0.96 ± 0.06^{a}	0.99 ± 0.01^{a}	0.97 ± 0.05^{a}	0.99 ± 0.04^{a}	0.88 ± 0.21^{a}	0.88 ± 0.22^{a}	0.89 ± 0.19^{a1}	0.89 ± 0.19^{a}	1.00 ± 0.00^{1}

Table 4. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Scyliorhinus canicula* across the seasons sampled. N: sample size for each group. Different superscript letters and numbers show significant differences across seasons for MA and P% of common parasites, respectively. Dashes indicate absence of the parasite.

	Besós winter		**Besós spring**		**Besós summer**		**Besós autumn**	
N	**7**		**9**		**13**		**12**	
	P(%)	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**
Monogenea								
Hexabothrium appendiculatum	14.3^1	0.29 ± 0.76^a	11.1^1	0.22 ± 0.67^a	30.8^1	0.62 ± 1.19^a	8.3^1	0.58 ± 2.02^a
Cestoda								
Nybelinia lingualis	14.3	0.14 ± 0.38	–	–	–	–	–	–
Nematoda								
Hysterothylacium aduncum	14.3^1	0.14 ± 0.38^a	22.2^1	0.22 ± 0.44^a	–	–	–	–
Anisakis type II	–	–	11.1^1	0.22 ± 0.67^a	7.7^1	0.08 ± 0.28^a	8.3^1	0.08 ± 0.29^a
Proleptus obtusus	100.0^1	28.57 ± 22.04^a	100.0^1	79.89 ± 58.76^a	100.0^1	47.31 ± 29.42^a	100.0^1	40.83 ± 18.52^a

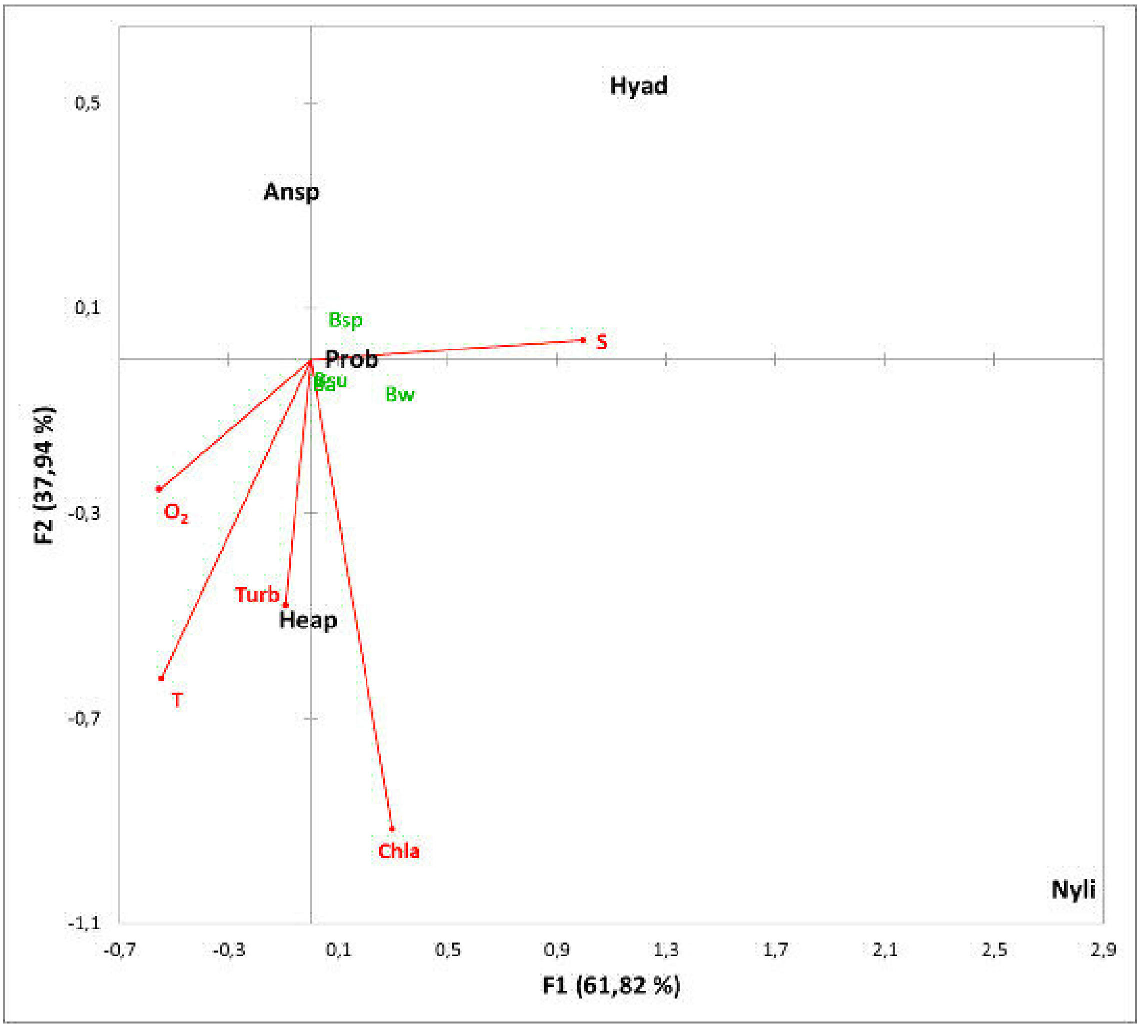

Figure 1. Canonical correspondence analysis (CCA) showing associations between the common parasites of *Scyliorhinus canicula* and environmental variables. Abbreviations for parasite names: Ansp, *Anisakis* Type II; Heap, *Hexabothrium appendiculatum*; Hyad, *Hysterothylacium aduncum*; Nyli, *Nybelinia lingualis*; Prob, *Proleptus obtusus*. Abbreviations for locality-season groups: Ba, Besós autumn; Bsp, Besós spring; Bsu, Besós summer; Bw, Besós winter. Abbreviations for environmental variables: Chla, chlorophyll a concentration; O_2, oxygen concentration; S, salinity; T, temperature; Turb, turbidity.

The parasite community of G. melastomus

A total of 13 parasite species comprising five nematodes, three cestodes, two monogeneans, two digeneans and one copepod were recovered from the specimens of *G. melastomus* examined (Table 2). Of these, ten species constitute new host records for this host: *Erpocotyle* sp., *Leptocotyle minor* (Monticelli, 1888), *Otodistomum cestoides* (Van Beneden, 1871) Odhner, 1911, Accacoelidae gen. sp., *Grillotia* sp., *H. aduncum*, *Anisakis* sp. ascribed to morphotype *Anisakis* Type II *sensu* Berland (1961), *P. obtusus, Piscicapillaria baylisi* Moravec, 1987 and *Cucullanus* sp.

The most frequent parasites were the cestodes *Ditrachybothridium macrocephalum* Rees, 1959 and *Grillotia* sp. Overall prevalence of infection in *G. melastomus* was 46%. Infracommunity richness and the abundance of the cestodes *D. macrocephalum* and *Grillotia* sp. were significantly correlated with fish TL (r_s= 0.17, p= 0.04; r_s= -0.16, p= 0.04 and r_s= 0.27, p= 0.001, respectively), with the abundance of *D. macrocephalum* being higher in juvenile sharks and infracommunity richness and the abundance of *Grillotia* sp. reaching higher values in adult hosts. Furthermore, the abundance of *D. macrocephalum* and *P. obtusus* showed significant positive and negative relationships, respectively, with K (r_s= 0.19, p= 0.019 and r_s= -0.23, p= 0.004, respectively), and the abundance of *D. macrocephalum* and *Grillotia* sp. were negatively and positively related, respectively, to HSI (r_s= -0.28, p= 0.004 and r_s= 0.20, p= 0.042, respectively). Parasite infracommunity descriptors were not significantly related to K or HSI (p>0.05 in all cases).

The abundance of *Grillotia* sp. showed significant differences among seasons, being maximum in summer and autumn (χ^2= 10.643, p= 0.014). In the cases of infracommunity mean abundance and abundance of *D. macrocephalum* interactions were found between the factor season and fish TL (χ^2= 16.478, p= 0.001 and χ^2=11.386, p= 0.003, respectively) and the analyses were thus repeated considering the two size-groups of hosts separately. Infracommunity mean abundance and abundance of *D. macrocephalum* displayed significant seasonal variability in juvenile sharks, (χ^2=21.737, p<0.001 and χ^2= 22.870, p<0.001, respectively), both being highest in spring than in the rest of seasons (Tables 3 and 5).

No seasonal differences were detected for infracommunity richness, diversity or dominance, or for the prevalence of common parasites (p>0.05 in all cases).

Concerning geographical variability, infracommunity richness and abundance were significantly higher in samples from off Besós than in those from off Vilanova in summer (χ^2= 6.031, p= 0.014 and χ^2= 5.679, p= 0.017, respectively) (Table 3). No significant differences were detected between localities for abundance or prevalence values of common parasites, although *Erpocotyle* sp., and *P. obtusus* were absent from the samples from off Vilanova (Table 5).

Table 5. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Galeus melastomus* across the seasons and localities sampled. N: sample size for each group, S1: size 1, S2: size 2. Different superscript letters and numbers show significant differences across seasons for MA and P% of common parasites, respectively. Dashes indicate absence of the parasite.

	Besós winter		**Besós spring**		**Besós summer**		**Besós autumn**		**Vilanova summer**	
N	**53**		**29**		**34**		**21**		**22**	
	P(%)	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**
Monogenea										
Erpocotyle sp.	5.7^{1}	0.08 ± 0.33^{a}	3.6^{1}	0.04 ± 0.19^{a}	2.9^{1}	0.03 ± 0.17^{a}	19.1^{1}	0.24 ± 0.54^{a}	–	–
Leptocotyle minor	1.9	0.02 ± 0.14	–	–	2.9	0.03 ± 0.17	–	–	–	–
Digenea										
Otodistomum cestoides	3.8	0.04 ± 0.19	–	–	–	–	4.8	0.05 ± 0.22	–	–
Accacoelidae gen. sp.	1.9	0.02 ± 0.14	3.6	0.04 ± 0.19	–	–	–	–	–	–
Cestoda										
Ditrachybothridium macrocephalum	28.3^{1}	0.43 ± 0.84	32.1^{1}	2.04 ± 5.47	–	–	14.3^{1}	0.14 ± 0.36	–	–
Ditrachybothridium macrocephalum-S1		0.44 ± 0.84^{a}		2.71 ± 6.20^{b}		–		–		–
Ditrachybothridium macrocephalum-S2		0.42 ± 0.90^{a}		–		–		0.18 ± 0.39^{a}		–
Sphyriocephalus viridis	–	–	–	–	2.9	0.09 ± 0.51	9.5	0.10 ± 0.30	–	–
Grillotia sp.	7.6^{1}	0.08 ± 0.27^{a}	10.7^{1}	0.11 ± 0.32^{ab}	23.5^{1}	0.32 ± 0.68^{b}	28.6^{1}	0.48 ± 0.87^{b}	9.1	0.14 ± 0.47
Nematoda										
Hysterothylacium aduncum	1.9	0.02 ± 0.14	7.1	0.07 ± 0.26	–	–	–	–	–	–
Anisakis type II	5.7	0.06 ± 0.23	–	–	2.9	0.03 ± 0.17	–	–	–	–
Proleptus obtusus	3.8^{1}	0.04 ± 0.19^{a}	–	–	14.7^{1}	0.18 ± 0.46^{a}	4.8^{1}	0.05 ± 0.22^{a}	–	–
Piscicapillaria baylisi	7.6	0.08 ± 0.27	–	–	8.8	0.09 ± 0.29	4.8	0.05 ± 0.22	4.6	0.05 ± 0.21
Dychelyne (*Cucullanellus*) sp.	–	–	–	–	–	–	4.8	0.05 ± 0.22	–	–
Copepoda										
Eudactylina sp.	1.9	0.02 ± 0.14	–	–	–	–	–	–	–	–

Fish TL and HSI were significantly lower in autumn samples than in the rest of seasons ($F_{(3,\ 132)}$= 10.937, p<0.001 and $F_{(3,\ 96)}$= 5.446, p= 0.002, respectively), while fish K reached minimum values in summer ($F_{(3,\ 130)}$= 3.861, p= 0.011). Among fish biological descriptors, only K showed significant differences between localities ($F_{(1,\ 54)}$= 6.845, p= 0.012), being higher in fishes from off Besós than in those from off Vilanova (Table 3).

The PERMANOVA analyses applied on infracommunity abundance data revealed significant differences in the structure of parasite infracommunities among the four seasons sampled off Besós (Pseudo-$F_{(3,\ 67)}$= 3.1495, $p_{(perm)}$<0.001, unique perms= 9,928). Post-hoc pairwise comparisons separated winter and spring samples, which grouped together, from summer and autumn ones, which grouped together as well. In contrast, no geographical differences between infracommunities collected off Besós and Vilanova in summer were detected ($p_{(perm)}$> 0.05).

The CCA relating common parasites of *G. melastomus* and environmental variables accumulated 95.0% of the total variance (Fig. 2). The abundance of the cestode *D. macrocephalum* was associated to high near-bottom turbidity coinciding with some hauls from off Besós in winter and spring. The parasites *Erpocotyle* sp. and *Grillotia* sp. were linked to high O_2 concentration, partly associated to hauls from off Besós in autumn. Finally, the nematode *P. obtusus* was associated to high levels of salinity and temperature, in this case associated to hauls from off Besós, although of any particular season.

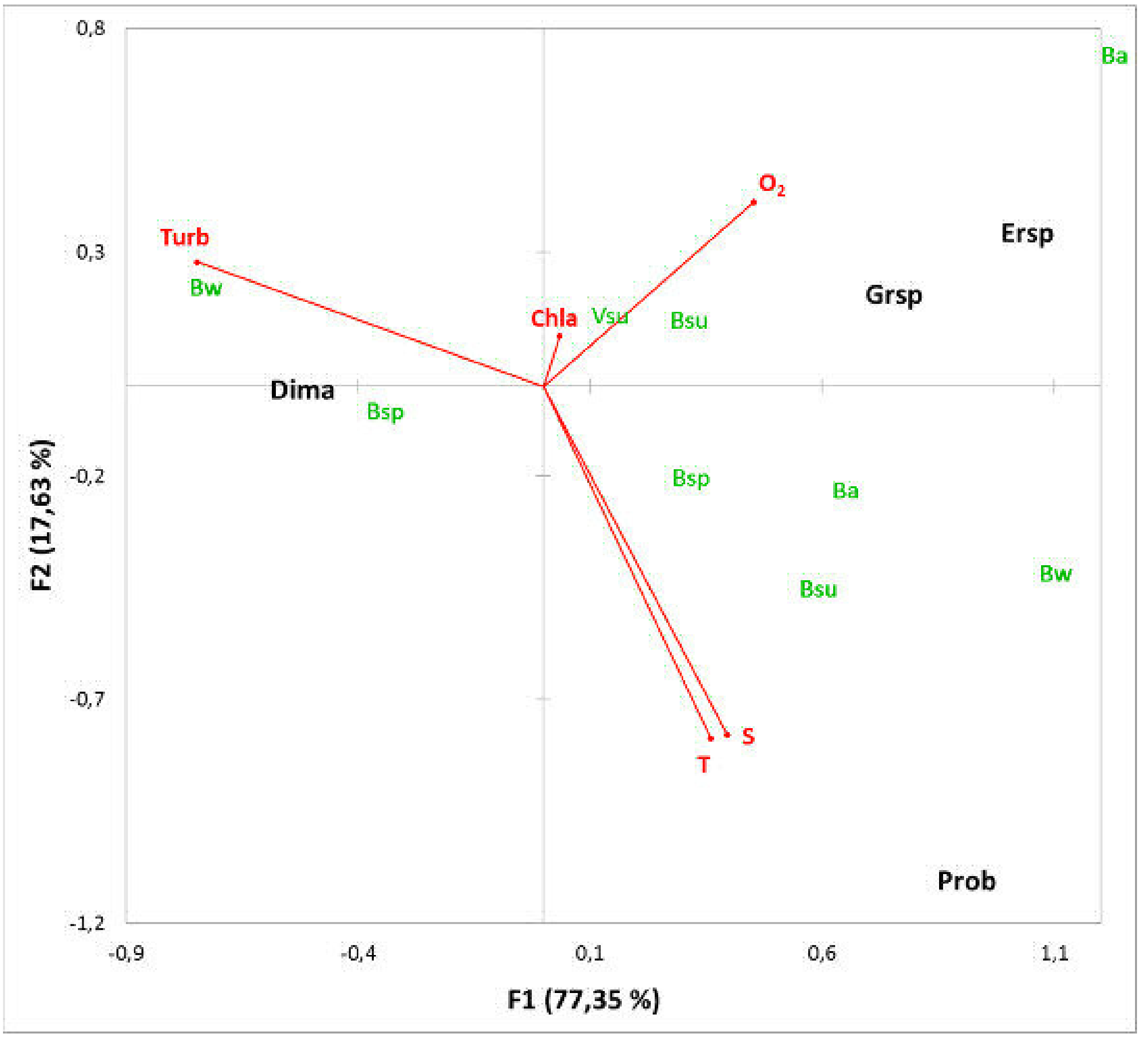

Figure 2. Canonical correspondence analysis (CCA) showing associations between the common parasites of *Galeus melastomus* and environmental variables. Abbreviations for parasite names: Dima, *Ditrachybothridium macrocephalum*; Ersp, *Erpocotyle* sp.; Grsp, *Grillotia* sp.; Prob, *Proleptus obtusus*. Abbreviations for locality-season groups: Ba, Besós autumn; Bsp, Besós spring; Bsu, Besós summer; Bw, Besós winter; Vsu, Vilanova summer. Abbreviations for environmental variables: Chla, chlorophyll a concentration; O_2, oxygen concentration; S, salinity; T, temperature; Turb, turbidity.

Comparison between the two sharks addressed

The MDS providing an ordination of parasite infracommunities of both hosts evidenced a clear differentiation between samples of *S. canicula* and *G. melastomus* (Stress= 0.04, Fig. 3).

Overall parasite infracommunity richness and dominance were significantly higher in *S. canicula* than in *G. melastomus* (t= -5.540, p<0.001 and t= -3.851, p<0.001, respectively), as also were total infracommunity abundance and the abundance of the nematode *P. obtusus* (χ^2= 365.441, p<0.001 and χ^2= 305.229, p<0.001, respectively, see Table 2). Infracommunity diversity and the abundance of the other two shared parasites (i.e. *H. aduncum* and *Anisakis* Type II) showed no significantly different values between hosts (p>0.05 in both cases).

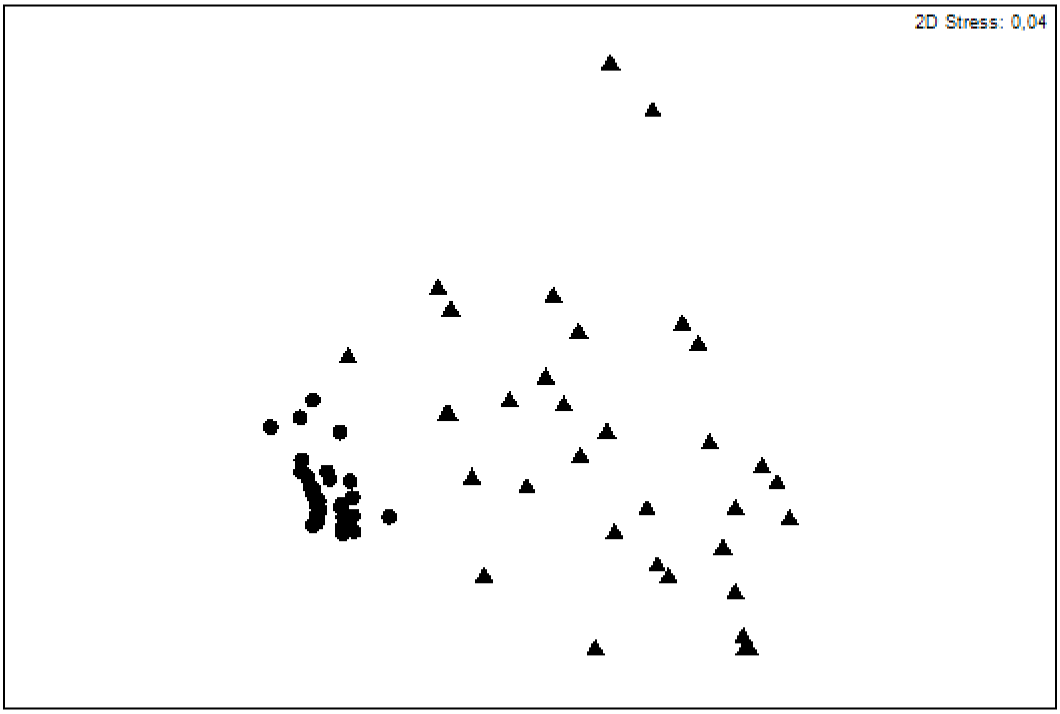

Figure 3. Non-parametric Multi-dimensional scaling (MDS) showing ordination of infracommunities of *Scyliorhinus canicula* (●) and *Galeus melastomus* (▲).

DISCUSSION

The present study represents the first attempt to describe the complete parasite communities of shark species in the Mediterranean Sea.

In the NW Mediterranean, *S. canicula* is characterized by an impoverished parasite community, displaying low richness and diversity and strongly dominated by a single species (i.e. the nematode *P. obtusus*). *Galeus melastomus*, for which the parasite community is described herein for the first time, shows in comparison markedly higher total parasite richness (13 vs. 5 parasite taxa), although parasite infracommunities are still depauperate, with lower mean richness and similar diversity and dominance values than *S. canicula*.

In spite of these similarities, structure of infracommunities is rather different between both species, as evidenced by their distinct ordination in the MDS. While infracommunities of *S. canicula* grouped together reflecting a high degree of similarity, those corresponding to *G. melastomus* appeared scattered throughout the plot, indicating a much higher heterogeneity among them. Actually, although values of Berger-Parker's dominance index were high in both species, in *G. melastomus* the dominant parasite was not consistently the same in all infracommunities, and relative abundances of the different parasites were generally modest. In contrast, in *S. canicula* the nematode *P. obtusus* showed very high abundance levels compared to the rest of parasite species, clearly dominating all infracommunities and displaying a homogenizing effect. Such effect was also appreciated in the CCA plot relating *S. canicula* parasite abundance levels with environmental variables, where all seasons appeared centralized, close to *P. obtusus*, since this parasite characterizes all seasonal groups in this host.

These differences in the parasite community composition and structure of both hosts are likely due to their different dietary habits (Carrassón et al. 1992; Valls et al. 2011; Mnrasi et al. 2012), largely influenced by the different availability of benthopelagic prey assemblages in the continental shelf (i.e. sampling depth of *S. canicula*) and in the upper slope (i.e. sampling depth of *G. melastomus*). Variations in the benthopelagic faunal assemblages occur along depth gradients in response to different environmental conditions (Cartes et al. 2006, 2013) and lead to different transmission dynamics for trophically-transmitted parasites and, therefore, to different composition of parasite communities.

The low mean richness and diversity, and high dominance of infracommunities observed for *S. canicula* and, to a lesser extent, for *G. melastomus,* have also been reported from other small-sized sharks from different areas. According to Isbert et al. (2015), infracommunities of *Etmopterus spinax* (L.) from the NE Atlantic were also characterized by low mean richness and high dominance values. Moore (2001) and Henderson et al. (2002) also reported depauperate infracommunities in *S. canicula* and *Squalus acanthias* L., respectively, from the NE Atlantic as well.

As far as we are concerned, available studies on shark parasite communities have focused on small-sized sharks from North-Atlantic waters, in which the total number of parasite taxa is usually low. Henderson and Dunne (1998) found five different parasites in *S. canicula*, Chambers (2008) reported eight parasites from *Centroscyllium fabricii* (Reinhardt), Henderson et al. (2002) recovered 10 parasites from *S. acanthias* and Moore (2001) and Isbert et al. (2015) found 11 different taxa in *S. canicula* and *E. spinax*, respectively. In larger, although still small-sized sharks, Palm and Schröeder (2001) reported six parasites from *Heptranchias perlo* (Bonnaterre), three from *Deania calcea* (Lowe), seven from *Deania profundorum* (Smith and Radcliffe) and nine from *Deania histricosa* Garman. Isbert et al. (2015) made the suggestion that poor parasite faunas, with low richness and diversity and high dominance values could represent features of small sharks, which would be to some extent supported by present results. However, there are few data available for comparison and the suggested trends could be a characteristic pattern of sharks in general, regardless of their size.

Different studies on teleost parasite communities have repeatedly reported lower parasite richness in Mediterranean with respect to Atlantic populations (e.g. Pérez-del-Olmo et al. 2009; Mattiucci et al. 2014; Constenla et al. 2015). A smaller fish size, lower food consumption and lower biomass and abundance of animal communities in the Mediterranean have been suggested as possible explanations for such pattern (see Constenla et al. 2015 and references therein). Actually, maximum size of *S. canicula* in the Mediterranean is lower than elsewhere (Compagno, 1984), and a similar pattern has been observed for Mediterranean specimens of *G. melastomus* with respect to their Atlantic counterparts (Compagno, 1984; Carrassón et al. 1992; present results). However, results available to date are far from sufficient to conclude whether elasmobranch parasite communities are more diverse and abundant in Atlantic than in Mediterranean waters. Parasitological data for *S. canicula* are scarce (only Henderson and Dunne (1998) and Moore (2001) have analysed the parasite community of this shark in the Atlantic, and present results constitute the first report from the Mediterranean) and the range of variability reported for its total number of parasites is wide and thus not feasible for comparison. In the case of *G. melastomus*, present results represent the first description of its parasite community, and comparative data are thus completely absent. Undoubtedly, additional parasitological studies in sharks from Mediterranean and Atlantic waters will open the possibility to test if the same trend observed in teleosts is applicable to shark parasite communities.

Fish condition indices vary in response to multiple factors and clear relationships between them and parasitological infections are usually difficult to determine (Heins and Baker, 2008). Parasites can be detrimental to their hosts and consequently alter condition indices, but fish hosts with reduced health condition may show higher susceptibility to parasite infections (Dallarés et al. 2016). It is also possible that parasite infections do not reach the threshold needed to affect condition indices or even that healthier fishes harbour more abundant and rich parasite communities, as suggested by Dallarés et al. (2014). In any case, unless the impact of a given parasite on host fitness is strong (i.e. Heins and Baker (2008) detected heavily reduced reproductive fitness in the three-spine stickleblack *Gasterosteus aculeatus* infected with the cestode *Schistocephalus* sp.), observations are often inconsistent, and must therefore be carefully considered.

In this sense, the contrary associations detected in *G. melastomus* between the parasites *D. macrocephalum* and *P. obtusus* and fish condition factor, or between the cestodes *D. macrocephalum* and *Grillotia* sp. and fish hepatosomatic index, do not allow stating any general trend. Such relationships, observed for a few individual parasites only, may reflect variations in parasite loads in response to specific biological aspects of their host (i.e. dietary trends) or environmental patterns that are coupled with variations in fish condition parameters, instead of an impact of parasites on fish health.

These observations, alongside with the absence of noticeable effects of the parasite load on *S. canicula* general condition, point to a negligible repercussion of the parasite burden in general fish condition indices in the two sharks addressed.

In the present study, seasonal variations of the parasite burden of the two species of sharks addressed are assessed throughout the whole length of the year for the first time.

In the case of *S. canicula*, the absence of significant differences among seasons either for the abundance or prevalence of the different parasites recovered or for infracommunity descriptors and structure might be attributed to the low number of hosts available for each season group. Further studies with increased number of specimens are needed in order to confirm the observed lack of seasonal patterns.

The much lower infracommunity richness and abundance observed in Vilanova with respect to Besós samples of *G. melastomus* could be possibly explained by the vicinity of the Besós submarine canyon to the latter locality. Submarine canyons, formed as a result of river discharge (the Besós River in this case), favour aggregation of zooplankton and more complex invertebrate communities (Macquart-Moulin and Patriti, 1996; Rumolo et al. 2015), which can presumably enhance parasite transmission. A similar pattern has been observed in the case of the parasite communities of the teleost *Phycis blennoides* (Brünnich) in the same area, where the more abundant, rich and diverse parasite composition of samples collected off the mainland vs. the insular slope in the Balearic basin are partly explained by the higher availability of benthic prey linked to submarine canyons in samples off the mainland slope (Dallarés et al. 2016).

Of the total number of parasites recovered from *S. canicula* and *G. melastomus*, the digenean *O. cestoides* (Plagiorchiida: Azygiidae) and the nematode *P. baylisi* (Enoplida: Capillariidae) are reported from the Mediterranean Sea for the first time and thus constitute new geographic records. While *O. cestoides* had been recovered in North-Atlantic and Pacific waters (Gibson and Bray, 1997), *P. baylisi* was only previously known from the NE Atlantic (Moravec, 1987).

Parasites with direct life cycles, such as monogeneans, are subjected to environmental rather than to biotic factors, in contrast with trophically-transmitted parasites. Although the response of larval and adult monogeneans to environmental variables has been repeatedly documented (Kearn, 1993 and references therein; Raymond et al. 2006; Marchiori et al. 2015), few studies have addressed the response of these parasites to O_2 levels, for which this relationship is not well understood yet. Monni and Cognetti-Varriale (2002) and Raymond et al. (2006) found negative correlations between monogenean prevalence and abundance and oxygen concentration, either explained by toleration to hypoxia allowing less competition with other parasites or by an enhanced antibody response of the host towards the parasite. These results contrast with the opposite trend observed in the present study, in which *Erpocotyle* sp. recovered from *G. melastomus* was linked to high O_2 concentration partly associated to hauls from off Besós in autumn, where the prevalence and abundance of this parasite were highest. *Hexabothrium appendiculatum* was also linked to high O_2 levels, to a lesser extent though, in samples of *S. canicula*. The response to environmental factors may vary depending on the biology and/or ecology of the parasite, of its host and on the relationship between them. Although infection levels by both monogeneans were low and the obtained results should thus be taken with caution, an enhancement of monogenean reproduction and infection success in an environment with higher O_2 levels, as has been documented for other invertebrates (Cheung et al. 2008), could be suggested. Contrary to O_2 levels, effects of higher temperature and/or turbidity levels in increasing monogenean infection success have been demonstrated (Skinner, 1982; Brazenor and Hutson, 2015) and are consistent with the association observed between *H. appendiculatum* and these environmental parameters in *S. canicula*. While higher temperatures can enhance monogenean hatching success and reduce time to maturity (Brazenor and Hutson, 2015), aquatic environments with increased turbidity due to high levels of suspended materials can provoke irritation and inflammation of gill filaments accompanied by an inhibition of fish defense mechanisms, increasing their susceptibility to infection by gill parasites, such as monogeneans (Skinner, 1982; Moles and Wade, 2001; Madi and Ueta, 2009).

Ditrachybothridium macrocephalum uses *G. melastomus* as definitive host (Dallarés et al. 2015), where it was the most prevalent and abundant parasite. No complete life-cycle is known for any cestode of the order Diphyllidea, but Tyler (2006) hypothesized that these cestodes use two invertebrate intermediate hosts (a filter-feeding crustacean as first and a shrimp or crab as second) before reaching the elasmobranch final host where they will develop into adults. Among the known prey of *G. melastomus* (Carrassón et al. 1992), amphipods and different decapods have been found to host larval stages of diphyllideans (see Bray and Olson (2004) and references therein). The higher abundance of *D. macrocephalum* in juvenile compared to adult sharks is in all likelihood related to an ontogenetic diet shift of this host, as already highlighted by Dallarés et al. (2015). According to Carrassón et al. (1992) the decapod *C. macandreae* is an important prey in the sampled area, and its presence in guts decreases with age and depth. The rest of decapods increase in importance with age and amphipods are only relevant below 1,000 m. Therefore, *C. macandreae* could be a transmitter for this parasite. The lower rate of infection by this parasite in adult sharks further suggests either a reduced lifespan of these cestodes in their definitive host, or that sharks develop an immunitary response towards the parasites, as already pointed out by Tyler (2006).

Ditrachybothridium macrocephalum was found linked to high near-bottom turbidity levels, which, according to Cartes et al. (2013), suggests more food availability for zooplankton. The consequent increase in biomass of these crustaceans enhances aggregation of benthopelagic fish (Cartes et al. 2013) and probably stimulates parasite transmission. The close association observed between water turbidity and Besós winter samples in the CCA plot (further corroborated by Rumolo et al. (2015) with the environmental data recorded in the present study) suggests that the transmission of larval stages of *D. macrocephalum* in their invertebrate hosts is enhanced in winter. The parasites must reach their final host with some temporal delay, which can explain the maximum abundance levels attained by this parasite in spring samples of *G. melastomus*.

Grillotia sp. is one of the most frequent genera recovered from fishes within the cestode order Trypanorhyncha (Beveridge and Campbell, 2007). Although copepods act as first intermediate hosts for trypanorhynchs (Palm, 2004), these are not abundant in the diet of *G. melastomus* and other prey, such as teleosts, in turn preying on copepods, could be the transmitters of the parasite. Its higher abundance in adult sharks responds to the accumulation of the larval forms of the parasite until the host is consumed by a larger predator, where the plerocerci excyst and develop into the adult form. The kitefin shark *Dalatias licha*, which is known to prey on *G. melastomus* in the NW Mediterranean Sea (Navarro et al. 2014), or *Hexanchus griseus*, which preys upon smaller sharks (Ebert, 1994), could be potential final hosts for the *Grillotia* specimens recovered.

The association observed between the abundance of *Grillotia* sp. and high levels of O_2 is in accordance with what is known about the life cycle of this parasite. High O_2 levels are known to enhance copepod biomass (Keister et al. 2000; Moon et al. 2006; Cartes et al. 2013), and specifically Cartes et al. (2013) found a significant association between O_2 levels in the benthic boundary layer (i.e. the layer of water immediately above the sea-floor) and copepod biomass in the Balearic Sea. These crustaceans are first intermediate hosts for trypanorhynch cestodes, as commented above, and higher copepod biomass likely favours parasite transmission to the subsequent hosts, *G. melastomus* among them. Oxygen levels off Besós at ca. 700 m depth increase in winter and spring according to Cartes et al. (2011), and maximum abundance values of *Grillotia* sp. in *G. melastomus* were observed in summer and autumn, which might be explained by the time needed by the parasite to reach higher trophic levels, in a similar way as suggested for *D. macrocephalum*.

The different abundance patterns across seasons displayed by *D. macrocephalum* and *Grillotia* sp., in turn associated to the temporal dynamics of the populations of their intermediate hosts (as explained above), likely explain the differences in the structure of parasite infracommunities of this host across the distinct periods of the year, which was further evidenced by the results of the PERMANOVA analysis.

Proleptus obtusus was the preferential parasite of *S. canicula*. The presence of this nematode in all the examined sharks in the Mediterranean Sea is consistent with the previous results in the Atlantic by Moore (2001). Although very few information is available about the life cycle of physalopterid nematodes, *P. obtusus* is known to have a two-host life cycle, with sharks being final and crustaceans intermediate hosts. In the marine environment, larval forms of this nematode have been recovered from the decapods *Carcinus maenas*, *Eupagurus bernhardus*, *Pachygrapsus marmoratus* and *Hyas araneus* (Moravec, 2007 and references therein). Accordingly, Valls et al. (2011) reported reptantian decapods as the most important prey of *S. canicula* on the continental shelf off the slope of the Balearic Islands (NW Mediterranean Sea) (36% IRI). Therefore, the great importance of reptantian decapods in the diet of *S. canicula* is in accordance with the high prevalence and abundance of *P. obtusus* found in the present and in previous studies (Henderson and Dunne, 1998; Moore, 2001). In the case of *G. melastomus*, Carrassón et al. (1992) found that the reptantian decapod *Calocaris macandreae*, although important in juveniles, is only a casual prey in adult sharks at 371–667 m off the continental slope of the Balearic Sea. Valls et al. (2011) also reported that reptantian decapods seem to be of minor importance at depths between 500–750 m in the slope of the Balearic Islands. Hence, the low prevalence and abundance values attained by this parasite in *G. melastomus* are likely due to the low presence of such crustaceans in its diet.

Although no seasonal patterns have been observed for *P. obtusus* in any of the two hosts addressed, the abundance of this parasite has been associated to high temperature and salinity levels in the CCA performed with data of *G. melastomus*. In contrast, *P. obtusus* was not linked to any specific environmental variable in the CCA with data from *S. canicula*, probably because, within this host, this parasite generally reached maximum abundances in hauls with low values of the environmental parameters addressed (see Tables 1 and 4). This inconsistency, coupled with the low abundance of *P. obtusus* in samples of *G. melastomus*, make us reluctant to provide an explanation for the abundance patterns of this parasite based on the environmental variables addressed.

In a similar way as *P. obtusus* in *G. melastomus*, the raphidascaridid nematode *H. aduncum* appeared also linked to high water salinities in present samples of *S. canicula*.

This trend was also observed in the teleost *P. blennoides* off the same waters (Dallarés et al. 2016). In spite of these coincidendes, the abundance of *H. aduncum* in *S. canicula* was low and more consistent results should be obtained before any generalizations are made.

In conclusion, the parasite communities of *S. canicula* and *G. melastomus* in the NW Mediterranean Sea are characterized by low infracommunity richness and diversity, and high dominance. However, significant differences exist in the infracommunity structure and composition between both species, likely due to different feeding habits in turn influenced by differential availability of benthopelagic prey assemblages along the distinct depth ranges inhabited by these two sharks. In general, the parasite faunas of *S. canicula* and *G. melastomus* are comparable to those reported from other small-sized sharks from different areas. Seasonal and geographical variability has been observed in the parasite community of *G. melastomus*, with different parasite composition in winter and spring with respect to summer and autumn and with higher parasite burden in samples from off Besós than off Vilanova, probably due to the vicinity of the Besós submarine canyon to the latter locality. Possible intermediate hosts have been suggested for the more frequent parasites with heteroxenous life cycles based on parasite abundance patterns, the existing knowledge on their life cycles and, when available, previous dietary studies conducted in the sampled area.

Different environmental variables have been linked to the abundance of some parasites, mainly near-bottom turbidity and temperature levels to monogeneans, as these parameters enhance infection and reproductive success of these parasites, and O_2 and turbidity levels, which are known to enhance zooplankton biomass and thus favour parasite transmission, to heteroxenous parasites.

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CHAPTER 6 - THE PARASITE COMMUNITY OF *GALEUS MELASTOMUS* RAFINESQUE, 1810 AND TWO ADDITIONAL SHARKS FROM THE NW MEDITERRANEAN DEEP-SEA IN RELATION TO FEEDING ECOLOGY AND HEALTH CONDITION OF THE HOST AND ENVIRONMENTAL GRADIENTS AND VARIABLES

The parasite community of *Galeus melastomus* Rafinesque, 1810 and two additional sharks from the NW Mediterranean deep-sea in relation to feeding ecology and health condition of the host and environmental gradients and variables

Sara Dallarés[a], María Constenla[a], Francesc Padrós[a], Joan E. Cartes[b], Montserrat Solé[b] and Maite Carrassón[a,]*

[a]Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain
[b]Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37–49, 08003 Barcelona, Spain

*Corresponding author: E-mail address: Maite.Carrasson@uab.cat Tel: +34 935814637

Abstract: The parasite communities of sharks have been largely neglected despite the ecological importance and vulnerability of this group of fish. The main goal of the present study is to describe the parasite communities of three deep-dwelling shark species in the NW Mediterranean. A total of 120 specimens of *Galeus melastomus*, 11 *Etmopterus spinax* and 10 *Centroscymnus coelolepis* were captured at 400–2,200 m depth at two seasons and three localities off the mainland and insular slopes of the Balearic Sea. Environmental and fish biological, parasitological, dietary, enzymatic and histological data were obtained for each specimen, and the relationships among them tested. For *G. melastomus*, *E. spinax* and *C. coelolepis* a total of 15, two and eight parasite species were respectively recovered. The parasite community of *G. melastomus* is characterized by high abundance, richness and diversity, and the cestodes *Ditrachybothridium macrocephalum* and *Grillotia adenoplusia* dominate the infracommunities of juvenile and adult specimens, respectively. A differentiation of parasite communities, linked to a diet shift, has been observed between ontogenic stages of this species. *E. spinax* displays a depauperate parasite community, and that of *C. coelolepis*, described for the first time, shows moderate richness and diversity. Detailed parasite-prey relationships have been discussed and possible transmission pathways suggested for the three hosts. Parasites were mostly related to high water turbidity and O_2 levels, which enhance zooplankton proliferation and could thus enhance parasite transmission. The nematodes *Hysterothylacium aduncum* and *Proleptus obtusus* were linked to high salinity levels, as already reported by previous studies, which are associated to high biomass and diversity of benthic and benthopelagic crustaceans. A decrease of acetylcholinesterase activity and lower hepatosomatic index, possibly linked to infection-related stress, have been observed. Lesions associated to encapsulated larvae of *G. adenoplusia* have been observed in the muscle of *G. melastomus*, especially in the tail region, which can be indicative of the hunting strategy of its final host and may compromise the escape response of *G. melatomus* thus facilitating parasite transmission.

Keywords: *Galeus melastomus*, *Etmopterus spinax*, *Centroscymnus coelolepis*, Parasites, Mediterranean, Deep-sea

1. Introduction

From the late 1960s, bottom-trawl fisheries have progressively spread into deeper grounds as fishing resources became scarce in the continental shelf and pelagic waters (Morato et al., 2006; Norse et al., 2012). This global trend has raised concerns on the status and future perspectives of deep-water fishes (Morato et al., 2006), and chiefly on the still poorly understood deep-dwelling elasmobranchs (Carbonell et al., 2003), whose biological and reproductive characteristics make them very susceptible to population mining (Stevens et al., 2000). Because of their important role as top predators and modellers of the structure and dynamics of marine ecosystems (Stevens et al., 2000), the understanding of their vulnerability and exact role in deep-sea habitats is essential.

In the Balearic Sea, the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 (Carcharhiniformes: Scyliorhinidae) is the most relevant shark in the upper and middle slopes (c.a. 400–800 m and 800–1,400 m, respectively) in terms of abundance and biomass (Carrassón et al., 1992; D'Onghia et al., 2004). Another frequent species, the lanternshark *Etmopterus spinax* (Linnaeus, 1758) (Squaliformes: Etmopteridae) shows its peak of abundance within the same depth range (Stefanescu et al., 1992; D'Onghia et al., 2004). In contrast, the sleeper shark *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello, 1864 (Squaliformes: Somniosidae) represents the most abundant selachian in the lower slope (below c.a. 1,400 m) (Carrassón et al., 1992; Moranta et al., 1998; D'Onghia et al., 2004). It is the only abundant shark and one of the most important contributors to biomass below 2,000 m (Carrassón et al., 1992; Stefanescu et al., 1993; D'Onghia et al., 2004).

Galeus melastomus shows a rather diversified diet, with crustaceans and fishes being preferential prey, while that of *E. spinax* and *C. coelolepis* is largely based on cephalopods (Carrassón et al., 1992). Marked ontogenic diet shifts are known for the three species (Carrassón et al., 1992).

Although none of these species is currently endangered, *G. melastomus* and *E. spinax* are an important by-catch in the sampled area (Carbonell et al., 2003) and *C. coelolepis* bears the status of "Near threatened" according to The Red List of the IUCN (IUCN, 2016).

In spite of being often disregarded, parasites are an important part of all ecosystems (actually, parasite organisms are believed to outnumber free-living species (Price, 1980)) and are informative on many different aspects of their habitats and hosts (Williams et al., 1992). Many parasites use more than one host to complete their life cycle, and can be effectively used to infer dietary habits and trophic interactions of their hosts (Valtonen et al., 2010; Münster et al., 2015). Furthermore, parasites can reflect host phylogenetic relationships (Locke et al., 2013), respond to environmental impacts (Pérez-del-Olmo et al., 2007, 2009a) and their use as discriminators of fish populations has been widely recommended (MacKenzie and Abaunza, 1998). For all these reasons, recent studies have stressed that parasites should be incorporated into food webs (Lafferty et al., 2008) and ecotoxicological studies (Marcogliese et al., 2009), among others.

While the parasite assemblage infecting *G. melastomus* in the NW Mediterranean Sea is fairly well-known (Dallarés et al., in press), very few parasite data is available for *C. coelolepis* and *E. spinax* in this area (Guiart, 1935; Euzet, 1959). For the two latter species, a relevant number of single parasite records exist from the Atlantic Ocean (among others, Guiart, 1935; Gibson and Bray, 1977; Pascoe, 1987; Bates, 1990; Caira and Pickering, 2013 for *C. coelolepis* and Pintner, 1930; Williams, 1959; Hennemann, 1985; Noever et al., 2010; Caira and Pickering, 2013 for *E. spinax*) and two studies have addressed the parasite community of *E. spinax* in Atlantic waters (Klimpel et al., 2003 (only for juvenile specimens); Isbert et al., 2015).

Parasite-host relationships are mainly characterized by damage inflicted to the host by the parasite, which would be expected to alter stress markers or induce histological alterations. In this sense, enzymatic activities respond in a natural way to biological factors such as size, sex or swimming behaviour (Drazen and Seibel, 2007; Koenig and Solé, 2014), but have also proved to be effective biomarkers of ecosystem alterations or stressing conditions in fishes (Chatterjee et al., 2010). Effects of fish parasites on enzymatic markers have barely been addressed and have never been attempted in sharks. Furthermore, the few existing studies in teleosts have yielded contradicting results (Podolska and Napierska, 2006; Pérez-i-García et al., 2015; Dallarés et al., 2014, 2016). Actually, information on enzymatic activities of deep-dwelling chondrichthyans is overall scarce and, in relation to the species addressed in the present work, only *G. melastomus* and *E. spinax* have received attention (Totland et al., 1978; Fänge et al., 1979; Solé et al., 2008, 2010), while *C. coelolepis* remains essentially unstudied regarding this aspect.

In a similar way, histopathological changes in different organs and systems have been used as markers of fish health status and as indicators of environmental changes (Carrassón et al., 2008; Carreras-Aubets et al., 2011; Fricke et al., 2012). Quantitative variations in numbers of melano-macrophages (MM) or MM aggregates are also known to be driven by physiological changes, pathological conditions or environmental pollution in teleosts (see Agius and Roberts, 2003 and references therein; Carrassón et al., 2008). In the last years, these structures have been characterized in a few shark species (Borucinska et al., 2009), but data are still extremely limited for this group and lacking for the three species addressed in the present study.

The aim of this study is, first and foremost, to carry out the first complete description of the parasite communities of *G. melastomus, E. spinax* and *C. coelolepis* in the Balearic basin. Secondly, ontogenic and environmental variability (eight distinct locality-season-depth combinations) on the composition of such parasite community is assessed for *G. melastomus*. With the aim of explaining infection patterns of the different parasites, their associations to environmental variables (water temperature, salinity, oxygen content and turbidity) and to the abundance of host prey identified in guts are tested. In order to assess the possible effects of parasite load on health condition of the hosts addressed, relationships between parasite abundance and fish general condition indices, activity of enzymatic biomarkers and density of hepatic MM (only for *G. melastomus*) are explored. Moreover, the presence of parasite-induced histological alterations and pathological conditions is evaluated in different organs.

2. Materials and methods

2.1. Sampling area and specimen collection

A total of 120 specimens of *G. melastomus*, 11 *E. spinax* and 10 *C. coelolepis* were captured in summer (July) 2010, summer (June) 2011 and autumn (October) 2011 at 400–2,200 m depth in the Balearic Sea (north-western Mediterranean Sea) (Fig. 1). Hauls were carried out on board of the research vessels Garcia del Cid and Sarmiento de Gamboa using a semi-balloon otter-trawl (OTSB 14) at three different localities: one off the mainland slope (Barcelona) and two off the slope of the Balearic Islands (Mallorca and Ibiza) (Fig. 1, Table 1).

Environmental data: temperature (T) in ºC, salinity (S) in psu, O_2 concentration in ml/l and turbidity (voltage), the latter representing organic and inorganic suspended material, were taken at 5 m above the sea-bottom by deployment of a CTD simultaneously (same data, same depth) to fish samplings (Table 1).

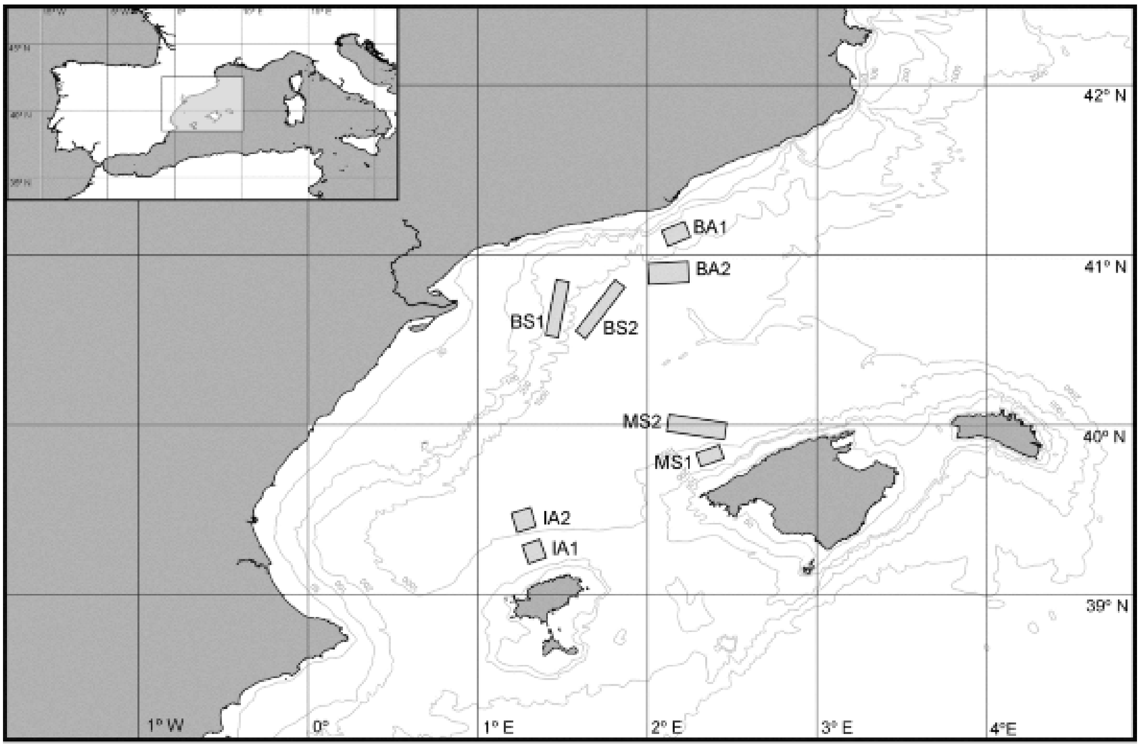

Figure 1. Study area showing the sampling localities in the Balearic Sea. BA1, Barcelona autumn at depth 1; BA2, Barcelona autumn at depth 2; BS1, Barcelona summer at depth 1; BS2, Barcelona summer at depth 2; IA1, Ibiza autumn at depth 1; IA2, Ibiza autumn at depth 2; MS1, Mallorca summer at depth 1; MS2, Mallorca summer at depth 2. Depth 1 (upper slope): 400–1,000 m; depth 2 (middle and lower slopes): > 1,000 m.

Table 1. Sampling data of the three species of sharks addressed. n: number of specimens; T ºC: temperature; S: salinity in psu; O: oxygen concentration in ml/L; Turb: Turbidity in V. Abbreviations for locality-season-depth groups (habitats): BS2: Barcelona summer, depth 2; MS2: Mallorca summer, depth 2; MS1: Mallorca summer, depth 1; BS1: Barcelona summer, depth 1; BA1: Barcelona autumn, depth 1; BA2: Barcelona autumn, depth 2; IA1: Ibiza autumn, depth 1; IA2: Ibiza autumn, depth 2.

Habitat	**Haul**	**Date**	**Depth (m)**	**Coordinates**		**n**			**Environmental variables**			
				Latitude (deg, min, N)	**Longitude (deg, min, E)**	***G. melastomus***	***C. coelolepis***	***E. spinax***	**T ºC**	**S (psu)**	**O (mL/L)**	**Turb (V)**
BS2	A1–3	08/07/2010	1,048	40º 58.06	2º 5.30	5			13.08	38.48	4.41	0.06
BS2	A1–4	08/07/2010	1,024	40º 58.69	2º 1.14	9			13.20	38.51	4.18	0.06
BS2	A1–6	10/07/2010	1,308	40º 53.85	2º 4.00	3			13.08	38.48	4.41	0.06
MS2	A1–13	16/07/2010	2,057	40º 38.82	3º 6.70		1		13.20	38.48	4.35	0.05
MS2	A1–14	16/07/2010	2,194	40º 37.59	3º 27.82		1		13.22	38.48	4.34	0.06
MS1	A1–15	17/07/2010	682	39º 48.24	2º 20.55	9			13.07	38.49	4.14	0.90
MS1	A1–16	17/07/2010	457	39º 46.73	2º 21.85	5			13.09	38.50	4.12	0.07
MS2	A1–17	19/07/2010	1,006	39º 52.39	2º 20.26	5			13.06	38.48	4.33	0.08
MS2	A1–18	19/07/2010	1,006	39º 53.37	2º 18.66	15			13.06	38.48	4.33	0.08
MS2	A1–19	19/07/2010	1,232	39º 55.16	2º 8.25	1	1		13.08	38.48	4.38	0.07
BS2	A1–24	22/07/2010	2,197	41º 4.31	3º 16.74		2		13.22	38.48	4.34	0.06
BS1	A2–1	18/06/2011	639	40º 34.50	1º 26.51	3			13.14	38.50	4.16	1.03
BS1	A2–2	18/06/2011	646	40º 34.45	1º 26.44	1			13.14	38.50	4.16	1.03
BS1	A2–4	19/06/2011	627	40º 54.40	1º 34.61	3			13.12	38.50	4.14	0.24
BS1	A2–5	19/06/2011	628	40º 54.72	1º 34.80	1			13.12	38.50	4.14	0.24
BS1	A2–6	19/06/2011	648	40º 54.32	1º 34.83	3			13.12	38.50	4.14	0.24
BS1	A2–8	20/06/2011	632	40º 40.86	1º 26.44	3			13.08	38.49	4.20	0.00
BS2	A2–11	23/06/2011	1,043	40º 51.97	1º 44.29	4			13.10	38.49	4.17	0.13
BS2	A2–12	23/06/2011	1,06	40º 47.23	1º 35.24	4			13.10	38.49	4.17	0.13
BS2	A2–13	23/06/2011	1,052	40º 55.86	1º 50.32	1			13.10	38.49	4.17	0.13
BA1	A3–1	14/10/2011	661	41º 5.88	2º 13.34	8			13.28	38.54	3.91	0.33
BA1	A3–2	14/10/2011	581	41º 7.85	2º 5.32	9			13.38	38.55	3.86	0.64
BA2	A3–3	15/10/2011	1,051	40º 50.80	1º 43.94	2			13.11	38.49	4.21	0.24
BA2	A3–4	15/10/2011	1,236	40º 41.96	1º 37.46	12		2	13.12	38.48	4.25	0.28
BA2	A3–6	16/10/2011	1,751	40º 9.65	2º 0.23		4		13.18	38.49	4.32	0.21
IA1	A3–7	17/10/2011	508	39º 11.63	1º 18.88	1		3	13.07	38.49	4.10	0.38
IA1	A3–8	17/10/2011	508	39º 13.92	1º 23.52	4		6	13.07	38.49	4.10	0.38
IA2	A3–9	17/10/2011	573	39º 13.92	1º 23.52	1			13.07	38.49	4.29	0.25
IA2	A3–10	18/10/2011	1,272	39º 25.31	1º 16.84	8			13.10	38.49	4.16	0.23
IA2	A3–11	19/10/2011	1,626	39º 56.20	1º 37.91		1		13.15	38.49	4.31	0.20

Immediately upon capture, records of total length (TL) in mm and total weight (TW) in g were obtained for each fish. A portion of the axial muscle was dissected out and kept at –20 ºC for biochemical purposes, and samples of right gills, liver, spleen, gonads and spiral valve (the latter for *G. melastomus* only) were immediately fixed in 10% buffered formalin for histological analyses. The rest of the specimen was frozen at –20 ºC for parasitological examination.

2.2. Parasitological study

Once in the laboratory, fish were thawed and external surfaces, buccal cavity and olfactory organs were examined macroscopically and under stereomicroscope in search of ectoparasites. All organs and musculature were carefully removed and inspected for endoparasites. Liver and gonads were weighed prior to examination. Parasites collected were counted and preserved in 70% ethanol. Nematodes, copepods and everted tentacles of trypanorhynch cestodes were studied as temporary mounts in glycerine. Cestodes were stained with iron acetocarmine, dehydrated through a graded ethanol series, cleared in clove oil and examined as permanent mounts in Canada balsam. Molecular analyses were also performed to confirm the identity of the cestode *Ditrachybothridium macrocephalum* Rees, 1959 (see Dallarés et al. (2015) for methodological details). All parasites were identified to the lowest possible taxonomic level. Nomenclature of tapeworm larval stages follows Chervy (2002).

The following voucher material was deposited in the Helminthological Collection of the Universitat Autònoma de Barcelona (UABhc) under the following accession numbers: *G. melastomus*: C22, 23 (*D. macrocephalum*), C24 (*Grillotia adenoplusia* (Pintner, 1903) Palm, 2004), N7 (*Proleptus obtusus* Dujardin, 1845), N8 (*Piscicapillaria baylisi* Moravec, 1987) and Co3 (*Eudactylina vilelai* Nuñes-Ruivo, 1956); *C. coelolepis*: C25 (*Clistobothrium* sp.) and C26 (*Monorygma* sp.). Material of *Sphyriocephalus viridis* (Wagener, 1854) from present specimens of *G. melastomus* and *C. coelolepis* were deposited in the same collection in a previous study (see Dallarés et al., 2017).

2.3. Diet analysis

Stomach contents of 104 specimens of *G. melastomus* previously examined for the occurrence of parasites were further analysed for diet determination. For each fish, prey recovered in guts were identified to the lowest possible taxonomic level using a stereomicroscope, counted and weighed to the nearest 0.001 g. Diet was considered by prey number, hence being directly comparable with parasitological indices. In previous articles (see Dallarés et al., 2014, 2016) details on methodological limitations, e.g. difficulties in identifying and counting soft animals, have been provided. In parallel, stomach contents of *E. spinax* and *C. coelolepis*, rare at the usual depth range inhabited by *G. melastomus*, were analysed. Results of nine specimens of *E. spinax* and seven of *C. coelolepis* were considered for further prey-parasite analyses (see below). In the case of *C. coelolepis*, some prey items (e.g. carrion) were identified by means of molecular techniques (see Cartes et al., 2016).

2.4. Enzymatic determinations

A muscle portion of about 0.3 g was used for acetylcholinesterase (AChE), lactate dehydrogenase (LDH) and citrate synthase (CS) determinations. The tissue was homogenised in a 50 mM buffer phosphate (pH 7.4) in a 1:5 (weight:volume) ratio using a polytron$^{®}$ blender. The homogenate was centrifuged at 10,000 g × 30 min and the supernatant (S10) was used for biochemical determinations.

A range of six concentrations of acetylthiocholine iodide (ATC) from 0.05 to 10 mM was used to determine kinetic constants such as V_{max} and K_m of AChE in each species and catalytic efficiency measured as V_{max}/K_m. For AChE determination, the concentration of the substrate (ATC) selected was 1 mM, as described in Solé et al. (2010). Acetylcholinesterase activity was assayed according to the principle of Ellman et al. (1961) at 405 nm. Lactate dehydrogenase activity was determined at 340 nm according to the method developed by Vassault (1983), modified to 96-well microplate format, with the reactive quantities described in Dallarés et al. (2014) and using 1:40, 1:80 (for samples from *G. melastomus*) or 1:100 (for samples from *C. coelolepis* and *E. spinax*) diluted original sample. For CS assay, the protocol used was based on Childress and Somero (1979) at 412 nm and final conditions were: 0.1mM of dithiobis-nitrobenzoic acid solution in 50 mM Tris-HCl buffer (pH 8) were mixed with 25 µl of 1:5 diluted (for samples from *G. melastomus*) or undiluted sample (for samples from *C. coelolepis* and *E. spinax*), 0.1 mM of acetyl CoA and 0.5 mM of oxaloacetate in each microplate well.

In all determinations, reading was performed in triplicate in a microplate reader (TECAN Infinite 200) during 5 min at 25 ºC. Activity was expressed in nmol/min/mg protein.

Total protein content in the S10 fraction was determined by the Bradford (1976) method using bovine serum albumin as standard (BSA 0.05–0.5 mg/ml).

2.5. Histological assessment

Fixed samples of gills, liver, spleen, gonads and spiral valve (the latter for *G. melastomus* only) from the three elasmobranch species were embedded in paraffin, sectioned at 4 µm, stained with Haematoxylin and Eosin and examined microscopically. A quantitative analysis of melano-macrophages (MM) in liver sections of *G. melastomus* was carried out using a MicroComp Integrated Image Analysis System. Melano-macrophages were counted in five randomly-selected fields of view from each liver section at 100× of magnification (0.92 mm^2/screen), and MM/mm^2 was calculated for each field.

Protist parasites (i.e. intestinal coccidians) were only detected in histological sections. In this case, prevalence and intensity were calculated. Intensity was determined by counting the number of oocysts in five randomly-selected fields of view on each section of the spiral valve of the infected sharks at 400× of magnification (0.06 mm^2/screen), and was expressed as number of oocysts (OC) per square millimetre. Only developing and mature oocysts which could be clearly identified were considered.

2.6. Data Analyses

Two distinct size-groups for specimens of *G. melastomus*, i.e. size 1 (TL < 34 cm for males and TL < 40 cm for females) and size 2 (TL ≥ 34 cm for males and TL ≥ 40 cm for females), corresponding to juvenile and adult sharks (Capapé and Zaouali, 1977), were established prior to analyses.

Parasitological terms such as prevalence (P) and mean abundance (MA) were calculated following Bush et al. (1997) and using data from all specimens. Parasite taxa with total P $\geq 8\%$ within any size-group of *G. melastomus* and $> 30\%$ in the total sample of *E. spinax* and *C. coelolepis* were considered not-accidental and are henceforth called common (P threshold was increased in the two latter species due to the low number of specimens available). Diversity and dominance of parasite infracommunities (i.e. all parasites of all taxa in an individual fish) were estimated using Brillouin's diversity index (PRIMER v6; Anderson et al., 2008) and Berger-Parker dominance index (B-P, calculated as the number of individuals of the most abundant parasite species divided by the total number of parasites in a given fish host), respectively. Fish condition was assessed by condition factor (K, calculated as $(TW/TL^3) \times 100$), hepatosomatic index (HSI, calculated as (liver weight/TW) × 100) and gonadosomatic index (GSI, calculated as (gonad weight/TW) × 100). In all tests, GSI was not considered for juvenile sharks and was addressed separately for adult females and males. Analyses involving MM/mm^2 and OC/mm^2 from *G. melastomus* were performed using the average value of the five field-measurements calculated for each section.

2.6.1. Galeus melastomus

Firstly, a non-parametric Multi-Dimensional Scaling (MDS) was applied on infracommunity data using the factor “size” in order to visualize the ordination of parasite infracommunities of *G. melastomus* with respect to the maturity stage of their host. A permutation multivariate analysis (PERMANOVA) was also carried out using infracommunities as replicate samples in order to test an age-related parasitological differentiation. Both analyses were applied on a Bray-Curtis similarity matrix derived from logarithmically transformed (log(x+1)) abundance data (PRIMER v6; Anderson et al., 2008). For the PERMANOVA analysis, permutation p-values were obtained under unrestricted permutation of raw data (9,999 permutations). Generalized Models (GZM) were applied to test possible associations between fish TL or condition indices (K, HSI and GSI) (set as covariates) and individual total parasite abundance, abundance of common parasites and parasite richness. Spearman rank correlation tests were used to assess relationships between fish TL or condition indices and diversity and dominance of parasite infracommunities. In view of the results obtained in the analyses above described, the description of the parasite community of *G. melastomus* was performed in the two size-groups of hosts separately.

Differences among the eight locality-season-depth groups (each of the following subdivided into depths 1 (i.e. upper slope: 400–1,000 m) and 2 (i.e. middle and lower slopes: 1,000–2,200 m): Barcelona summer (BS1 and BS2), Barcelona autumn (BA1 and BA2), Mallorca summer (MS1 and MS2) and Ibiza autumn (IA1 and IA2)) were tested for infracommunity parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (Brillouin’s index) (MD) and mean dominance (Berger-Parker’s index) (B-P)), fish TL and condition indices (K, HSI and GSI), enzymatic activity levels (AChE, LDH and CS), density of hepatic melano-macrophages (MM/mm^2) and intensity of intestinal coccidia (OC/mm^2) of *G. melastomus* by means of General Linear Models (GLM) followed by post hoc tests, GZM (for TMA and MSR) or Kruskal-Wallis tests (for TL, MD, B-P and OC/mm^2). Differences among groups on parasitological descriptors were tested in the two size-groups of hosts separately. In all contrasts among categorical groups, only groups with n ≥ 5 host specimens were considered. No differences were tested for male GSI data because only one group showed n ≥ 5. Enzymatic and MM data were log-transformed prior to analyses to comply for normality and homoscedasticity requirements.

In order to visualize the parasite abundance patterns in relation to the categorical groups, factorial correspondence analyses (FCA) were applied on data matrices containing component population (i.e. all parasites of the same taxa and developmental stage in a particular group of hosts) abundance data of the common parasites in juvenile and adult sharks separately. Hierarchical cluster analyses were simultaneously performed based on the coordinates of the first two axes obtained in the corresponding FCA to define host groups clearly. In both cases, all-zero samples were removed. Then, using individual fish as replicate samples, differences in MA and P of the common parasites of each size-group of hosts were tested across locality-season-depth groups by GZM (applying the log-binomial model for abundance and the logistic model for prevalence).

Possible relationships between the most abundant parasites and prey were analysed by Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). A matrix was generated including individuals for which parasitological and dietary information was available. To build this matrix, parasitological and dietary information were grouped by haul in the case of *G. melastomus*, and exceptionally, when the number of specimens per haul was low, some hauls were grouped. Grouped hauls (i.e. A201/02/08 and A204/06) included specimens caught at the same locality, season and depth. Number of specimens analysed per haul (or group of hauls) ranged between four and 15. Bearing in mind that *G. melastomus* hosts a moderate parasite richness and diversity, four specimens were considered representative enough of the diet of this species for our objectives. Parasites with occurrences ≥ 2 were included in the analysis, accumulating a total of 10 parasites (and 15 prey) in the CCA matrix. The canonical correspondence analysis related in this case the abundance of main parasites (using infracommunity data) with prey found in guts. In CCA plots, arrows represent explanatory variables and are proportional in length to their importance on the explained variable. The same analysis was repeated in order to assess the relationships between the abundance of main parasites and environmental variables (T, S, O_2 and turbidity).

Enzymatic activity values and MM/mm^2 were used as covariates to assess their possible association with individual total parasite abundance, abundance of common parasites and parasite richness by means of GZM. The relationship of enzymatic activity values and MM/mm^2 with individual parasite diversity and dominance was assessed by Spearman rank correlation tests.

The correlations between log-transformed enzymatic activity levels and fish TL, condition indices and MM/mm^2 were tested by means of GLM. In a similar way, possible associations between log-transformed MM data and fish TL and condition indices were assessed by GLM.

Possible associations between OC/mm^2 and fish TL, condition indices (GSI was considered only for females, due to the low number of male sharks infected by intestinal coccidians), enzymatic activity levels and MM/mm^2 were tested by Spearman Rank correlation tests.

2.6.2. Etmopterus spinax and Centroscymnus coelolepis

Parasite infracommunity richness, diversity and dominance data of *E. spinax* were not used in the data analyses due to their almost uniform value in all host specimens. Gonadosomatic index was not considered for *E. spinax* because most specimens were juveniles, nor for males of *C. coelolepis* due to low sample numbers.

Nine specimens of *E. spinax* and seven *C. coelolepis* were included in the CCA relating the most important parasites with prey found in guts (described above, see section 2.6.1.). The two assemblages of *C. coelolepis* were grouped as a function of season (A1, A3) including hauls from different depths (between 1,626–2,224 m). For *E. spinax* only the group A3/4, containing three sharks, was finally included in the CCA, since the parasitological diversity and abundance of the rest of specimens was very low. Parasite abundance was much higher in the specimens of the group A3/4, although parasite diversity was also low. Actually, the diet of *E. spinax* at > 1,000 m, where the specimens of the group A3/4 were sampled, is known to be also poorly diversified, exclusively based on cephalopods (Carrassón et al., 1992, Cartes et al., 2016).

The same specimens of *E. spinax* and *C. coelolepis* were included in the CCA assessing relationships between the abundance of main parasites and environmental variables.

Fish TL, condition indices and enzymatic activity values were set as covariates to assess their relationship with individual total parasite abundance, the abundance of common parasites and parasite richness in the case of *C. coelolepis*, and with the abundance of larval tetraphyllidean cestodes (Tetraphyllidea fam. gen. sp., collectively known as *Scolex pleuronectis* Müller, 1788) in the case of *E. spinax* by means of GZM. Total parasite abundance data of *E. spinax* were not used due to the meaningless contribution of the only parasite apart from Tetraphyllidea fam. gen. sp. (i.e. *Aporhynchus norvegicus* (Olsson, 1868)).

Spearman Rank correlation tests were applied to test the association of fish TL and condition indices with individual parasite diversity and dominance of *C. coelolepis*. General linear model analyses were used to test the relationship between square-root-transformed enzymatic activity levels of both species and fish individual parasite diversity and dominance (for *C. coelolepis* only), fish TL and condition indices.

3. Results

Unless otherwise stated, no interaction was found between fish TL and factors or covariates tested in any of the above described analyses.

3.1. Fish biological factors

For *G. melastomus*, TL of the sampled fish ranged between 100 and 610 mm. Significant differences among groups for fish TL (Kruskal-Wallis, χ^2= 36.382, $p< 0.001$) and K (GLM, $F_{(4,\ 38)}$= 3.108, p= 0.026) were detected, with fish from off Mallorca in summer at depth 1 showing lower values (Table 2). No differences among groups were detected for HSI or GSI ($p > 0.05$).

For *E. spinax* and *C. coelolepis*, TL of the sampled fish ranged between 152 and 435 mm and between 243 and 660 mm, respectively.

Table 2. Means and standard deviations of fish total length (TL), condition factor (K), hepatosomatic index (HSI), adults gonadosomatic index (GSI), acetylcholinesterase (AChE), lactate dehydrogenase (LDH) and citrate synthase (CS) activities and density of hepatic melano-macrophages (MM/mm^{2}) in the different categorical groups of *Galeus melastomus*. N: sample size of *G. melastomus*; (*): number of females. Different superscript letters show significant differences among categorical groups. Dashes indicate non-available data.

	Mainland slope				**Insular slope**			
	Barcelona summer		**Barcelona autumn**		**Mallorca summer**		**Ibiza autumn**	
	400-1,000 m	**1,000-1,400 m**	**400-1,000 m**	**1,000-1,400 m**	**400-1,000 m**	**1,000-1,400 m**	**400-1,000 m**	**1,000-1,400 m**
N(*)	14(8)	26(23)	17(8)	14(13)	14(12)	21(21)	5(2)	9(7)
TL	38.24 ± 19.60^{A}	45.65 ± 13.39^{A}	34.00 ± 17.92^{A}	38.40 ± 19.52^{A}	13.60 ± 3.38^{B}	43.15 ± 13.53^{A}	39.98 ± 16.97^{A}	39.38 ± 19.37^{A}
K	0.29 ± 0.06^{A}	0.29 ± 0.03^{A}	0.27 ± 0.04^{A}	0.29 ± 0.07^{A}	0.23 ± 0.03^{B}	0.28 ± 0.03^{A}	0.29 ± 0.04^{A}	0.27 ± 0.02^{A}
HSI	5.20 ± 3.10^{A}	4.44 ± 1.77^{A}	3.97 ± 1.41^{A}	3.57 ± 1.38^{A}	4.34 ± 2.27^{A}	5.05 ± 1.25^{A}	4.20 ± 1.31^{A}	3.60 ± 1.80^{A}
GSI (females)	4.28 ± 2.83^{A}	2.45 ± 1.97^{A}	2.54 ± 0.90^{A}	2.26 ± 2.19^{A}	–	2.87 ± 2.07^{A}	2.00 ± 2.55^{A}	2.50 ± 2.00^{A}
GSI (males)	2.04 ± 0.50	0.99 ± 0.78	0.91 ± 0.36	–	–	1.13 ± 1.20	1.76	2.08
AChE	22.84 ± 19.79^{AB}	10.72 ± 8.11^{B}	22.91 ± 16.15^{AB}	32.55 ± 27.14^{A}	26.73 ± 4.69	12.33 ± 5.95^{B}	18.31 ± 8.07	18.85 ± 8.92^{AB}
LDH	1,728 ± 445^{BC}	1,330 ± 397^{C}	1,860 ± 676^{BC}	2,925 ± 1,298^{A}	1,437 ± 46	1,483 ± 328^{C}	1,628 ± 517	2,398 ± 889^{AB}
CS	24.19 ± 6.01^{C}	24.51 ± 7.15^{C}	30.92 ± 17.92^{C}	64.68 ± 31.54^{A}	18.70 ± 0.37	34.91 ± 12.88^{BC}	32.58 ± 13.28	59.40 ± 37.19^{AB}
MM/mm^{2}	138.89 ± 171.97^{A}	187.95 ± 170.71^{A}	–	344.96 ± 251.31^{A}	34.11 ± 20.00^{A}	112.41 ± 96.62^{A}	91.00 ± 19.82	383.18 ± 358.53^{A}

3.2. Composition of the parasite communities and their relationship with fish biological parameters and environmental gradients and variables

3.2.1. Galeus melastomus

Globally, percentage of uninfected sharks was 10.83%. In the parasitized specimens, a total of 15 different metazoan parasite taxa were recovered: one monogenean, one digenean, five cestodes, seven nematodes and one copepod (Tables 3 and 4). Of these, the cestodes *G. adenoplusia* and Tetraphyllidea fam. gen. sp., and the nematodes Anisakidae gen. sp. and *Collarinema collaris* (Petter, 1970) constitute new host records. The MDS providing an ordination of parasite infracommunities with respect to host size evidenced a differentiation of such communities between juvenile and adult specimens of *G. melastomus* (Stress= 0.06, Fig. 2). The PERMANOVA analysis testing the effect of host size on the composition of parasite infracommunities showed a significant difference between juvenile and adult sharks on such composition (Pseudo-$F_{(1,\ 104)}$= 42.715, $p_{(perm)}$= 0.0001; 9,955 unique permutations).

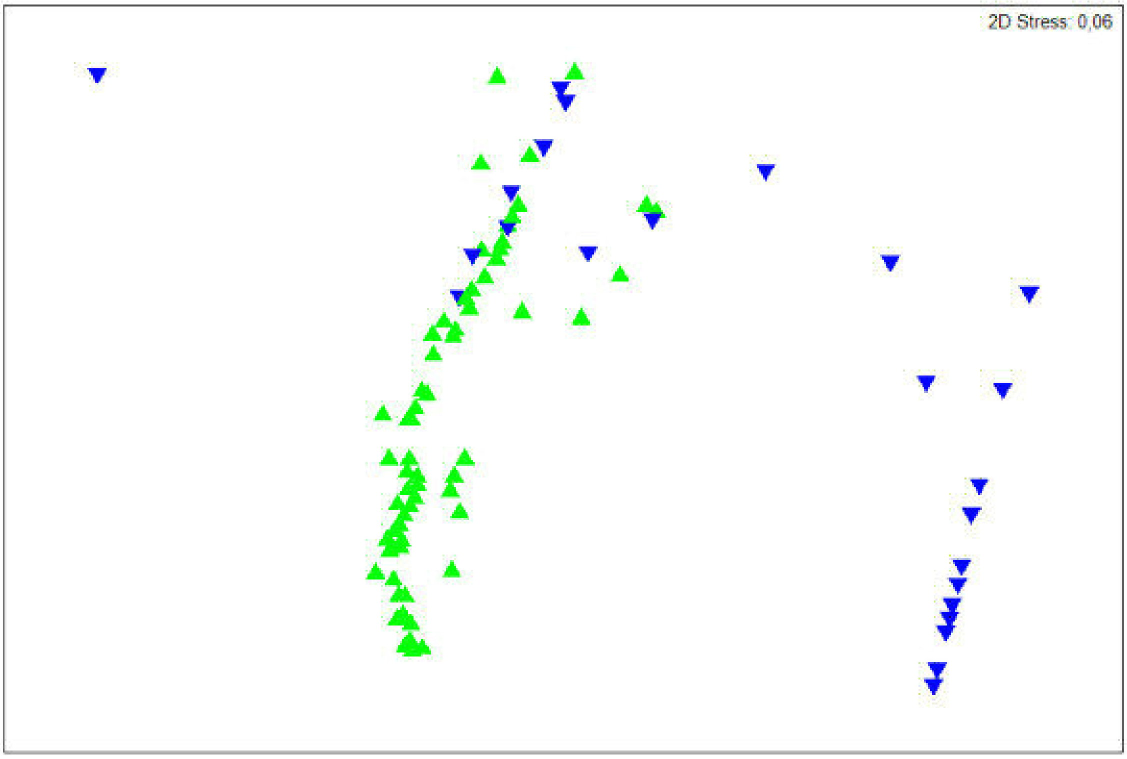

Figure 2. Multi-dimensional scaling (MDS) showing ordination of infracommunities of juvenile (▼) and adult (▲) specimens of *Galeus melastomus*.

Table 3. Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in juvenile specimens of *Galeus melastomus,* and means and standard deviations of fish parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)) in the different categorical groups of juvenile specimens of *G. melastomus*. N: sample size of *G. melastomus*; SR: species richness. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Mt, metacercaria; Pd, plerocercoid; Ps, plerocercus. Abbreviations for locations within host: G, gills; I, intestine; M, muscle; S, stomach; SW, stomach wall (encysted). Different superscript letters and numbers show significant differences among categorical groups in abundance and prevalence, respectively. Dashes indicate absence of the parasite.

	Stage	**Location**	**Mainland slope**								**Insular slope**							
			Barcelona summer				**Barcelona autumn**				**Mallorca summer**				**Ibiza autumn**			
			400-1,000 m		**1,000-1,400 m**		**400-1,000 m**		**1,000-1,400 m**		**400-1,000 m**		**1,000-1,400 m**		**400-1,000 m**		**1,000-1,400 m**	
N			**5**		**3**		**10**		**6**		**14**		**9**		**2**		**1**	
			P(%)	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**
Digenea																		
Otodistomum sp.	Mt	S, SW	-	-	-	-	-	-	-	-	-	-	11	0.11 ± 0.33	50	0.50 ± 0.71	-	-
Cestoda																		
Ditrachybothridium macrocephalum	Ps, J, A	S, I	40^{1}	0.40 ± 0.55^{A}	67	1.00 ± 1.00	60^{1}	1.2 ± 1.55^{A}	50^{1}	1.17 ± 1.94^{A}	71^{1}	6.00 ± 5.68^{B}	33^{1}	1.89 ± 3.48^{A}	50	0.50 ± 0.71	100	1.00
Grillotia adenoplusia	Ps	M	-	-	-	-	20^{1}	1.00 ± 2.31^{A}	17^{1}	0.17 ± 0.41^{A}	-	-	78^{2}	7.67 ± 6.38^{B}	50	7.50 ± 10.61	-	-
Tetraphyllidea fam. gen. sp.	Pd	I	-	-	-	-	-	-	-	-	-	-	11	0.22 ± 0.67	-	-	-	-
Nematoda																		
Hysterothylacium aduncum	L3	S	-	-	-	-	10	0.10 ± 0.32	-	-	-	-	-	-	-	-	-	-
Hysterothylacium sp.	L3/L4	S	20	0.20 ± 0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proleptus obtusus	L3/L4/A	S, I	-	-	-	-	10	0.40 ± 1.26	-	-	-	-	-	-	-	-	-	-
Collarinema collaris	L3/L4	S	-	-	-	-	-	-	-	-	-	-	-	-	50	0.50 ± 0.71	-	-
Piscicapillaria baylisi	A	I	-	-	-	-	-	-	-	-	-	-	11	0.11 ± 0.33	-	-	-	-
Copepoda																		
Eudactylina vilelai	A	G	-	-	67	0.67 ± 0.58	-	-	-	-	-	-	11	0.11 ± 0.33	50	0.50 ± 0.71	-	-
TMA			0.60 ± 0.55^{A}		1.67 ± 1.15		2.70 ± 3.43^{AB}		1.33 ± 1.97^{A}		6.00 ± 5.68^{BC}		10.11 ± 5.06^{C}		9.50 ± 12.02		1.00	
SR			1		3		6		2		1		3		4		1	
MSR			0.60 ± 0.55^{A}		1.67 ± 1.15		1.40 ± 1.90^{A}		0.67 ± 0.82^{A}		0.71 ± 0.47^{A}		1.56 ± 0.73^{A}		2.50 ± 2.12		1.00	
MD (Brillouin's index)			0.00 ± 0.00^{A}		0.12 ± 0.21		0.20 ± 0.31^{A}		0.12 ± 0.20^{A}		0.00 ± 0.00^{A}		0.13 ± 0.17^{A}		0.24 ± 0.33		0.00	
B-P (Berger-Parker's index)			1.00 ± 0.00^{A}		0.89 ± 0.19		0.88 ± 0.21^{A}		0.83 ± 0.29^{A}		1.00 ± 0.00^{A}		0.94 ± 0.09^{A}		0.92 ± 0.12		1.00	

Table 4. Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in adult specimens of *Galeus melastomus,* and means and standard deviations of fish parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)) in the different categorical groups of adult specimens of *G. melastomus*. N: sample size of *G. melastomus*; SR: species richness. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Mt, metacercaria; Pd, plerocercoid; Ps, plerocercus. Abbreviations for locations within host: Ca, Abdominal cavity; G, gills; I, intestine; M, muscle; S, stomach; SW, stomach wall (encysted). Different superscript letters and numbers show significant differences among categorical groups in abundance and prevalence, respectively. Dashes indicate absence of the parasite.

	Stage	**Location**	**Mainland slope**								**Insular slope**					
			Barcelona summer				**Barcelona autumn**				**Mallorca summer**		**Ibiza autumn**			
			400-1,000 m		**1,000-1,400 m**		**400-1,000 m**		**1,000-1,400 m**		**1,000-1,400 m**		**400-1,000 m**		**1,000-1,400 m**	
N			**9**		**23**		**7**		**8**		**12**		**3**		**8**	
			P(%)	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**
Monogenea																
Monogenea indet.	A	G	-	-	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Digenea																
Otodistomum sp.	Mt	S, SW	-	-	-	-	-	-	50^{1}	1.75 ± 1.91^{A}	8^{1}	1.17 ± 4.04^{A}	-	-	25^{1}	2.13 ± 5.25^{A}
Cestoda																
Ditrachybothridium macrocephalum	Ps, J, A	S, I	-	-	26^{1}	0.35 ± 0.65^{A}	29^{1}	0.29 ± 0.49^{A}	13^{1}	0.25 ± 0.71^{A}	8^{1}	0.08 ± 0.29^{A}	-	-	-	-
Grillotia adenoplusia	Ps	M, S, Ca	100^{1}	84.67 ± 66.34^{A}	100^{1}	53.04 ± 55.93^{A}	100^{1}	35.29 ± 38.66^{A}	100^{1}	79.13 ± 61.30^{A}	100^{1}	70.67 ± 67.27^{A}	100	32.00 ± 29.87	100^{1}	78.88 ± 63.16^{A}
Sphyriocephalus viridis	Pd	S	11	0.11 ± 0.33	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Hepatoxylon trichiuri	Pd	I	-	-	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Tetraphyllidea fam. gen. sp.	Pd	I, S	11^{1}	0.11 ± 0.33^{A}	13^{1}	0.35 ± 1.27^{A}	-	-	-	-	25^{1}	0.42 ± 0.90^{A}	-	-	13^{1}	0.75 ± 2.12^{A}
Nematoda																
Anisakis Type ll	L3	S	-	-	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Hysterothylacium aduncum	L3	I	-	-	-	-	14	0.29 ± 0.76	-	-	-	-	-	-	-	-
Anisakidae gen. sp.	L3	I	-	-	-	-	-	-	13	0.13 ± 0.35	-	-	-	-	-	-
Proleptus obtusus	L3	S	11	0.11 ± 0.33	-	-	14	0.14 ± 0.38	-	-	-	-	-	-	-	-
Collarinema collaris	A	S	11	0.11 ± 0.33	-	-	-	-	-	-	-	-	-	-	-	-
Piscicapillaria baylisi	A	I	22	0.22 ± 0.44	4	0.04 ± 0.21	-	-	-	-	8	0.42 ± 1.44	-	-	-	-
Copepoda																
Eudactylina vilelai	A	G	100^{1}	2.56 ± 1.81^{A}	52^{1}	1.61 ± 2.31^{A}	57^{1}	1.57 ± 1.90^{A}	88^{1}	2.00 ± 2.14^{A}	42^{1}	0.92 ± 1.51^{A}	67	2.33 ± 3.21	100^{1}	2.88 ± 3.00^{A}
TMA			87.89 ± 68.27^{A}		55.57 ± 56.12^{A}		37.57 ± 37.39^{A}		83.25 ± 61.08^{A}		73.67 ± 67.74^{A}		34.33 ± 32.93		84.63 ± 69.01^{A}	
SR			5		4		4		7		5		2		4	
MSR			2.67 ± 1.12^{A}		2.17 ± 1.11^{A}		2.14 ± 1.21^{A}		3.38 ± 1.85^{A}		2.08 ± 1.51^{A}		1.67 ± 0.58		2.50 ± 0.76^{A}	
MD (Brillouin's index)			0.16 ± 0.11^{A}		0.21 ± 0.22^{A}		0.28 ± 0.32^{A}		0.24 ± 0.32^{A}		0.14 ± 0.21^{A}		0.16 ± 0.14		0.21 ± 0.17^{A}	
B-P (Berger-Parker's index)			0.95 ± 0.06^{A}		0.91 ± 0.12^{A}		0.85 ± 0.19^{A}		0.91 ± 0.16^{A}		0.95 ± 0.08^{A}		0.94 ± 0.05		0.94 ± 0.05^{A}	

Significant positive relationships with host size were detected for individual total parasite abundance (GZM, χ^2= 208.819, $p < 0.001$), abundance of *Otodistomum* sp. (GZM, χ^2= 15.757, $p < 0.001$), Tetraphyllidea fam. gen. sp. (GZM, χ^2= 8.304, p= 0.004), *G. adenoplusia* (GZM, χ^2= 233.982, $p < 0.001$) and *E. vilelai* (GZM, χ^2= 34.712, $p< 0.001$) and for individual parasite richness (GZM, χ^2=14.350, $p < 0.001$) and diversity (r_S= 0.32, p= 0.001). In contrast, abundance of *D. macrocephalum* and individual dominance were significantly higher in juvenile sharks (GZM, χ^2= 66.678, $p < 0.001$ and r_S= -0.278, p= 0.004, respectively). Significant positive associations with host K were found for individual total parasite abundance (GZM, χ^2= 10.360, p= 0.001), abundance of *E. vilelai* (GZM, χ^2= 8.759, p= 0.003) and *G. adenoplusia* (GZM, χ^2= 14.071, $p < 0.001$) and Brillouin's diversity index (r_S= 0.364, $p < 0.001$). In contrast, abundance of *D. macrocephalum* and dominance index were negatively correlated with K (GZM, χ^2= 13.939, $p < 0.001$ and r_S= -0.367, $p < 0.001$, respectively). Negative correlations were detected between HSI and individual total abundance (GZM, χ^2= 24.288, $p < 0.001$), abundance of *E. vilelai* (GZM, χ^2= 6.182, p= 0.013), *Otodistomum* sp. (GZM, χ^2= 20.686, $p < 0.001$), Tetraphyllidea fam. gen. sp. (GZM, χ^2= 6.910, p= 0.009) and *G. adenoplusia* (GZM, χ^2= 26.048, $p < 0.001$). Individual total abundance and abundance of *G. adenoplusia* were positively linked to male GSI (GZM, χ^2= 5.554, p= 0.018 and χ^2= 5.993, p= 0.014, respectively), and individual total abundance and abundance of *Otodistomum* sp. and *G. adenoplusia* showed a positive relationship with female GSI (GZM, χ^2= 5.103, p= 0.024; χ^2= 4.131, p= 0.042 and χ^2= 5.282, p= 0.022, respectively).

Based on the observed differences on the parasite composition of the communities infecting juvenile and adult sharks, the description of the parasite assemblages of the two host size-groups is performed separately.

Within juvenile sharks, 26% of the fish were free of parasites. The parasites recovered from the infected hosts were grouped in a total of ten different parasite taxa (Table 3). Parasites most frequently found and classified as "common" (total $P \geq 8\%$) were *D. macrocephalum*, *G. adenoplusia* and *E. vilelai.* The most abundant parasites were the cestodes *D. macrocephalum* (TMA= 2.54) and *G. adenoplusia* (TMA= 1.90).

All adult specimens were infected by at least one parasite, and a total of 14 parasite taxa were identified (Table 4). Common parasites (total $P \geq 8\%$) were *Otodistomum* sp., *D. macrocephalum*, Tetraphyllidea fam. gen. sp., *G. adenoplusia* and *E. vilelai*. The most abundant parasite was, by far, the cestode *G. adenoplusia* (TMA= 63.39) followed by the copepod *E. vilelai* (TMA= 1.83).

A significant effect of the factor locality-season-depth was found for TMA in juvenile sharks (GZM, χ^2= 20.873, $p < 0.001$). No differences among categorical groups were found for MSR, MD or B-P in the case of juvenile sharks or for TMA, MSR, MD and B-P in the case of adult sharks (GZM/Kruskal-Wallis, $p > 0.05$).

Figure 3 shows a plot of the first factorial plane of co-inertia analysis covering 99.996% of the total variance, mainly on the first axis (99.86% of the total inertia) of the FCA performed using component population data of the common parasites in juvenile sharks. Component populations of the three parasites strongly correlated with the first FCA axis (Cosine^2= 0.873–1.000). None of them was strongly correlated with the second FCA axis. From FCA and cluster analyses, three distinct assemblages of juvenile sharks could be distinguished depending on their parasite load:

Group A: Comprises samples from off Barcelona and Mallorca in summer at depth 1 and from off Barcelona in autumn at depth 2. These were characterized by the cestode *D. macrocephalum*, which was significantly more abundant in samples from off Mallorca in summer at depth 1 (GZM, χ^2= 19.271, p= 0.001). No significant differences among groups were detected for prevalence (GZM, $p > 0.05$).

Group B: Comprises samples from off Barcelona in autumn at depth 1, which were not characterized by any particular parasite.

Group C: Comprises samples from off Mallorca in summer at depth 2, which were characterized by the cestode *G. adenoplusia* and the copepod *E. vilelai*. The former parasite showed a significantly higher abundance and prevalence in this group (GZM, χ^2= 20.186, $p < 0.001$ and GZM, χ^2= 7.025, p= 0.03, respectively). *Eudactylina vilelai* was absent from all other groups.

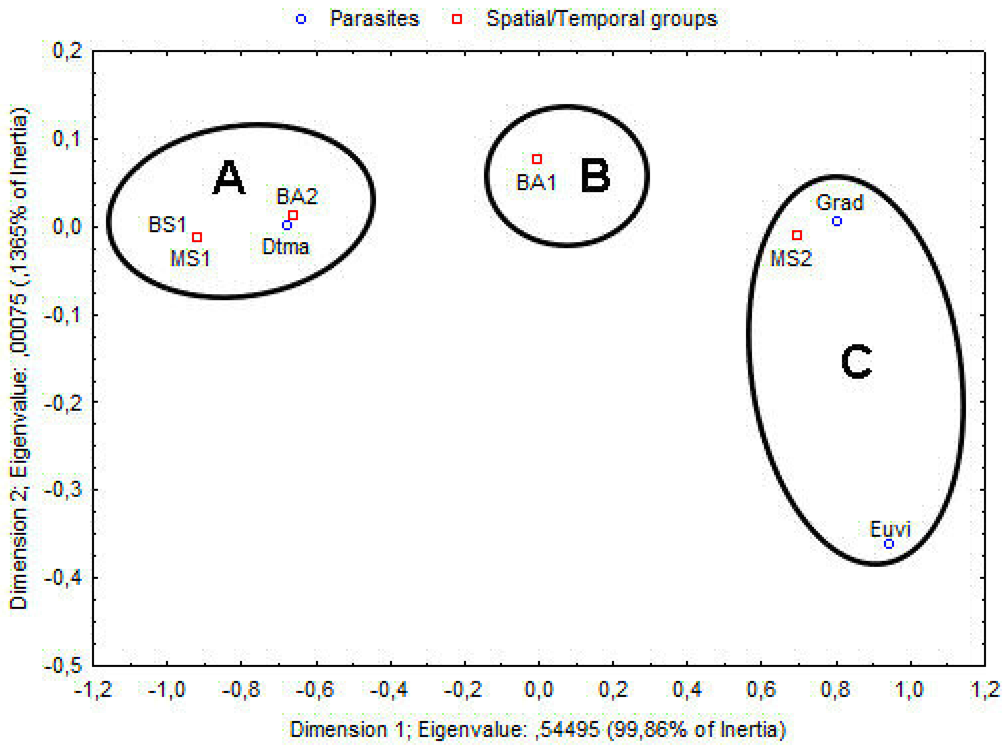

Figure 3. Plot of the first factorial plane of co-inertia of the factorial correspondence analysis (FCA) performed using component population data of the three common parasites (P > 10%) in juvenile specimens of *Galeus melastomus*. A/B/C refers to the groups established in the description of the parasite fauna. Abbreviations for locality-season groups (BA1, etc.) are defined in Fig. 1. Abbreviations for parasite names: Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*.

Figure 4 shows a plot of the first factorial plane of co-inertia analysis covering 82.81% of the total variance, mainly on the first axis (66.88% of the total inertia) of the FCA performed using component population data of the common parasites in adult sharks. Component populations of two parasites strongly correlated with the first FCA axis: *Otodistomum* sp. and *G. adenoplusia* (Cosine^2= 0.505–0.970). Only Tetraphyllidea fam. gen. sp. was significantly correlated with the second FCA axis: (Cosine^2= 0.509). From FCA and cluster analyses, two distinct assemblages of adult sharks could be distinguished depending on their parasite load:

Group A: Comprises samples from off Mallorca in summer and from off Barcelona and Ibiza in autumn at depth 2, which were characterized by the digenean *Otodistomum* sp. and the cestodes Tetraphyllidea fam. gen. sp. Although both parasites reached maximum abundances in samples from off Ibiza in autumn at depth 2, no significant effects were detected neither for abundance or prevalence (GZM, p > 0.05).

Group B: Comprises samples from off Barcelona in autumn at depth 1 and from off Barcelona in summer at both depths, which were characterized by the cestodes *D. macrocephalum* and *G. adenoplusia* and by the copepod *E. vilelai*. The two former parasites were most abundant in samples from off Barcelona in summer at depths 2 and 1, respectively. No significant effects were detected neither for abundance or prevalence (GZM, p > 0.05).

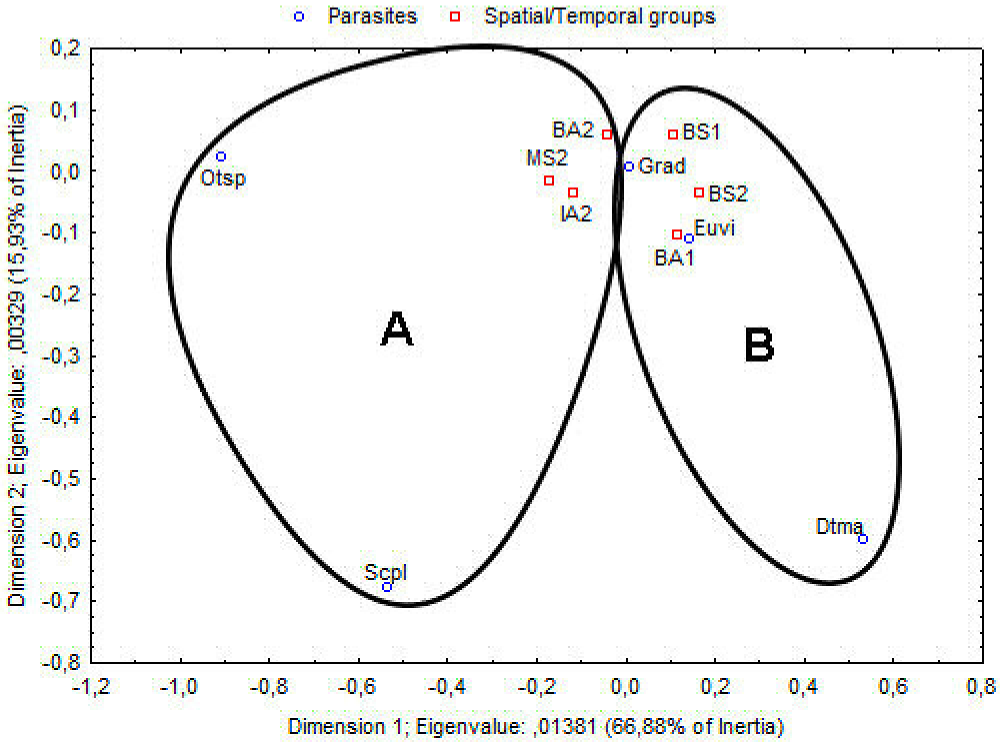

Figure 4. Plot of the first factorial plane of co-inertia of the factorial correspondence analysis (FCA) performed using component population data of the five common parasites (P > 10%) in adult specimens of *Galeus melastomus*. A/B refers to the groups established in the description of the parasite fauna. Abbreviations for locality-season groups (BA1, etc.) are defined in Fig. 1. Abbreviations for parasite names: Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*; Otsp, *Otodistomum* sp.; Scpl, *Scolex pleuronectis* (Tetraphyllidea fam. gen. sp.).

The CCA assessing the relationships between the abundance of main parasites and environmental variables explained 84.9% of the constrained variance in the first two axes (Fig. 5). At the right-lower part of the plot, the strongest associations were found between high turbidity levels and the abundance of the parasites *D. macrocephalum* and *P. baylisi*, linked to hauls from 500–700 m depth. High salinity levels were related to the nematodes *H. aduncum* and *P. obtusus*. At the right-upper part of the plot and more weakly, O_2 levels were linked to the cestodes Tetraphyllidea fam. gen sp., *S. viridis* and larval anisakid nematodes.

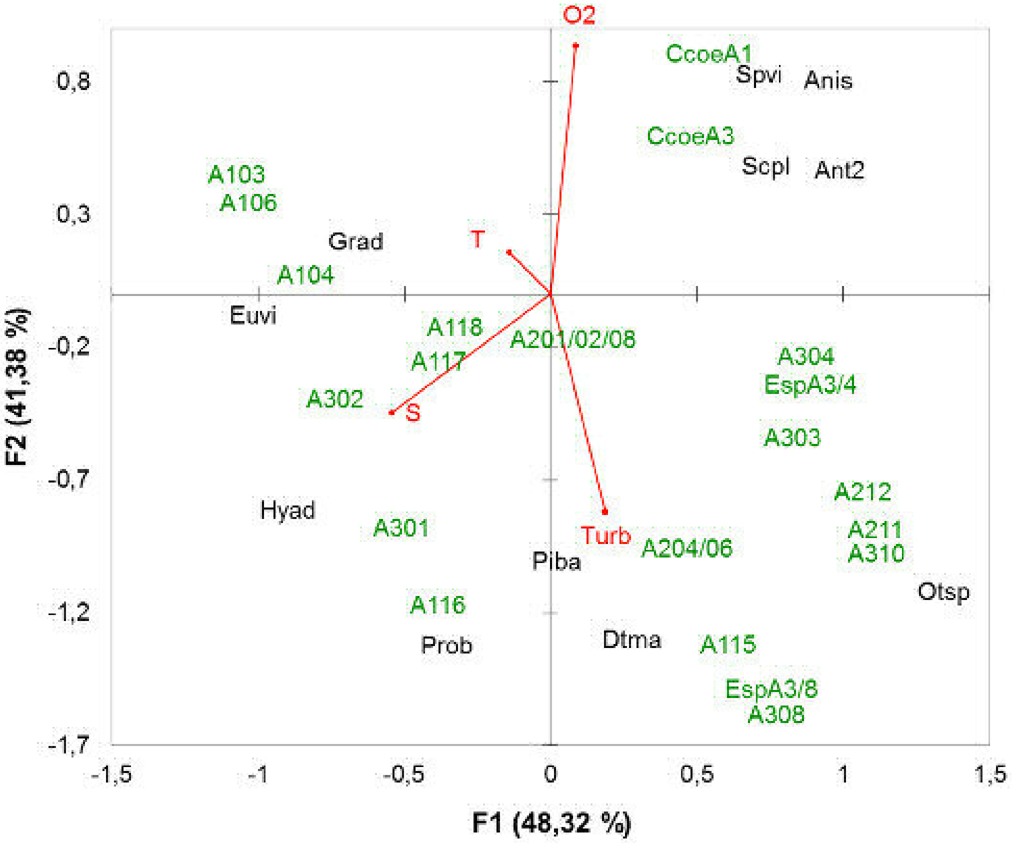

Figure 5. Canonical correspondence analysis (CCA) showing relationships between the abundance of parasites infecting *Galeus melastomus*, *Etmopterus spinax* and *Centroscymnus coelolepis* and environmental data. Abbreviations for parasites names: Anis, Anisakidae gen. sp; Ant2, *Anisakis* Type II; Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*; Hyad, *Hysterothylacium aduncum*; Otsp, *Otodistomum* sp.; Piba, *Piscicapillaria baylisi*; Prob, *Proleptus obtusus*; Scpl, *Scolex pleuronectis* (Tetraphyllidea fam. gen. sp.); Spvi, *Sphyriocephalus viridis*. Abbreviations for environmental variables: O2, oxygen concentration; S, salinity; T, temperature; Turb, turbidity.

3.2.2. *Etmopterus spinax*

The percentage of uninfected sharks was 36%. In infected specimens, a total of two parasite taxa were found (Table 5), of which only one of them (i.e. Tetraphyllidea fam. gen. sp.) was considered common.

Table 5. Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Etmopterus spinax* and *Centroscymnus coelolepis*, and means and standard deviations of fish parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)), fish total length (TL), condition factor (K), hepatosomatic index (HSI), adults gonadosomatic index (GSI) and acetylcholinesterase (AChE), lactate dehydrogenase (LDH) and citrate synthase (CS) activities. N: sample size of *C. coelolepis* and *E. spinax*, (*): number of females. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Pd, plerocercoid; Ps, plerocercus. Abbreviations for locations within host: Ca, Abdominal cavity; I, intestine; K, kidney; L, liver; M, muscle; S, stomach; SW, stomach wall (encysted). Dashes indicate absence of the parasite.

	Etmopterus spinax				***Centroscymnus coelolepis***			
N(*)	**Stage**	**Location**	**Total 11(6)**		**Stage**	**Location**	**Total 10(7)**	
			P(%)	**MA±SD**			**P(%)**	**MA±SD**
Cestoda								
Aporhynchus norvegicus	A	I	9	0.09 ± 0.30	–	–	–	–
Grillotia adenoplusia	–	–	–	–	Ps	M, SW, Ca, K	30	29.70 ± 93.22
Sphyriocephalus viridis	–	–	–	–	Pd	S	50	2.20 ± 3.08
Clistobothrium sp.	–	–	–	–	J	I	10	0.10 ± 0.32
Monorygma sp.	–	–	–	–	A	I	20	0.20 ± 0.42
Tetraphyllidea fam. gen. sp.	Pd	I, L	64	10.36 ± 28.58	Pd	I	100	896.00 ± 2,665.50
Cestoda indet.	–	–	–	–	A	I	10	0.10 ± 0.32
Nematoda								
Anisakidae gen. sp.	–	–	–	–	L3	S	10	0.10 ± 0.32
Anisakis Type II	–	–	–	–	L3	M, SW	20	0.30 ± 0.67
TMA			10.45 ± 28.55				928 ± 2,658	
SR			2				8	
MSR			0.73 ± 0.65				2.50 ± 1.18	
MD (Brillouin's index)			0.05 ± 0.13				0.25 ± 0.25	
B-P (Berger-Parker's index)			0.93 ± 0.19				0.85 ± 0.18	
TL			23.59 ± 9.69				38.58 ± 14.34	
K			0.43 ± 0.07				0.71 ± 0.20	
HSI			9.28 ± 3.75				10.68 ± 7.06	
GSI			5.90 ± 8.75				3.51 ± 7.02	
AChE			78.61 ± 37.52				19.15 ± 12.71	
LDH			5,170 ± 796				4,877 ± 1,043	
CS			13.98 ± 16.75				1.92 ± 1.87	

Abundance of Tetraphyllidea fam. gen. sp. showed positive correlations with fish TL (GZM, χ^2= 5.678, p= 0.017) and HSI (GZM, χ^2= 5.911, p= 0.015). No relationship between the abundance of Tetraphyllidea fam. gen. sp. and K was detected (GZM, $p > 0.05$).

No particular associations were detected between the parasites of *E. spinax* and any environmental variable (Fig. 5).

3.2.3. *Centroscymnus coelolepis*

All examined sharks were infected by at least one parasite. A total of eight different parasite taxa were recovered (Table 5), of which the following three were considered common: *G. adenoplusia*, *S. viridis* and Tetraphyllidea fam. gen. sp. Furthermore, *G. adenoplusia*, Anidakidae gen sp. and *Anisakis* Type II constitute new host records.

Significant positive correlations with fish size and HSI were detected for total individual parasite abundance (GZM, χ^2= 4.801, p= 0.028 and GZM, χ^2= 4.712, p= 0.03, respectively), abundance of Tetraphyllidea fam. gen. sp. (GZM, χ^2= 7.497, p= 0.006 and GZM, χ^2= 7.787, p= 0.005, respectively) and *S. viridis* (GZM, χ^2= 7.862, p= 0.005 and GZM, χ^2= 7.256, p= 0.007, respectively). No effects of total individual parasite abundance, abundance of Tetraphyllidea fam. gen. sp. or *S. viridis* were detected on fish K or GSI (GZM, $p > 0.05$). Abundance of *G. adenoplusia* and individual richness, diversity and dominance were not related to fish TL or condition factors (GZM/r_s, $p > 0.05$).

The CCA assessing the relationships between the abundance of main parasites and environmental variables evidenced an association between the abundance of Tetraphyllidea fam. gen. sp., *S. viridis* and anisakid larval nematodes and near-bottom O_2 levels (Fig. 5, right-upper part of the plot).

3.3. *Dietary composition of sharks and its relationship with the parasite fauna*

Diet of *G. melastomus*, which will not be described in detail herein, was highly diversified (69 different prey items were identified, most of them to genus/species level). For the CCA on parasite-prey relationships (Fig. 6) only 16 prey-groups were considered. These groups represented 87.9% of the 499 prey identified (excluding items like scales and foraminiferans) in terms of number, and a higher proportion in terms of wet weight.

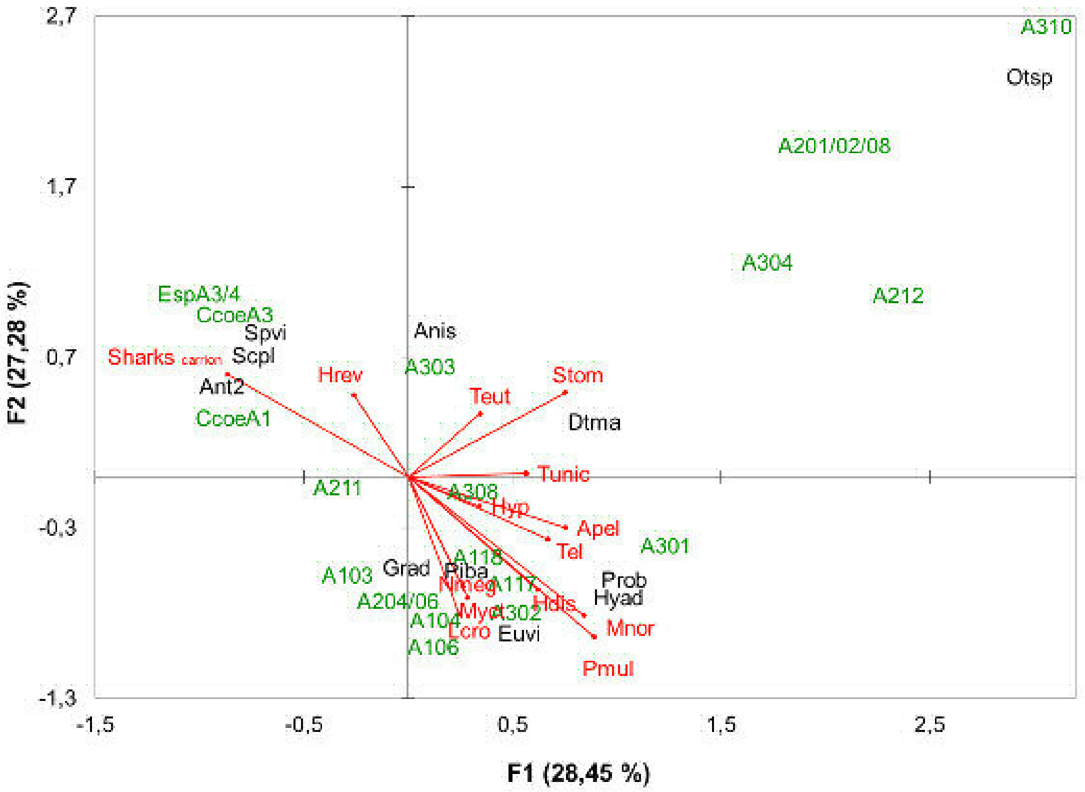

Figure 6. Canonical correspondence analysis (CCA) showing relationships between the abundance of main parasites and main prey-items found in *Galeus melastomus*, *Etmopterus spinax* and *Centroscymnus coelolepis*. Abbreviations for parasites names: Anis, Anisakidae gen. sp; Ant2, *Anisakis* Type II; Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*; Hyad, *Hysterothylacium aduncum*; Otsp, *Otodistomum* sp.; Piba, *Piscicapillaria baylisi*; Prob, *Proleptus obtusus*; Scpl, *Scolex pleuronectis* (Tetraphyllidea fam. gen. sp.); Spvi, *Sphyriocephalus viridis*. Abbreviations for prey names: Apel, *Acantephyra pelagica*; Hdis, *Heteroteuthis dispar*; Hrev, *Histioteuthis reversa*; Hyp, Hyperiidea; Lcro, *Lampanyctus croccodilus*; Mnor, *Meganyctiphanes norvegica*; Myct, Myctophidae; Nmeg, *Nematoscelis megalops*; Pmult, *Pasiphaea multidentata*; Stom, Stomiidae; Tel, Teleostei; Teut, Teutoidea; Tunic, Tunicata.

The diet of this shark was mainly based on benthopelagic-mesopelagic prey, including also some benthic prey. In terms of number, decapods (*Pasiphaea multidentata* and *Acanthephyra pelagica*), euphausiids (mainly *Meganyctiphanes norvegica*) and myctophid fish (*Lampanyctus croccodilus*) were dominant among pelagic prey, but tunicates were also included among the groups considered in the CCA. The main benthopelagic prey were squids (*Histioteuthis* sp., *Heteroteuthis dispar*) and fish (among those identified, *Lepidion lepidion* and some macrourids). Among benthic (not swimming) prey, crabs/lobsters (e.g. *Monodaeus couchi*, *Calocaris macandreae*, *Munida tenuimana*) were dominant. In terms of weight, squids, fish and decapods were the most important prey.

In *G. melastomus* diet changed as a function of fish size, although the rather low number of small specimens, mainly juveniles, available (n=23) did not allow to include size as factor in the CCA. Diet of small specimens was mainly based on *M. norvegica* (44.1% of prey), with *P. multidentata* and squids (both 8.9% of prey) as secondary prey. Meso-bathypelagic shrimps (*P. multidentata*, *A. pelagica*: 29.0% of prey) and squids (*H. dispar*, unidentified: 16.3% of prey) were comparatively more important in the diet of large (adult) specimens, in which *M. norvegica* only represented 14.9% of the total prey.

Etmopterus spinax preyed on mesopelagic decapods (*Sergestes* spp., *Pasiphaea* spp.), euphausiids and myctophids at < 1,000 m, while the diet at > 1,000 m was almost exclusively based on cephalopods (*Histioteuthis* spp.).

Centroscymnus coelolepis is the deepest distributed species (see Table 1), and its diet was essentially based on squids and carrion.

The CCA relating the abundance of individual parasites and prey explained 55.7% of the constrained variance in the first two axes (Fig. 6). Most of the parasites linked to diet items recovered from *G. melastomus* grouped at the right part of the plot. Hauls were not ordered as a function of depth, but as a function of season and of prey swimming capacity. At the right-upper part of the plot, the digenean *Otodistomum* sp., the cestode *D. macrocephalum* and unidentified larval anisakid nematodes were related to squids (Teuthoidea) and Stomiid fish associated to samples collected in October (A3 hauls). At the right-lower part of the plot, the cestode *G. adenoplusia*, the nematode *P. baylisi* and the copepod *E. vilelai* were most related to the euphausiid *Nematoscelis megalops* and to myctophid fish. The nematodes *H. aduncum* and *P. obtusus* were associated to mesopelagic (migratory) prey such as decapods (*P. multidentata*) and euphausiids (*M. norvegica*).

The larval cestodes Tetraphyllidea fam. gen. sp. were the only parasites found in the specimens of *E. spinax* included in the CCA (all from hauls at > 1,000 m depth), and appeared linked to the squids *Histioteuthis* spp., practically the only prey recovered from *E. spinax* guts (Fig. 6).

Parasites from *C. coelolepis* (the cestodes Tetraphyllidea fam. gen. sp. and *S. viridis*, and the nematode *Anisakis* Type 2) were associated to carrion (shark food falls) at the left-upper part of the plot (Fig. 6).

3.4. Relationship of enzymatic markers with parasite infection and fish biological parameters

3.4.1. Galeus melastomus

Mean activity levels (in nmol/min/mg prot) ranged across groups from 10.72 to 32.55 for AChE, from 1,330 to 2,925 for LDH and from 18.70 to 64.68 for CS (Table 2). For AChE, V_{max} was 14.1 nmol/min/mg prot. and K_m was 0.112 mM, with an average catalytic efficiency of 125.

A significant effect of the factor locality-season-depth was found for AChE (GLM, $F_{(5, 78)}= 5.697$, $p < 0.001$), LDH ($F_{(5, 74)}= 7.568$, $p < 0.001$) and CS ($F_{(5, 75)}= 6.787$, $p < 0.001$) activity levels. Highest activity levels of the three enzymes assessed were recorded in samples from off Barcelona in autumn at depth 2 (Table 2).

Significant negative relationships were detected between AChE activity and fish TL (GLM, $F_{(1, 88)}= 46.192$, $p < 0.001$) and between LDH activity and fish K (GLM, $F_{(1, 83)}= 7.551$, $p= 0.007$). Acetylcholinesterase activity levels were negatively related to total parasite abundance (GZM, $\chi^2= 23.588$, $p < 0.001$), abundance of *E. vilelai* ($\chi^2= 8.032$, $p= 0.005$), *Otodistomum* sp. ($\chi^2= 4.112$, $p= 0.043$) and *G. adenoplusia* ($\chi^2= 22.366$, $p < 0.001$), as also were LDH and CS activity levels to the abundance of *Otodistomum* sp. (GZM, $\chi^2= 6.922$, $p= 0.009$ and $\chi^2= 13.959$, $p < 0.001$, respectively).

3.4.2. Etmopterus spinax

Acetylcholinesterase, LDH and CS mean activity levels were 78.61, 5,170 and 13.98 nmol/min/mg prot, respectively (Table 5). For AChE, V_{max} was 54.1 nmol/min/mg prot and K_m was 0.119 mM, with an average catalytic efficiency of 454.6.

Acetylcholinesterase activity levels were negatively correlated with HSI (GLM, $F_{(1, 7)}= 5.693$, $p= 0.048$) and with the abundance of Tetraphyllidea fam. gen. sp. (GZM, $\chi^2= 4.850$, $p= 0.028$). No other significant associations were found between the parameters assessed for this species.

3.4.3. Centroscymnus coelolepis

Acetylcholinesterase, LDH and CS mean activity levels were 19.15, 4,877 and 1.92 nmol/min/mg prot, repectively (Table 5). For AChE, V_{max} was 43.3 nmol/min/mg prot and K_m was 0.043 mM, with an average catalytic efficiency of 1,007.

Acetylcholinesterase activity was negatively associated with fish TL (GLM, $F_{(1, 8)}$= 18.544, p= 0.003) and HSI (GLM, $F_{(1, 8)}$= 13.053, p= 0.007), while CS showed a positive relationship with the same two biological parameters (GLM, $F_{(1, 8)}$= 75.503, p= 0.0001 and $F_{(1, 8)}$= 29.250, p= 0.001, respectively). Total individual parasite abundance, abundance of Tetraphyllidea fam. gen. sp. and *S. viridis* correlated negatively with AChE (GZM, χ^2= 9.279, p= 0.002; χ^2= 14.386, p= 0.0001 and χ^2= 7.100, p= 0.008, respectively). Abundance of Tetraphyllidea fam. gen. sp. and *S. viridis* showed a negative association with LDH as well (GZM, χ^2= 6.225, p= 0.013 and GZM, χ^2= 5.938, p= 0.015, respectively). No other significant associations were found between the parameters assessed for this species.

3.5. Relationships of histological observations with parasite infection, enzymatic activities and fish biological parameters

3.5.1. Galeus melastomus

Frequent presence of coccidian oocysts was detected in histological sections of the spiral valve (Fig. 7A). Oocysts with four sporocysts were heterogeneously distributed and preferentially located basally in the epithelium of the intestinal mucosa, reaching in some cases the underlying lamina propria. Oocysts wall was colourless, while sporocysts showed a refringent surface and contained two eosinophilic sporozoites, each with a conspicuous granule of basophilic material. Oocysts were round or oval in shape, measuring from 11.2 to 15.9 µm in diameter (mean diameter= 13.5, n= 21). Although the methodology used in the present study made accurate identification of these microparasites not possible, the presence of four sporocysts, each containing two sporozoites, within each oocyst readily allows placing them within the family Eimeridae. Overall prevalence of oocysts was 55%. In infected specimens, OC/mm^2 ranged between 3 and 200 (mean number= 32). No significant differences in OC/mm$_2$ were detected across locality-season-depth groups (Kruskal-Wallis, $p > 0.05$). No associations were found between OC/mm^2 and fish TL, condition indices, enzymatic activity levels or MM/mm^2 (r_s, $p > 0.05$ in all cases).

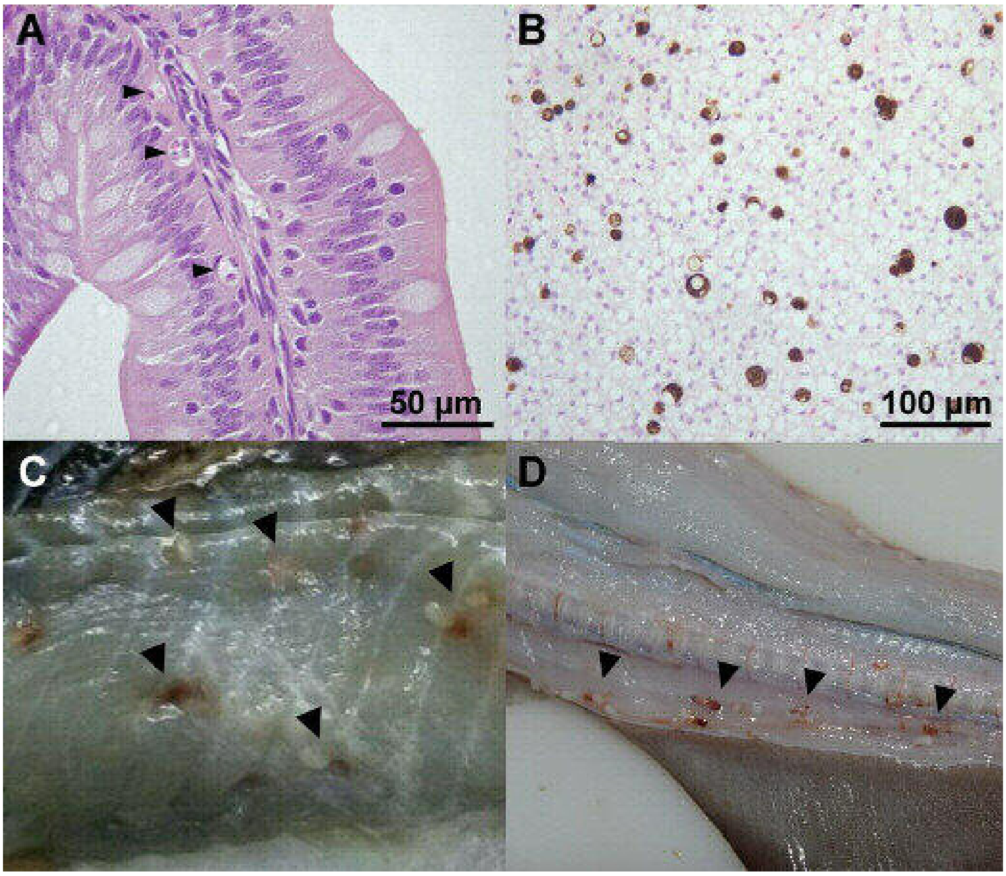

Figure 7. Photomicrographs of histological pathologies and alterations found in histological sections of different organs of *Galeus melastomus*. A, Coccidian oocysts in the intestinal mucosa (arrowheads); B, Melano-macrophages in hepatic tissue; C, Larval stages of the cestode *Grillotia adenoplusia* encapsulated in the abdominal musculature (arrowheads); D, Larval stages of the cestode *G. adenoplusia* encapsulated in the tail musculature (arrowheads).

Melano-macrophages were homogenously distributed across all hepatic and some splenic sections (Fig. 7B). Their mean densities across groups ranged from 34.11 to 383.18 MM/mm^2 (Table 2). Density of melano-macrophages significantly increased with fish size and female GSI (GLM, $F_{(1,\ 77)}$= 88.941, $p < 0.001$ and $F_{(1,\ 46)}$= 4.723, p= 0.035, respectively), but was inversely associated to HSI (GLM, $F_{(1,\ 77)}$= 43.061, $p < 0.001$). Positive relationships between MM/mm^2 and total parasite abundance (GZM, χ^2= 33.362, $p < 0.001$), abundance of *E. vilelai* (GZM, χ^2= 12.346, $p < 0.001$) *Otodistomum* sp. (GZM, χ^2=27.481, $p < 0.001$) and *G. adenoplusia* (GZM, χ^2= 36.971, $p < 0.001$) and the dominance index (r_s= -0.312, p= 0.007) were detected. In contrast, MM/mm^2 correlated negatively with the abundance of *D. macrocephalum* (GZM, χ^2= 17.163, $p < 0.001$) and Tetraphyllidea fam. gen. sp. (GZM, χ^2= 10.403, p= 0.001) and with the diversity index (r_s= 0.350, p= 0.002). An interaction with fish TL was detected in the analyses assessing the association between MM/mm^2 and the abundances of Tetraphyllidea fam. gen. sp. and *G. adenoplusia*. Finally, significant positive relationships were detected between MM/mm^2 and LDH (GLM, $F_{(1, 59)} = 13.582$, $p < 0.001$) and CS ($F_{(1, 58)} = 10.589$, $p = 0.002$) activities.

Macroscopical lesions associated to encapsulated larval stages of the parasite *G. adenoplusia* were detected in the muscular tissues of all adult specimens of *G. melastomus* (Fig 7C, D). These parasites were sometimes recovered from the stomach walls, but were mainly found in the musculature of the abdominal and tail regions. In the latter area they concentrated in large numbers. Damage of the caudal musculature was observed in highly parasitized sharks. In these regions, the affected tissue was much softer than normal.

No other relevant histological alterations were detected in the mentioned organs or in gill or gonad sections.

3.5.2. Etmopterus spinax and Centroscymnus coelolepis

No relevant alterations were observed in histological sections of the examined organs of *E. spinax* and *C. coelolepis*. No quantitative analysis of MM was carried out for these species since, in contrast with *G. melastomus*, only a very slight pigmentation was detected in some liver sections.

4. Discussion

4.1. General features of the parasite communities of G. melastomus, E. spinax and C. coelolepis in the northwestern Mediterranean Sea

Shark species have been barely addressed as regards their parasite communities, and *G. melastomus* from the Balearic Sea shows the highest total richness values reported to date (15 vs. 2–11 different parasite taxa, see Henderson and Dunne, 1998; Moore, 2001; Palm and Schröeder, 2001; Henderson et al., 2002; Chambers, 2008; Isbert et al., 2015; Dallarés et al., in press). As regards small-sized demersal sharks examined for parasites, they show moderate to high richness values compared to the rest of sharks addressed (Henderson and Dunne, 1998; Moore, 2001; Henderson et al., 2002; Dallarés et al., in press). This trend is likely related to their benthopelagic feeding habits: the higher availability of potential intermediate hosts close to the seafloor results in more complex and diverse fish parasite communities in this environment (Campbell et al., 1980; Marcogliese, 2002).

The parasite community of *G. melastomus* in the Balearic Sea, which is herein described in full for the first time, is thus characterized by high abundance, richness and diversity values when compared to other sharks. Furthermore, high dominance values are attained by most infracommunities as a result of the high preponderance of a single parasite species (i.e. *D. macrocephalum* in small and *G. adenoplusia* in large fish).

A recent study (Dallarés et al., in press) has also addressed the parasite community of this species in the mainland slope of the Balearic Sea; however, despite having been conducted in the same area and at similar depth ranges, the structure and composition of parasite infracommunities described in the cited and present studies show marked differences. Overall mean parasite abundance of present specimens is extremely higher (40.83 vs. 1.04), the main reason being that in the first description of the parasite community of *G. melastomus* the most important parasite in terms of abundance in adult and large-sized juvenile sharks (i.e. the larval trypanorhynch cestode *G. adenoplusia*) was not collected. As described by Dallarés et al. (in press), all organs were thoroughly examined with the exception of the musculature, where, as evidenced by present results, *G. adenoplusia* is almost exclusively located. Mean infracommunity richness and diversity are somewhat higher in present specimens (1.85 vs. 0.6 and 0.16 vs. 0.09, respectively). Since no differences exist between both samples concerning size-ranges of the sharks examined or localities, seasons and depths sampled, these differences might be due either to stochastic factors or to long-term changes in benthic and/or suprabenthic faunal assemblages (as those reported by Cartes et al., 2009) in turn affecting parasite availability and transmission.

This represents the first attempt to describe the parasite community infecting *E. spinax* in the Mediterranean Sea. With a total of only two different parasites recovered, parasite infracommunities of *E. spinax* from the present study are characterized by extremely low richness and diversity values and a very high dominance index, which may be related with the pelagic feeding habits of this species (Carrassón et al., 1992, present results). As a rule, pelagic fish possess impoverished parasite communities compared to those living close to the seafloor, as has been for instance observed in mediterranean myctophid teleosts (Mateu et al., 2015), due to the generalized scarcity of potential intermediate hosts compared to the benthic environment (Campbell et al., 1980; Marcogliese, 2002).

Present parasitological results for *E. spinax* contrast with the outcomes by Klimpel et al. (2003), who recovered seven different parasite taxa from 37 juvenile specimens of *E. spinax* in the North Sea. Apparently, these dissimilarities could not be attributed to dietary differences between Atlantic and Mediterranean sharks (i.e. a more diverse diet of the former that would presumably favour the acquisition of more parasite taxa), since juvenile specimens of *E. spinax* from the Atlantic essentially consumed one euphausiid crustacean and one teleost species (Klimpel et al., 2003), while those from the Mediterranean feed onto a wider array of prey (present results). The absence of ectoparasites, which do not rely on host feeding patterns for transmission, in Mediterranean specimens further suggests that other factors than diet may be responsible for the lower parasite richness and diversity observed in present sharks. Some authors have reported a generalized decrease in parasite richness and abundance in Mediterranean with respect to Atlantic teleosts (Pérez-del-Olmo et al. 2009b; Mattiucci et al. 2014; Constenla et al., 2015), and factors such as lower fish size, feeding intensity, dietary diversity and general faunal biomass and abundance in the Mediterranean Sea have been suggested as responsible for this pattern (Constenla et al., 2015). However, comparative data for condrichthyans are at present scarce (parasite communities of only two shark species have been described in the Mediterranean to date, see Dallarés et al., in press), and the low number of specimens of *E. spinax* available in the present study does not allow any generalization.

The parasite community infecting *C. coelolepis* is addressed herein for the first time, and seems characterized by moderate richness and diversity, and overall lower dominance values than those reported for the rest of sharks studied in the Mediterranean (Dallarés et al., in press; present results). The total parasite richness observed in *C. coelolepis* from the Mediterranean Sea resembles that reported for other bathydemersal sharks of similar size in the Atlantic: Palm and Schröder (2001) reported six, seven and nine different parasite taxa from *Heptranchias perlo*, *Deania profundorum* and *Deania histricosa*, respectively, in eastern Atlantic waters. However, parasitological data for *C. coelolepis* presented herein should be considered as representative of mainly juvenile hosts, as these constituted the majority of specimens sampled, and those reported by Palm and Schröder (2001) are from adult specimens (H.W. Palm, personal comm.). The positive correlation between total parasite abundance and body length, coupled with the fact that the largest shark examined harboured the parasite infracommunity with highest richness, indicates that parasite richness and abundance in adult C. *coelolepis* will probably reach higher values than those presented herein. As outlined for *E. spinax*, the number of specimens available for *C. coelolepis* was low and the outcomes of the present study should thus be considered preliminary and wait to be confirmed by future additional studies.

4.2. The parasite community of G. melastomus in relation to host ontogeny and environmental gradients

A marked differentiation in the parasite communities between ontogenic stages of *G. melastomus* can be clearly appreciated, as highlighted by the MDS and PERMANOVA results. As pointed out by Campbell et al. (1980), changes in parasite fauna during the life history of the host can be directly attributed to changes in its feeding habits. Accordingly, an important ontogenic diet shift has been reported for *G. melastomus* in the study area (Carrassón et al., 1992; present results), with juveniles preying on smaller prey, mainly euphausiids, and adults preferring larger ones including decapod crustaceans, cephalopods and fish. Overall, parasite infracommunities of adult specimens of *G. melastomus* are more abundant, richer and more diverse than those of juvenile hosts. On the one hand, the much higher parasite abundance observed in adult specimens of *G. melastomus* in the present study is mainly explained by the accumulation of histozoic parasites (mainly *G. adenoplusia* in the muscular tissue, see section 4.3.) throughout the lifespan of the fish. On the other hand, the increased parasite richness and diversity may be related to a diversification of the diet in adult specimens, as reported by Carrassón et al. (1992), which implies the consumption of a greater variety of potential intermediate hosts and, therefore, the exposure to a larger diversity of parasites. The preferential consumption of larger prey belonging to higher trophic levels that may harbour more diverse parasite assemblages than the small crustaceans consumed by juvenile sharks is also a plausible explanation.

The generalized homogeneity of parasite communities in juvenile and adult specimens of *G. melastomus* across environmental gradients (i.e. seasons, localities and depth ranges) is altogether surprising. However, although general infracommunity structure does not show environmental-related variations, composition of infracommunities does differ across environmental gradients as a consequences of differences in prevalence and abundance of individual parasite species (see section 4.3.), which is further highlighted by the outcomes of the FCAs.

4.3. Patterns on individual parasites in relation to fish size, diet and environmental gradients and variables

The diphyllidean cestode *D. macrocephalum* was the most important parasite in terms of abundance in juvenile specimens of *G. melastomus*, and steadily decreased in number in adult hosts. This pattern can be explained by the ontogenic diet shift undergone by *G. melastomus* (Carrassón et al., 1992, present results). For diphyllideans, a filter-feeding crustacean is believed to act as first and a shrimp or crab as second intermediate host before the parasite infects the elasmobranch final host (Tyler, 2006). Indeed, larval stages of diphyllideans have been recovered from euphausiid and decapod crustaceans, among others (see Bray and Olson (2004) and references therein), and Dallarés et al. (in press) suggested that the decapod *C. macandreae*, more abundant in the diet of juvenile sharks according to Carrassón et al. (1992), could be the main transmitter of this parasite to *G. melastomus*. Present diet results suggest that the euphausiid *M. norvegica*, the most abundant prey in juvenile hosts, is the most probable source of larval *D. macrocephalum* infecting *G. melastomus* in the Balearic Sea, although *C. macandreae* was also identified among shark's prey and should not be discarded as potential intermediate host.

Ditrachybothrdium macrocephalum was strongly associated to high turbidity levels in the CCA relating parasites and environmental variables. This association was already found by Dallarés et al. (in press) in samples of *G. melastomus* from the same area and 549–809 m depth. Increased water turbidity generally involves more food availability for zooplankton and an increased abundance of these organisms enhance the aggregation of benthopelagic fish (Cartes et al., 2013), which could favour the transmission of this parasite.

The trypanorhynch cestode *G. adenoplusia* was the most abundant and prevalent parasite in adult specimens of *G. melastomus* (note that prevalence reached 100% in all groups), and was also recovered from the largest *C. coelolepis*.

Trypanorhynchs use copepods as first and other invertebrates or small fish as second intermediate hosts (Palm, 2004), and those belonging to the genus *Grillotia* are commonly found in elasmobranchs (Beveridge and Campbell, 2007). *Grillotia adenoplusia* from *G. melastomus* was linked to myctophid fish, which are known to prey on copepods in the sampled area (Bernal et al., 2015). This result is consistent with the outcomes of Dallarés et al. (2014), where the taxon Trypanorhyncha fam. gen. sp. (which grouped specimens of three different species, of which more than 90% belonged to the genus *Grillotia* (unpublished results)) was linked to myctophids as well. Dallarés et al. (2016) reported an association between *Grillotia* cf. *erinaceus* and the teleost *Gaidropsarus biscayense*. These coincidences suggest that the infection of benthopelagic predatory fish with plerocerci of *Grillotia* is likely to occur, in many cases, through the consumption of fish prey in turn feeding on copepods.

Grillotia adenoplusia was essentially recovered from the musculature, where it was specially located in the caudal region. The progressive acquisition of this parasite throughout the lifespan of the host results in the accumulation of large numbers of plerocerci in the muscular tissue. The preferential location of this parasite in the caudal fin may be explained by the hunting strategy followed by its final host, which would likely be a large shark such as *Dalatias licha* or *Hexanchus griseus*, larger species inhabiting the same depths as *G. melastomus* in the Mediterranean and that feed on other chondrychtians, especially the former (Matallanas, 1982; Stefanescu et al., 1993; Ebert, 1994). Furthermore, *G. adenoplusia* has been recorvered from *H. griseus* in the Atlantic (Beveridge and Campbell, 2013). Indeed, Seamone et al. (2014) demonstrated that the optimal attack strategy for large sharks preying onto smaller relatives is a tail-on approach, i.e. attacking from behind, which would presumably enhance the transmission of parasites located in the tail region. The same pattern of distribution of plerocerci of *Grillotia* has been observed in *C. coelolepis* from the present study and in *E. spinax* from the Atlantic (W. Isbert, personal comm.), which suggests that the tail-on approach is a common hunting strategy among shark predators.

An additional trypanorhynch, *S. viridis*, was recovered from *G. melastomus* and *C. coelolepis* and was found linked to high near-bottom O_2 levels in the latter, which are known to enhance biomass of copepod crustaceans (Cartes et al. 2013) and would presumably enhance the transmission of this parasite. Moreover, *S. viridis* was also found associated to carrion (of shark origin), which could point to an acquisition of this parasite through consumption of infected shark remains. As regards their final host, adult specimens of *S. viridis* have been mainly recovered from *D. licha* (Dallarés et al., 2017), which preys on small sharks in the study area (Matallanas, 1982). The high presence of larval stages of *S. viridis* recovered from *C. coelolepis* suggests that this shark could be an usual prey of *D. licha* in the lower slope of the Balearic Sea.

Similarly to *S. viridis*, Tetraphyllidean larval stages collectively known as *Scolex pleuronectis* are believed to use copepods as first intermediate hosts (Klimpel et al., 2010) and were linked to O_2 levels. These cestodes use different large invertebrates and fish as paratenic and second intermediate hosts before maturing in a chondrichthyan final host (Euzet, 1994; Klimpel et al., 2010). As suggested by present results, *E. spinax* becomes infected through consumption of cephalopods, while *C. coelolepis* may acquire Tetraphyllidea fam. gen. sp. through feeding on infected carrion (at least in part). The recovery of larger numbers of *S. viridis* and Tetraphyllidea fam. gen. sp. in the biggest specimens of *C. coelolepis* could point to a higher consumption of carrion by larger sharks.

Digenean metacercariae of the genus *Otodistomum* were mostly found encysted in the stomach wall of *G. melastomus* and were more abundant in larger hosts due to accumulation through time (in the same way as described for *G. adenoplusia*), as is characteristic of histozoic parasites (Dallarés et al., 2014). Knowledge on the life cycle of *Otodistomum* is scarce. These digeneans are known to use fish as second intermediate hosts and to mature in elasmobranchs (Rocka, 2006). Although first intermediate hosts are not known for *Otodistomum*, molluscs act as such for digeneans (Gibson, 2002) and, in this sense, *Otodistomum* was associated to squids in the CCA with prey abundance data, which could shed some light on the infection pathway followed by this parasite.

Despite the low prevalence and abundance levels of the nematodes *H. aduncum* and *P. obtusus* in *G. melastomus*, it is worth to note the associations detected between their abundance and high salinity levels. *Hysterothylacium aduncum* has been also linked to salinity levels in the teleost *Phycis blennoides* and in the shark *Scyliorhinus canicula* in the Balearic Sea, in the same way as *P. obtusus* in *G. melastomus* from the same area (Dallarés et al., 2016; Dallarés et al., in press). According to Cartes et al. (2013), salinity levels decrease linearly from 400 m to 1,000–1,200 m depth in the mainland and insular slopes of the Balearic Sea, which is consistent with the association of high salinity levels with hauls of 600–700 m depth (i.e. A301, A302) in the CCA plot relating parasite abundance with environmental variables (actually, *H. aduncum* and *P. obtusus* were only recovered from specimens sampled on the upper slope). Relationships with higher salinity levels could be an indirect effect of higher salinity at shallower depths (associated to the Levantine Intermediate Waters, LIW) in our sampling. In addition, near-bottom salinity levels in the upper slope of the Balearic Islands have been found to correlate with suprabenthos biomass (Cartes et al., 2008). To these depths have been found associated euphausiids and hyperiid amphipods in the mainland slopes (Cartes et al., 2013), as well as the greatest decapod biomass and diversity of all the bathymetric range encompassed by the continental slope (Fanelli et al., 2013). *Proleptus obtusus* is believed to use decapod crustaceans as intermediate hosts (Moravec, 2007 and references therein; Dallarés et al., in press), while *H. aduncum* can use copepods, isopods, amphipods and mysids as first and larger invertebrates as second intermediate hosts (Køie, 1993; Klimpel and Rückert, 2005). Therefore, environmental conditions in the upper slope seem to favour the abundance of the crustaceans needed by *H. aduncum* and *P. obtusus* to complete their life cycles, and can explain the higher abundance of these nematodes.

The nematode *P. baylisi,* recovered from *G. melastomus,* is known to parasitize scyliorhinid sharks in the adult form (Moravec, 2001; Dallarés et al., in press, present results) but its biology and life cycle are essentially unknown. Nothing is known about its intermediate hosts (if any), but this nematode has been linked to the euphausiid *N. megalops* and myctophid fish in the CCA relating parasite abundance to host prey. Its association to high water turbidity levels could further suggest that its intermediate hosts are favoured by increased levels of organic matter.

In contrast to heteroxenous parasites (i.e. those with indirect life cycles), which are trophically transmitted, prevalence and abundance patterns of monoxenous parasites (i.e. those using a single host to complete their life cycle), such as copepods, are not explained by the feeding habits of their hosts. Other factors, such as abiotic parameters or host population dynamics, determine instead the infection levels of these parasites (Skinner, 1982; Bagge et al., 2004). In this sense, *E. vilelai* was to some extent linked to higher salinity levels, which could be related to the above-explained about depth-related trends at LIW levels. It is possible that the associated increased suprabenthos biomass favours the aggregation of specimens of *G. melastomus* due to higher prey availability. As demonstrated by Bagge et al. (2004), infection levels by directly transmitted parasites scale as a function of fish population size (i.e. overall availability of hosts). Such increase in the quantity of hosts could enhance transmission of *E. vilelai*, which would be translated into higher abundances of this parasite. In the same way as suggested for *G. melastomus*, myctophid fish could also aggregate as a result of increased biomass of benthopelagic fauna, and these coinciding favourable environmental conditions may explain the correlation observed between the abundance of *E. vilelai* and of myctophid fish. As regards the higher copepod abundance in larger hosts, it might indicate that these parasites accumulate in host gills with time, in a similar way as histozoic parasites in tissues.

Concerning the only microparasite detected (i.e. a coccidian of the family Eimeridae, in the intestinal mucosa of *G. melastomus*), it probably belongs to the species *Eimeria palavensis*, described from *G. melastomus* in the NW Mediterranean (Marquès and Capapé, 2001). The observed shape and size of oocysts are consistent with the description provided by the mentioned authors (11.2 to 15.9 µm in diameter in present specimens vs. 12.0 to 14.4 µm). However, some morphological features were impossible to observe due to the methodology used in sample processing and additional observations should be carried out in order to confirm the identity of this coccidian.

4.4. Enzymatic activity levels (AChE, LDH, CS) in relation to fish biological parameters and parasite abundance

Acetylcholinesterase is a key enzyme involved in neurotransmission (Solé et al., 2008, 2010), LDH is a glycolytic enzyme involved in anaerobic metabolism and burst swimming capacity and CS is a key regulating enzyme of the Krebs cycle, and its activity reflects oxygen consumption in aerobic metabolism (Bernal et al., 2003; Drazen and Seibel, 2007, Drazen et al., 2015).

To the best of our knowledge, no data for the muscular enzymatic activities measured in the present study is available in the literature for the three sharks addressed, except for AChE in *G. melastomus*.

A recent study with the blue shark *Prionace glauca* (Alves et al., 2015) reported a catalytic efficiency for muscular AChE similar to that of *E. spinax* and within the range observed considering the three sharks studied herein.

Parasites can be considered as natural stressors to their hosts and, as such, are expected to alter stress and/or metabolic biomarkers. To date, few studies have addressed the effects of parasite infections on enzymatic activity levels in fish (Gupta and Agarwal, 1985; Dautremepuits et al., 2003; Podolska and Napierska, 2006; Pérez-i-García et al., 2015; Dallarés et al., 2014, 2016) and general patterns in relation to this aspect are yet to be described. The negative associations consistently observed between AChE activity levels and parasite abundance (either total or of individual parasite species) in the three sharks addressed may suggest a certain compromise on this enzyme activity caused by parasite infection and its associated stress. Pérez-i-García et al. (2015) and Dallarés et al. (2016) reported similar trends in the teleosts *Alepocephalus rostratus* and *P. blennoides* off the same area, which supports this hypothesis. However, different contrasting trends have been reported by some authors in other fish species (Gupta and

Agarwal, 1985; Podolska and Napierska, 2006). The negative correlations also observed between LDH activity levels and abundance of some parasites have not been reported elsewhere and actually contradict the outcomes by Pérez-i-García et al. (2015), where an opposite trend was observed. These inconsistencies warn against a too simplistic interpretation of these results, especially considering that enzymes respond to many different factors (Solé et al., 2010) that might further interact among them yielding complex activity patterns.

A negative association between AChE activity levels and body size, as observed for *G. melastomus* and *C. coelolepis*, is a generalized trend commonly observed in many fish (Koenig and Solé, 2014; Dallarés et al., 2016) and it is considered to respond to a proportionality between AChE activity and muscular cell surface, which decreases with increasing body size (Lundin, 1962).

Although the comparison of enzymatic activities among the sharks addressed is not one of the main purposes of the present study, it is worth to note that mean CS activity values are much lower in *C. coelolepis* than in *G. melastomus* or *E. spinax* and that AChE activity values reach much higher values in *E. spinax* than in the other two species. Metabolic rates linked to oxygen consumption, in turn related to CS activity, are usually lower in deep-dwelling fishes than in those with a shallower distribution (Treberg et al., 2003; Drazen et al., 2005; Drazen et al., 2015). These trends have been explained on the basis of the visual-interactions hypothesis, which predicts declines in metabolism with depth for visual animals due to a reduction of the distances over which predators and prey interact coupled with lower need for rapid locomotory capacity to chase prey (Drazen and Seibel, 2007). The deeper distribution of *C. coelolepis* could thus account for the observed difference in CS activity. The overall higher AChE activity levels of *E. spinax* could be also explained on the basis of the different ecological habits of these species. Solé et al. (2008) and Solé et al. (2010) reported lower AChE activities in fish with lower mobility and linked to the benthos than in those with high swimming capacity and, therefore, more pelagic habits. These differences were attributed to the need for more mobile species of higher metabolic activities to hunt prey or escape predators (Solé et al., 2010). Of the three sharks addressed, *E. spinax* shows strongly pelagic habits while *G. melastomus* and *C. coelolepis* are demersal species feeding on benthic and benthopelagic prey (Carrassón et al., 1992), which can explain the higher AChE activity levels observed in the former species.

4.5. Fish condition indices in relation to parasites

Parasites showing positive relationships with the gonadosomatic index and the condition factor were more abundant in larger sharks and vice versa. Since these somatic indices also scaled with fish body length, the detected associations are most probably the consequence of these tendencies rather than of a true impact of parasite loads on fish condition. However, the repeated negative correlations detected between the hepatosomatic index and parasite loads, either total or of individual parasites, could be attributed to a detrimental effect of parasites on fish hepatic resources, as suggested by Dallarés et al. (2016) for the teleost *P. blennoides*. This pattern was not detected for *E. spinax* or *C. coelolepis*, and we hypothesize that only in cases in which parasites inflict severe damage to their host (as, for example, *G. adenoplusia* in the muscular tissues of *G. melastomus*, see sections 3.5.1. and 4.6. for details), the re-allocation of liver resources to face infection damage is clearly reflected in the hepatosomatic index.

4.6. Histological assessment in relation to parasites and fish condition indices

Melano-macrophages (MM) are pigmented specialized macrophagic cells occurring mainly in the haemopoyetic tissues of different organs and that contain heterogeneous materials, such as different pigments including melanin, cell debris or lipid droplets (Agius and Roberts, 2003). These structures accumulate degraded materials and metabolic wastes, and respond to the presence of chemicals, pollutants or pathogens (Fournie et al., 2001; Anderson et al., 2003; Carrassón et al., 2008) and to biological factors (e.g. age, size, reproduction or nutritional condition) (Agius, 1985; Montero et al., 1999; Jordanova et al., 2008). The characteristic features of MM in sharks have been described to date in a very low number of species (Pulsford et al., 1982; Agius and Agbede, 1984; Borucinska et al., 2009). In contrast to teleosts, in which the spleen is preferred, the liver is the most adequate organ to quantify these structures in the case of sharks (Borucinska et al., 2009). The observation that MM in *G. melastomus* do not aggregate forming the classical ‘centres’ described in teleosts (Carrassón et al., 2008; Dallarés et al., 2014) is in accordance with previous observations performed in shark species (Borucinska et al., 2009).

Since these structures accumulate throughout the lifespan of the fish (Agius and Roberts, 2003), their number in examined tissues usually scales with body size (Brown and George, 1985; Dallarés et al., 2014, 2016), which is further confirmed by present results from *G. melastomus*. The almost complete absence of MM in hepatic sections of *E. spinax* and *C. coelolepis* could be attributed to the fact that all examined sections of these species were obtained from juvenile individuals. Increases of hepatic melanomacrophages with reproductive maturity have been reported in teleosts (Elston et al., 1997; Jordanova et al., 2008) and explained on the basis of a liver remodelling phase after the spawning period (see Jordanova et al. (2008) and references therein). A similar pattern might occur in sharks, as suggested by the correlation (weak though) detected between density of MM and female gonadosomatic index in *G. melastomus*.

The correlations detected between density of MM and the activities of the two enzymatic indicators of metabolic activity assessed in the present study (i.e. LDH and CS) are meaningful, since increased metabolism should enhance the production of metabolic waste and, consequently, the formation of MM. The same trend was reported by Dallarés et al. (2014) in the teleost *M. moro* for LDH activity.

As highlighted by Dallarés et al. (2016), caution should be taken when attributing an increased density of MM to parasite infections. Despite the significant associations detected between the former and the abundance of some parasites in the present study, interactions with fish length occurred in some analyses. In the rest of cases, although an interaction was not detected, parasites displaying more abundance in larger sharks showed positive relationships with MM density and vice versa. Therefore, the results obtained suggest that fish size is far more determinant than parasite abundance in explaining MM quantitative variations and that these structures will probably respond to parasites only when these inflict appreciable damage to host tissues (as reported by Dezfuli et al., 2007).

We do not know to which extent muscular tissues infested with larval stages of *G. adenoplusia* lost their functionality. It is plausible that this occurred in the heavily affected tail musculature of adult sharks, taking into account the altered structure of the musculature affected. If this was the case, it could be hypothesized that this parasite would decrease the chance of escaping predators of *G. melastomus*, which would thus enhance the probability of parasite transmission.

Unfortunately, no muscular tissues were freshly fixed in formaline and an accurate histopathological analysis of the tissular alterations induced by *G. adenoplusia* was not possible in the present study. Montero et al. (2015) performed a preliminary histological assessment of these lesions, also in *G. melastomus* from the Mediterranean Sea, and concluded that the parasites were located within dilated muscular fibers and compressing the adjacent cells. However, no observations regarding the destruction of the muscular tissue were made, probably because the studied sharks were not large adults (as deduced from the prevalence of infection provided, 38.5%, when adult sharks show a prevalence of 100%) and the infestation levels by the parasite were still low.

5. Conclusions

In conclusion, the parasite community of *G. melastomus* is characterized by high abundance, richness and diversity. The cestodes *Ditrachybothridium macrocephalum* and *Grillotia adenoplusia* dominate the infracommunities of juvenile and adult specimens, respectively, in this host. A differentiation of parasite communities, linked to a diet shift, has been observed between ontogenic stages of this species. *Etmopterus spinax* displays a highly depauperate parasite community, which contrasts with results from the Atlantic Ocean. The parasite community of *C. coelolepis*, addressed herein for the first time, shows moderate richness and diversity.

Detailed parasite-prey relationships have been discussed and possible transmission pathways suggested for parasites of the three hosts. Parasites were mostly related to high water turbidity and O_2 levels, which enhance zooplankton proliferation and likely thus enhance parasite transmission. The nematodes *H. aduncum* and *P. obtusus* were in turn linked to high salinity levels, as already reported by previous studies, associated to high biomass and diversity of benthic and benthopelagic crustaceans, which may enhance nematode transmission. A decrease of acetylcholinesterase activity and lower hepatosomatic index, possibly linked to infection-related stress, have been observed. However, caution is recommended when using general fish condition indices and enzymatic activities to assess the impact of parasite infections on fish health. Lesions associated to encapsulated larvae of *G. adenoplusia* have been observed in the muscle of *G. melastomus*, especially in the tail region, which can be indicative of the hunting strategy of its final host and may compromise the escape response of *G. melatomus* thus facilitating parasite transmission.

Conflict of interest

The authors of the present study declare that they have no conflict of interest.

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CHAPTER 7 - MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *DITRACHYBOTHRIDIUM MACROCEPHALUM* REES, 1959 (CESTODA: DIPHYLLIDEA) FROM *GALEUS MELASTOMUS* RAFINESQUE IN THE WESTERN MEDITERRANEAN

CHAPTER 8 - REVISION OF THE FAMILY SPHYRIOCEPHALIDAE PINTNER, 1913 (CESTODA: TRYPANORHYNCHA), WITH THE DESCRIPTION OF *HETEROSPHYRIOCEPHALUS ENCARNAE* N. SP. AND REDESCRIPTIONS OF TWO SPECIES OF *SPHYRIOCEPHALUS*

CONCLUSIONS

CONCLUSIONS

1. The parasite communities of the teleosts *Mora moro* and *Phycis blennoides* are described for the first time, and are characterized by high parasite abundance, richness and diversity. *Mora moro* hosts 18 different endoparasite taxa, of which 17 are new host records, and *P. blennoides* hosts 20 different parasite taxa, of which 11 constitute new host records.

2. The nematodes Anisakidae gen. sp., *Anisakis* Type II and larval forms of tetraphyllidean cestodes are suggested as tags for differentiating populations of *M. moro* in the Balearic Sea.

3. Parasite infracommunities of *P. blennoides* vary with depth as a result of an ontogenic diet shift coupled to a bathymetric migration of this host, in turn associated to a different composition of macroinvertebrate communities along the continental slope.

4. Parasite infracommunities of *M. moro* and *P. blennoides* are more abundant, rich and diverse in the mainland than in the insular slope due to the existence of more complex and abundant benthopelagic faunal assemblages in the former, in turn associated to more favourable environmental and hydrographic conditions (i.e. higher O_2 levels and increased organic matter linked to submarine canyons).

5. Parasites communities of shark species are described for the first time from the Mediterranean Sea. To date, no shark parasite communities had been described in this area.

6. The parasite community of the shark *Scyliorhinus canicula* is characterized by low richness and diversity, and high dominance of the nematode *Proleptus obtusus*, which reaches very high abundance levels. A total of five parasites have been recovered, of which one constitutes a new host record.

7. The parasite community of the shark *Galeus melastomus* is described for the first time. It is characterized by high abundance, moderate richness and diversity values and high dominance of the cestodes *Ditrachybothridium macrocephalum*, in the case of juveniles, and *Grillotia adenoplusia*, in the case of adult sharks. A total of 15 metazoan parasites, of which 13 constitute new host records, and one microparasite have been recovered.

8. Parasite infracommunities of *G. melastomus* show lower richness and abundance off Vilanova than off Besós sampling sites, which is possibly due to the vicinity of the latter locality to the Besós submarine canyon, associated to more complex invertebrate communities as a result of higher nutrient availability.

9. Parasite infracommunities of adult specimens of *G. melastomus* are more abundant, rich and diverse than those of juveniles. This pattern is mainly associated to an ontogenic diet shift undergone by this shark, according to which adult sharks consume larger prey including crustaceans, cephalopods and fish.

10. The parasite community of juvenile specimens of the shark *Etmopterus spinax* is described for the first time in the Mediterranean Sea. It is characterized by extremely low richness and diversity values, with only two parasite species recovered, which contrasts with the parasite assemblages of juvenile *E. spinax* described from the Atlantic Ocean. The number of specimens available of this species was low and parasitological results should thus be considered preliminary.

11. The parasite community of the shark *Centroscymnus coelolepis* is described for the first time, and it is characterized by moderate richness and diversity. A total of eight parasites have been recovered, of which three constitute new host records. The number of specimens available of this species was low and parasitological results should thus be considered preliminary.

12. The abundance patterns of coelozoic parasites are mainly determined by feeding rates or dietary shifts of the host, while histozoic parasites accumulate in different tissues throughout the lifespan of the host and show a positive correlation with its size.

13. Multivariate Canonical Correspondence Analyses can be a powerful and reliable tool for inferring the environmental drivers of parasite abundance patterns, as suggested by the numerous coincidences found among the parasite communities of the hosts addressed regarding relationships between the abundance of individual parasites and environmental variables.

14. High O_2 concentration of water masses, linked to the mainland slope, enhances the abundance of parasites with indirect life cycles as a result of zooplankton proliferation and the associated aggregation of potential intermediate hosts. In a similar way, increased water turbidity favours parasite transmission and increases parasite loads due to an increase of zooplankton and suprabenthic invertebrate communities linked to higher nutrient availability.

15. High water salinity levels, associated to the upper slope, seem to be linked to higher abundance of the nematodes *Hysterothylacium aduncum* and *P. obtusus*, probably because salinity correlates with the abundance of decapods and mysids, among others, which are used by these nematodes as intermediate hosts. In turn, high water temperature levels increase the abundance of monogenean parasites, likely due to enhanced egg hatching success and reduced time to maturity, as previously reported for these parasites.

16. Multivariate Canonical Correspondence Analyses can be a useful tool for elucidating the transmission pathways of heteroxenous parasites, as suggested by the numerous coincidences found between the parasite-prey relationships determined in the different studies and what is known about the life cycles of the parasites addressed. Such detailed parasite-prey relationships provide a better understanding of the life cycles of the parasites addressed. Already known transmission patterns have been confirmed in many cases, and new ways of infection have been suggested in others.

17. A possible inhibition of acetylcholinesterase activity due to parasite infection-related stress has been detected in *P. blennoides*, *G. melastomus*, *E. spinax* and *C. coelolepis*. Furthermore, increased lipid peroxidation levels with higher abundance of the acanthocephalan *Echinorhynchus* sp. and the nematode *H. aduncum* have been detected in *P. blennoides*, possibly pointing to oxidative stress induced by parasitism. However, enzymes respond to many different factors that can interact among them yielding complex activity patterns, and inconsistencies observed for other enzymatic biomarkers or parasites, and in the literature, warn against a too simplistic interpretation of these results.

18. Lower citrate synthase activity has been observed in *C. coelolepis* compared to *G. melastomus* and *E. spinax*, possibly due to a deeper distribution of this shark. Higher acetylcholinesterase activity has been observed in *E. spinax* compared to *G. melastomus* and *C. coelolepis*, possibly explained by the more pelagic habits of this shark.

19. In general, parasite burden does not seem to have a significant effect on fish general condition indices, which points to a negligible impact of parasite loads on general fish condition when infection levels are not abnormally harmful or high. Caution should be taken when attributing variations of such indices, which respond to many different factors, to parasite infection.

20. Parasite burden does not seem to have a significant impact on the number and/or surface of splenic melano-macrophage or macrophage centres in the case of teleosts, or on the number of hepatic melano-macrophages in the case of *G. melastomus*. Since these structures respond to multiple factors, caution must be taken when attributing their variations to parasite infection.

21. The presence of cysts of unknown etiology is reported from *M. moro* and *P. blennoides*. In *P. blennoides*, these structures further reach the highest prevalences recorded to date in any fish from the study area.

22. Lesions associated to larval stages of the cestode *G. adenoplusia* have been observed in the musculature of all adult specimens of *G. melastomus*. These parasites accumulate on the tail region, possibly as a strategy to enhance their transmission onto the final host. Furthermore, the large accumulation of these larvae and associated destruction of the muscular tissues could compromise escape response of the host and favour its capture by the parasite final host.

23. Detailed morphological, molecular and ecological data of different developmental stages (plerocerci and adults) of the cestode *D. macrocephalum* are provided for the first time from its definitive host, the blackmouth catshark *G. melastomus*, in the Mediterranean Sea. Furthermore, morphological data on the eggs of *D. macrocephalum* are provided for the first time.

24. Mediterranean specimens of *D. macrocephalum* are conspecific with the material for the northeast Atlantic Ocean, as revealed by molecular results and morphological examination of type and voucher material. Morphological differences between specimens from the two localities are attributed to different developmental conditions and intraspecific phenotypic plasticity.

25. The higher abundance of *D. macrocephalum* in juvenile than in adult *G. melastomus*, and in the middle than in the upper slope, is in all likelihood related to ontogenic and bathymetric diet shifts undergone by its host.

26. The new species *Heterosphyriocephalus encarnae* n. sp. is described, and two already existing species, *Sphyriocephalus viridis* (the type species of the family Sphyriocephalidae) and S*phyriocephalus tergestinus* are further redescribed based on morphological and molecular data of newly-collected and museum material. *Heterosphyriocephalus encarnae* n. sp. can be readily distinguished from the rest of sphyriocephalid species by its small size, low number of proglottids, long velum with characteristically folded border, presence of cylindrical projections covering specific parts of the scolex, a typical heteroacanthous, heteromorphous, metabasal armature with eight hook per principal row, low number of testes in an exclusively pre-ovarian distribution and the absence of seminal vesicles.

27. The small cylindrical projections described in *H. encarnae*, only detectable by scanning electron microscopy, have also been observed in specific parts of the scolex of *S. tergestinus* and are reported for the first time in cestodes.

28. *Sphyriocephalus tergestinus* is allocated into *Heterosphyriocephalus* as *Heterosphyriocephalus tergestinus* n. comb. based on the results of a new phylogenetic analyses performed with available molecular data for sphyriocephalids and with new sequences generated from specimens from the Mediterranean Sea.

29. New generic dichotomous keys are provided for the family Sphyriocephalidae, as well as new generic diagnoses and keys for the determination of species for the genera *Sphyriocephalus* and *Heterosphyriocephalus* based on updated morphological descriptions and the results of the phylogenetic analysis.

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