Sentinels of the sea, from catsharks to their parasites: A multidisciplinary study using Scyliorhinus canicula (Linnaeus, 1758) as a model species for assessing the surrounding environment

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PhD thesis

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A multidisciplinary study using *Scyliorhinus canicula* (Linnaeus, 1758) as a model species for assessing the surrounding environment

A dissertation submitted by Andrea Higueruelo Fernández in fulfilment of the requirements for the degree of Doctor of Philosophy granted by the International Doctorate in Aquaculture in September 2025

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ABSTRACT/RESUM

Abstract

Catsharks, which includes the families Scyliorhinidae and Pentanchidae, is the most species-rich group of sharks, comprising numerous small-bodied, bottom-dwelling species that occupy relatively high trophic levels in marine ecosystems, displaying diverse life strategies.

Among them, the small-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758), is particularly abundant in European waters. Distributed throughout the northeastern Atlantic and the Mediterranean Sea, it inhabits sandy and muddy bottoms at depths from nearshore down to 800 m and is currently assessed as Least Concern by the International Union for Conservation of Nature. It is one of the most frequently caught elasmobranchs in the in the fisheries from the Mediterranean and the Balearic Sea, where it represents nearly two-thirds of upper-slope discards in terms of biomass. In Icelandic waters, about 20 demersal chondrychtians can be found, including the Iceland catshark *Apristurus laurussonii* (Saemundsson, 1922), the white ghost catshark *Apristurus aphyodes* Nakaya & Stehmann, 1998, and the mouse catshark *Galeus murinus* (Collett, 1904), three pentanchids from which many biological aspects are still poorly understood. All these catsharks, as is common within elasmobranchs, tend to harbour diverse parasite assemblages, shaped by a long history of co-evolution and holding the potential to serve as bioindicators of their host biology and ecology, as well as indicators of environmental changes. While extensive research has been carried out for decades in the Mediterranean basin, Icelandic waters remain relatively understudied.

Marine ecosystems face increasing impacts from human activities. The Mediterranean Sea, particularly the Balearic Sea, is a highly anthropized marine region. Among the pollutants of growing concern are marine litter and (micro)plastics that accumulate in the oceans, where they may be ingested by biota and cause potential harmful effects. Some heavy metals, which can bioaccumulate and biomagnify through marine food webs, also pose risks to both marine species and human health via seafood consumption due to their associated toxicity.

In this context, the present thesis provides a holistic study aimed to expand knowledge of the biology, ecology, health status, and parasite communities of catsharks, while assessing the potential risks of the consumption of species of this group to humans. It further evaluates their suitability as biomonitors of marine pollution and the use of their parasites as ecological indicators. *Scyliorhinus canicula* was selected as the model species, while comparative studies on Icelandic pentanchids aimed to offer insights into underexplored deepwater ecosystems.

In **Chapter 1**, a one-year-long in-depth study was conducted to gain insights into the reproductive biology of *S. canicula* in the Balearic Sea. A total of 1795 individuals were obtained from commercial fishing vessels off the Catalan coast in 24 samplings conducted between February 2022 and February 2023. Five female and three male reproductive stages were identified macroscopically and histologically and thoroughly described. The important role of the liver as an energy provider for reproduction was confirmed and liver colour variability was associated with reproductive stage rather than nutritional status. A continuous reproductive cycle was confirmed throughout the year, as well as the sperm storage capacity of females. A decreasing trend in length at 50% maturity was observed compared to previous estimates and further discussed.

In **Chapter 2**, an integrative assessment was conducted on the potential impacts affecting the population of *S. canicula* in three localities off the Catalan coast and on its consumption safety for humans. This was reached through the determination of distinct indicators such as biological data, ingested anthropogenic items (plastic and cellulose-like items), parasitological descriptors, trace metal concentrations and liver histopathology. Several small-scale differences among localities and depths were observed and discussed. Fibers were detected in 87% of *S. canicula* individuals, with a mean abundance of 5.47 items. Overall, analysed individuals did not appeared adversely affected by parasite infections, major pathologies or by the current levels of pollutants. While no zoonotic parasites were detected, encysted larvae of *Grillotia adenoplusia* found in the tail musculature may diminish the flesh quality. Finally, the high levels of mercury (Hg), well over European Comission (EC) limits, raised concerns regarding the consumption of *S. canicula* and other species from the region, warranting further investigation.

Consequently, in **Chapter 3**, a study was conducted with the aim to evaluate the biological and environmental factors influencing the accumulation of Hg, cadmium (Cd), and lead (Pb) (the three metals with limits in food regulated by the EC) in marine commercial species and the associated risks for consumers. To do so, trace metal concentrations were analysed in the muscle of ten marine species (seven teleosts, two elasmobranchs, and one crustacean) with varied habitat preferences caught off the Catalan coast. Geographical variability was also evaluated using *S. canicula* as a biomonitor, comparing Mediterranean results with data from Atlantic Spanish regions and published values. Mercury accumulation was size- and habitat-dependent, with benthic species showing the highest levels of this element. While Pb and Cd remained within European consumption limits, over one-third of benthic samples, and 92% of adult sharks (*S. canicula* and *G. melastomus*) exceeded Hg thresholds, compromising their commercialization.

The study highlighted the high methylation potential of the Mediterranean Sea and the suitability of *S. canicula* as a biomonitor.

Chapter 4 focused on exploring parasites as indicators of host ecology. In this study, the parasite assemblages of *A. aphyodes*, *A. laurussonii*, and *G. murinus* were characterised for the first time, based on 56 specimens collected in southern and western Icelandic waters. A total of 15 parasite taxa were identified, representing 27 new parasite-host records. Most common parasites were found in more than one host, suggesting similar feeding habits, niches, and trophic positions. Nonetheless, multivariate analyses revealed significant differences in the structure and composition of parasite assemblages, as well as small-scale geographic variability. Comparative analyses across a broader geographic scale indicated that North Atlantic pentanchids harbour more diverse and species-rich parasite communities, with lower dominance than their Mediterranean counterparts. A significant proportion of the identified parasite taxa may constitute species new to science.

Accordingly, in **Chapter 4.1**, a new monogenean parasiting Icelandic pentanchids, *Calicotyle carmenae* n. sp., was described from an integrative taxonomy approach (*i.e.*, combining morphological, molecular and ecological observations). *Calicotyle carmenae* is characterised by a haptor with one central and seven peripheral loculi, two hamuli, and several hooklets, and differs from its congeners by having a U-shaped double vagina, a male copulatory organ with a single loop, intercaecal vaginal pores, and the absence of eye spots. The study suggests that *Calicotyle* spp. rely heavily on the haptor for attachment and show host specificity at the family rather than species level. It also highlights the need for a revision of the Calicotylinae subfamily due to various inconsistencies and the lack of information on several species.

Resum

Els anomenats 'taurons gat', que inclouen membres de les famílies Scyliorhinidae i Pentanchidae, constitueixen el grup de taurons amb més espècies, configurat per individus de mida petita, bentònics, que ocupen nivells tròfics relativament alts en els ecosistemes marins i que presenten diverses estratègies de vida.

Entre elles, el gat, *Scyliorhinus canicula* (Linnaeus, 1758) és particularment abundant a les aigües europees. Distribuït al llarg de l'Atlàntic nord-est i la mar Mediterrània, habita fons sorrencs i fangosos a profunditats des de la costa fins als 800 m, i actualment està classificat com a "Preocupació Menor" per la Unió Internacional de Conservació de la Natura. És un dels elasmobranquis més capturats per pescadors de la Mediterrània i a la mar Balear, on representa gairebé dos terços dels descartes de la pendent superior en termes de biomassa. En aigües Islandeses, es poden trobar unes 20 espècies de condrictis demersals, entre els quals es troben *Apristurus laurussonii* (Saemundsson, 1922), *Apristurus aphyodes* Nakaya & Stehmann, 1998 i *Galeus murinus* (Collett, 1904), tres Pentanchids dels quals molts aspectes biològics en són encara poc coneguts. Tots aquests 'taurons gat', com és comú en elasmobranquis, tendeixen a comptar amb comunitats de paràsits diverses, modelades per una llarga història de coevolució, amb el potencial de servir com a bioindicadors de la biologia i l'ecologia del seu hoste, així com d'indicadors de canvis ambientals. Mentre que la conca Mediterrània ha estat objecte d'investigacions extenses durant dècades, les aigües islandeses han estat fins ara relativament poc estudiades.

Actualment, però, tots els ecosistemes marins es troben sotmesos als impactes creixents derivats de les activitats humanes. La mar Mediterrània, i en particular la mar Balear, és una regió marina altament antropitzada. Entre els contaminants de preocupació creixent hi ha la brossa marina i els (micro)plàstics que s'acumulen als oceans, on poden ser ingerits pels organismes i potencialment causar efectes adversos. Alguns metalls pesants, que poden bioacumular-se i biomagnificar-se a través de les xarxes tròfiques marines, també representen un risc tant per a les espècies marines com per a la salut humana quan aquestes són ingerides, a causa de la seva toxicitat associada.

En aquest context, la present tesi proporciona un estudi holístic amb l'objectiu d'ampliar el coneixement sobre la biologia, ecologia, estat de salut i comunitats parasitàries dels 'taurons gat', alhora que avalua els riscos derivats del consum d'aquestes espècies per als consumidors. Així mateix, s'hi avalua la seva idoneïtat com a biomonitors de la contaminació marina i l'ús dels

seus paràsits com a indicadors ecològics. Concretament, es va seleccionar *S. canicula* com a espècies model, mentre que les anàlisis comparatives amb pentànquids capturats a aigües Islandeses pretenen proporcionar coneixements sobre ecosistemes profunds relativament poc explorats.

En el **Capítol 1** es va dur a terme un estudi exaustiu d'un any de durada per aprofundir coneixements sobre la biologia reproductiva de *S. canicula* en el mar Balear. Es van obtenir un total de 1.795 individus de la costa catalana a través de vaixells de pesca comercial al llarg de 24 mostrejos realitzats entre febrer de 2022 i febrer de 2023. Es van identificar cinc estadis reproductius per femelles i tres per a mascles, tant macroscòpicament com histològicament, i es van descriure detalladament. Es va confirmar el paper important del fetge com a proveïdor d'energia per a la reproducció, i la variabilitat del color del fetge es va associar amb l'estadi reproductiu més que no pas amb l'estat nutricional. Es va confirmar un cicle reproductiu continu al llarg de l'any, així com la capacitat d'emmagatzematge d'esperma de les femelles. Per últim, es va observar una tendència decreixent en la talla de maduresa sexual en comparació amb estimacions prèvies, que es discutida.

En el **Capítol 2**, es va realitzar una avaluació integrada dels impactes potencials que afecten la població de *S. canicula* de tres localitats catalanes i els riscs derivats del seu consum per als humans. Això es va aconseguir mitjançant diversos indicadors com dades biològiques, ingestió d'ítems antropogènics (plàstics i fibres de cel·lulosa), descriptors parasitològics, concentracions de metalls traça i alteracions histològiques. Es van observar i discutir diverses diferències a petita escala entre localitats i fondàries. En total, es van detectar fibres en el 87 % dels individus de *S. canicula*, amb una abundància mitjana de 5,47 ítems. En genral, els individus analitzats no van presentar afectacions adverses per infeccions parasitàries, patologies greus ni pels nivells actuals de contaminants. Tot i que no es van detectar paràsits zoonòtics, la presència de larves enquistades de *Grillotia adenoplusia* a la musculatura de la cua redueixen la qualitat de la carn. Finalment, els nivells elevats de mercuri (Hg), molt per sobre dels límits de la Comissió Europea (CE), van generar preocupació sobre el consum de *S. canicula* i altres espècies de la regió, requerint investigacions addicionals.

En conseqüència, en el **Capítol 3**, es va dur a terme un estudi amb l'objectiu d'avaluar els factors biològics i ambientals que influeixen en l'acumulació de Hg, cadmi (Cd) i plom (Pb), els tres metalls amb límits regulats en aliments per la CE, en espècies marines comercials i els riscos associats per als consumidors. Per a això, es van analitzar les concentracions de metalls traça al múscul de deu espècies (set teleostis, dos elasmobranquis i un crustaci) amb preferències d'hàbitat i trets biològics variats, capturats davant la costa catalana. També es va avaluar la variabilitat geogràfica utilitzant *S. canicula* com a biomonitor, comparant els resultats mediterranis amb dades d'àrees atlàntiques espanyoles i amb valors publicats. L'acumulació de Hg va resultar depenent de la talla i de l'hàbitat, amb les espècies bentòniques mostrant els nivells més alts d'aquest element. Mentre que Pb i Cd es mantenien dins dels límits de consum europeus, més d'un terç de les mostres bentòniques i el 92% dels taurons adults (*S. canicula* i *G. melastomus*) van superar els llindars de Hg, comprometent la seva comercialització. L'estudi va destacar el gran potencial de metilació de la mar Mediterrània i la idoneïtat de *S. canicula* com a biomonitor.

El **Capítol 4** es centra en l'exploració dels paràsits com a indicadors de l'ecologia de l'hoste. En aquest estudi es van caracteritzar per primera vegada les comunitats parasites de *A. aphyodes*, *A. laurussonii* i *G. murinus*, basant-se en 56 exemplars recollits a aigües del sud i oest d'Islàndia. Es van identificar un total de 15 tàxons, representant 27 nous registres hoste-paràsit. Els paràsits més comuns es van trobar en més d'un hoste, suggerint hàbits alimentaris, nínxols i posicions tròfiques similars. No obstant això, les anàlisis multivariants van revelar diferències significatives en l'estructura i la composició de les comunitats parasitàries, així com variabilitat geogràfica a petita escala. Les anàlisis comparatives a una escala geogràfica més àmplia van indicar que els "taurons gat" de l'Atlàntic Nord allotgen comunitats parasitàries més diverses i riques en espècies, amb menor dominància que els seus homòlegs Mediterranis. Una proporció significative dels tàxons parasitaris identificats podrien constituir espècies noves per a la ciència.

Per aquest motiu, al **Capítol 4.1** es va descriure un nou monogeni que parasita Pentanchids d'aigües Islandeses, *Calicotyle carmenae* n. sp., a partir d'un enfocament taxonòmic integrador, combinant observacions morfològiques, moleculars i ecològiques. *Calicotyle carmenae* es caracteritza per un haptor amb un locul central i set perifèrics, dos 'hamuli' i diversos 'hooklets', i es diferencia dels seus congèneres per presentar una vagina doble en forma de U, un òrgan copulador masculí amb una sola volta, porus vaginals situats entre els cecs intestinals i l'absència de taques oculars. L'estudi suggereix que les espècies de *Calicotyle* depenen fortament del haptor per a l'adherència a l'hoste i mostren una especificitat d'hoste a nivell de família més que d'espècie. També posa de manifest la necessitat d'una revisió de la subfamília Calicotylinae a causa de múltiples inconsistències i de la manca d'informació sobre diverses espècies.

INTRODUCTION

1. Catsharks in marine ecosystems: diversity, biology and ecology

The class Chondrichthyes represents one of the most ancient extant vertebrate lineages, having persisted for approximately 440 million years (Ebert et al., 2021). Members of this class are distinguished by several morphological traits, including an endoskeleton composed of calcified cartilage, teeth in replicating rows rooted in the gum, and precaudal fins supported by proximal cartilaginous basals and distal radials (Compagno, 1999). Additional features characteristic of Chondrichthyes include five to seven pairs of uncovered gill slits, a skin covered with separate dermal placoid scales (dermal denticles), typically a pair of spiracles, and the absence of a swim bladder (Kotpal, 2010). Chondrichthyes encompass a wide range of ecological niches, displaying also variability in their feeding behaviour, size and reproductive strategies (Jabado et al., 2024).

This group is divided into two subclasses: Holocephali, which includes chimaeras, and Elasmobranchii, the larger and more diverse group comprising the infraclasses Selachii (*i.e.*, sharks) and Batoidea (*i.e.,* rays). To date, about 566 shark species have been described (FishBase, 2025), with this number continually increasing as new species are discovered. Of these, the order Carcharhiniformes, commonly known as ground sharks, is the most species-rich and widespread group, comprising 54.2% of all known shark species, distributed across 12 families (FishBase, 2025) **(Fig. 1)**.

Catsharks are part of this group and were originally all classified within the family Scyliorhinidae. However, subsequent taxonomic revisions divided this group, establishing Pentanchidae (deep-water catsharks) as a separate family, which has since become the most species-rich shark family (Iglésias et al., 2005a).

Sharks are normally associated with a K-selected life history strategy, producing a small number of large, energetically costly offspring with high survival potential. This strategy is associated with large body size, slow growth, late maturity, and long gestation periods, which in some species exceed those of mammals (Holden, 1974). In contrast, smaller shark species, as is the case of most catsharks, grow and reach maturity more rapidly.

While viviparity is the predominant mode of reproduction in sharks, oviparity allows smaller-bodied species, including those in the families Pentanchidae and Scyliorhinidae, to produce larger numbers of offspring more rapidly **(Fig. 2)**, although some exceptions exist (*e.g.,* aplacental viviparity in *Galeus polli* Cadenat, 1959) (Ebert et al., 2006, 2021). Catsharks may therefore represent an intermediate position between K- and r-selected strategies, characterised by the production of numerous, small, “low-cost” offspring (Jabado et al., 2024).

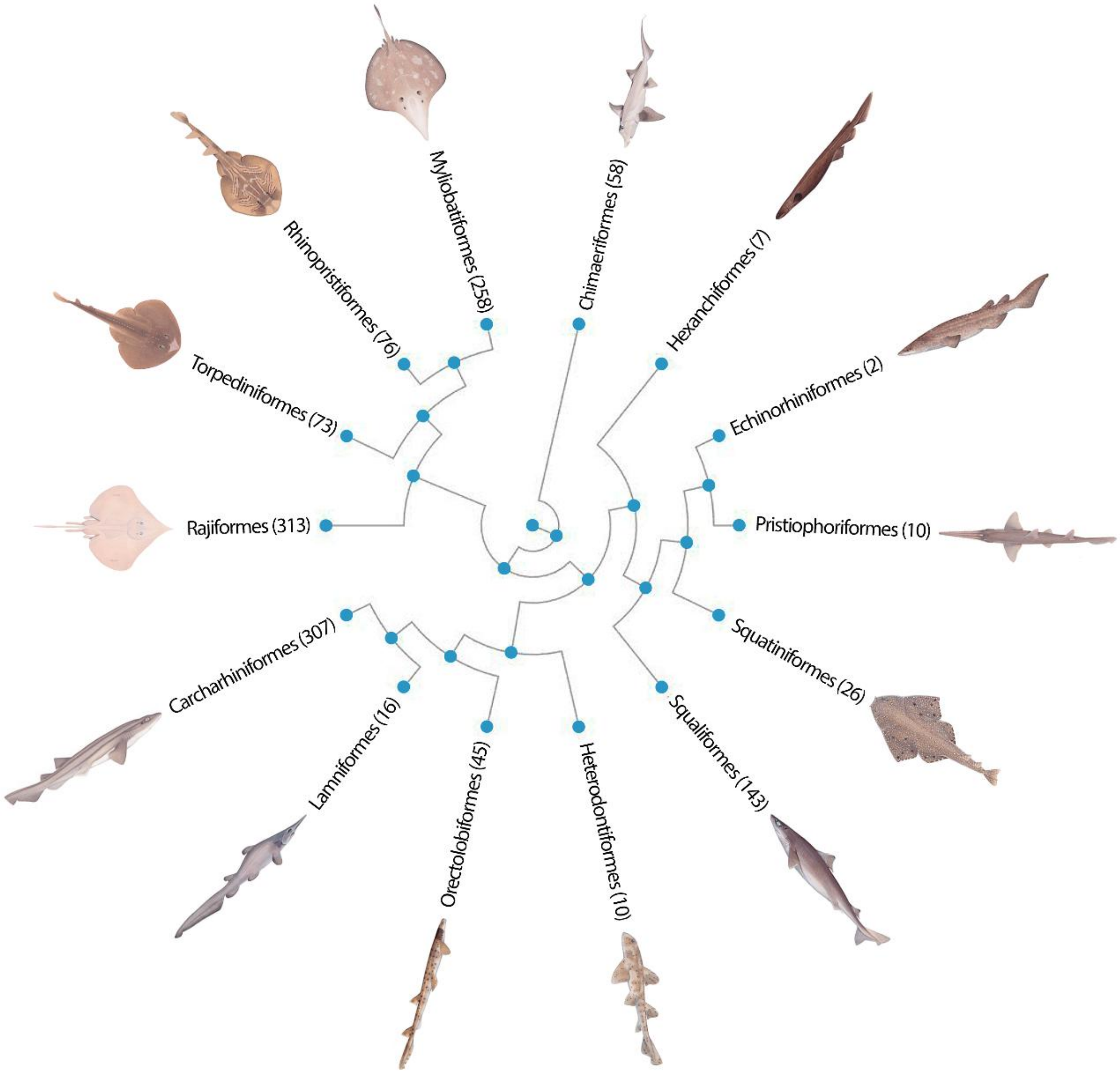


Figure 1. Phylogenetic estimate based on mitochondrial NADH2 sequence data derived from the Chondrichthyan Tree of Life project. Numbers in brackets refer to species numbers in the different orders. Source: Chondrichthyan Tree of Life (https://sharksrays.org/). Data updated from FishBase.

In most catsharks, only the right ovary is functional, and the left ovary is vestigial or absent (Hamlett & Koob, 1999). In addition, normal and abnormal hermaphroditism have also been reported in catshark species (Ellis & Shackley, 1997; Iglésias et al., 2005b).

Although much has been achieved in the last 40 years of research on chondrichthyan age and growth, age determination of chondrichthyans is often inaccurate and imprecise relative to many teleost fishes and limited to a fraction of the species described (Harry et al., 2022; Natanson et al., 2018).

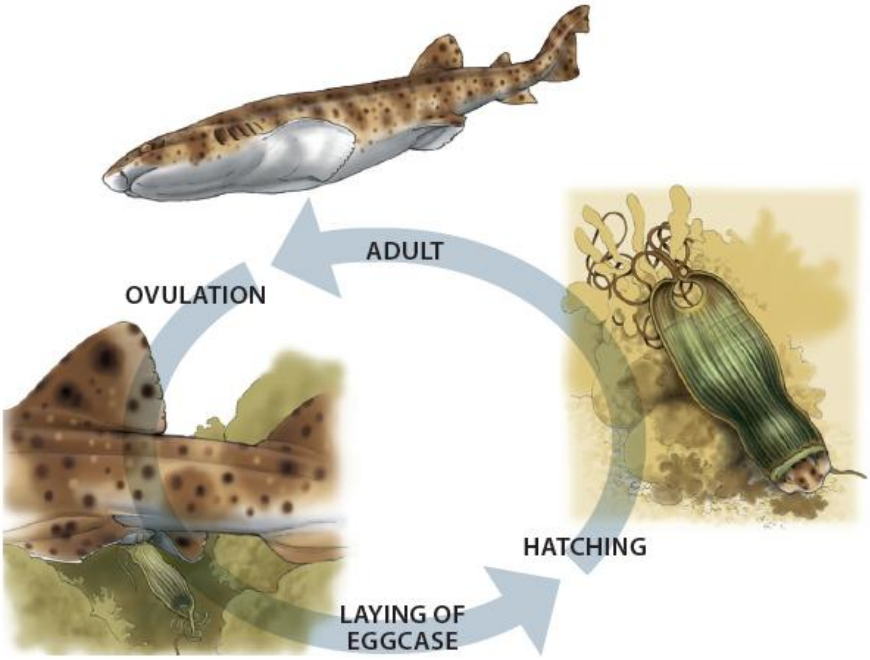


Figure 2. Oviparous reproductive cycle in a swellshark, *Cephaloscyllium ventriosum* (Garman, 1880) (Carcharhiniformes: Scyliorhinidae). Source: Ebert et al., 2021.

are found (Flammang et al., 2008). Since several shark species are commercially exploited, the study of age and growth is fundamental, with important applications in fisheries management and conservation, and in several branches of ecology (Harry et al., 2022).

Another field of ongoing research is the study of the ecological importance of sharks and their relatives in marine ecosystems. Historically, the literature has focused primarily on apex predators, which directly influence the abundance and distribution of prey species (Heithaus et al., 2022; Jabado et al., 2024). Through predation and risk effects, these predators help shaping community composition and regulate habitat structure, such as seagrass biomass and macroalgal establishment, which, in turn, supports carbon sequestration (Atwood et al., 2018; Dedman et al., 2024; Heithaus et al., 2022; Schmitz et al., 2023). Additionally, by feeding in one habitat and excreting in another, or through the falling of their carcasses after death, large sharks contribute to nutrient redistribution (*e.g.*, nitrogen and phosphorus), moving nutrients between habitats with varying productivity (Heithaus et al., 2022; Papastamatiou et al., 2015; Williams et al., 2018).

Nonetheless, very few sharks are truly apex predators, and they play a wide range of roles in marine trophic webs. Some sharks are scavengers and some of the largest ones, such as the Whale Shark (*Rhincodon typus* Smith, 1828), the Basking Shark [*Cetorhinus maximus* (Gunnerus, 1765)], or the Megamouth Shark (*Megachasma pelagios* Taylor, Compagno & Struhsaker, 1983), are filter-feeders on plankton and small fish. In addition, most small and medium-sized sharks, including most catsharks, are meso-predators or secondary consumers, located in the middle of the food web around levels 3–4 (Dedman et al., 2024; Ebert et al., 2021) **(Fig. 3).**

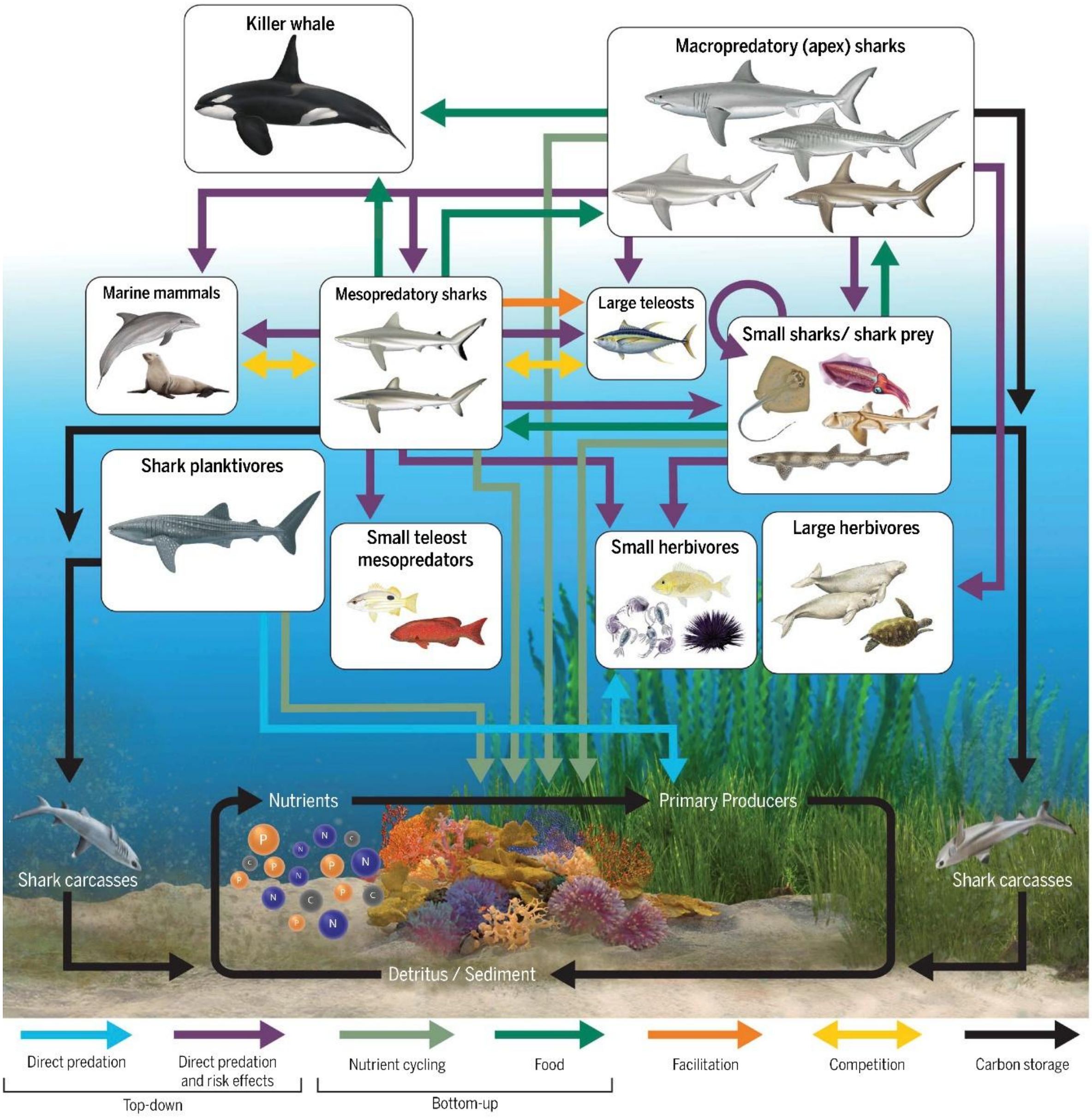


Figure 3. Conceptual model of the current state of knowledge of the ecological roles and importance of sharks in aquatic food webs. Source: Dedman et al., 2024.

In this sense, benthic small sharks, such as rays and catsharks, regulate populations of invertebrates and smaller fish and play a key role in linking lower and upper trophic levels, contributing to energy and nutrient transfer. Juveniles often feed in shallower, more specific niches, while adults have broader diets and sometimes move vertically in the water column, also connecting benthic and pelagic food webs (Barría et al., 2018).

Despite their ecological importance, shark populations across various ecosystems have undergone dramatic declines in their abundance (Dedman et al., 2024; Pacoureau et al., 2021; Roff et al., 2018). According to the International Union for Conservation of Nature (IUCN), more than third (37%) of all chondrichthyans are currently threatened with extinction, representing the most threatened vertebrate Class behind the amphibians (Díaz et al., 2019; IUCN, 2025). The primary threat that sharks are currently facing is overfishing, mainly due to bycatch, but also by targeted fishing for their fins, meat, and even liver oil. Additional threats include habitat loss and degradation, climate change and pollution (Dulvy et al., 2014; 2021). Rates of population declines consequence of these threats, however, are not universal. Large and slow-reproducing species are particularly vulnerable to anthropogenic pressures, showing prolonged population recovery times. In turn, smaller elasmobranchs with higher productivity are more resilient to fisheries associated impacts, and the collapse of larger sharks that prey on them can even favour their proliferation (Dedman et al., 2024; Jabado et al., 2024). Accordingly, the percentage of catshark species threatened with extinction (14%) is markedly lower than that of chondrichthyans as a whole (IUCN, 2025).

2. Assessment of marine ecosystems health

Marine ecosystems, covering over 70% of the Earth's surface, host a substantial portion of global biodiversity and play a critical role in regulating the global climate and biogeochemical cycles, among other fundamental ecosystem services (Crowe & Frid, 2015). However, these ecosystems are increasingly threatened by anthropogenic pressures including climate change, overfishing, and pollution (Halpern et al., 2008; Priya et al., 2023). The regular assessment of marine ecosystems and the species living within them is crucial for understanding the extent of human-induced changes and for guiding effective conservation, restoration, and management strategies to ensure the long-term sustainability of oceanic biodiversity and other ecosystem services (Crowe & Frid, 2015).

Adequately assessing the condition and health of wild fish populations, which is driven by complex and multifactorial influences, necessitates a holistic approach that integrates a diverse array of diagnostic indicators. This assessment should encompass general measures of overall fish condition through indicators such as Le Cren's relative body condition index or hepatosomatic and gonadosomatic indices (Rodríguez-Romeu et al., 2022), as well as more specific indicators, including the identification of potential histological alterations (*e.g.,* haemorrhagic lesions, necrosis and inflammation, epitheliocystis, macrophage aggregations) (Costa, 2018; Gajić et al., 2020; Stedman and Garner, 2018).

In addition, in the current era, known as the Anthropocene from the extensive influence of human beings on Earth natural dynamics (Lewis & Maslin, 2015), growing anthropogenic pressures have intensified the efforts on understanding the long-term effects of pollutant exposure in marine organisms. Traditionally, research and environmental assessments have focused on "legacy pollutants" such as heavy metals (*e.g.*, mercury, cadmium, or lead), polychlorinated biphenyls (PCBs), and organochlorine pesticides, substances known for their persistence, bioaccumulation, and toxicity (León & Bellas, 2023). These pollutants are relatively well studied and regulated due to their recognized environmental and health risks. However, increasing focus is being placed on a wider range of contaminants of emerging concern (CECs), including pharmaceuticals, personal care products, flame retardants, and perfluorinated compounds, many of which are potentially harmful and remain largely unregulated (Grobelak & Kowalska, 2022; León & Bellas, 2023). Among CECs, plastics and particularly microplastics (MPs) have received growing attention due to their widespread distribution, heterogeneous composition, and capacity to adsorb and transport hazardous substances, thereby raising concerns about their biological impacts on marine organisms (Pittura et al., 2023).

2.1. Heavy metals

Heavy metals are naturally present in marine ecosystems through natural processes such as weathering or volcanic activity, and some of them are essential for the ecosystems at low concentrations. Nonetheless, anthropogenic activities, such as stemming from industrial emissions, mining, agriculture, and urban runoff, have significantly contributed to increasing their concentrations, becoming a major global environmental concern (Briffa et al., 2020; Vardhan et al., 2019). In marine ecosystems, heavy metals are transported via rivers, atmospheric deposition, and coastal discharge, where they can accumulate in sediments and bioaccumulate in organisms (Piwowarska et al., 2024), entering food webs through ingestion, respiration, or adsorption. Once inside the organism, these metals may be subject to chemical and biological transformations, such as biotransformation and tissue deposition, thereby contributing to their accumulation (Lozano-Bilbao et al. 2024). Their accumulation in biota is determined by numerous physiological, biological and ecological factors, such as detoxification capacity, feeding habits, trophic level, age or habitat use (Madgett et al., 2021; Signa et al., 2017).

In addition, geographic location and, in turn, its associated environmental conditions (*i.e.,* pH, temperature, salinity or oxygen levels) are key determinants of metal uptake, as they influence metal bioavailability and speciation (Luoma & Rainbow, 2011).

These pollutants can cause a spectrum of adverse effects in marine life, including impaired growth, reproductive dysfunction, immunosuppression, and organ damage (Garai et al., 2021; Piwowarska et al., 2024). Among heavy metals, mercury (Hg), cadmium (Cd), and lead (Pb) are known as the toxic trio because of their highly hazardous characteristics (Chojnacka, 2010). These metals not only harm marine organisms (Garai et al., 2021) but also pose significant health risks to humans, and especially to vulnerable groups like pregnant women and children. Exposure to these elements has been linked to a range of health problems, including cancer, neurological damage, gastrointestinal, cardiovascular, and renal issues, as well as immunosuppression, reproductive defects, and endocrine disruption (WHO, 2019, 2023, 2025). Consequently, in the European Union these metals are strictly regulated under the Commission Regulation (EC) No 2023/915, which sets maximum levels and analytical protocols for Hg, Cd, and Pb in foodstuffs, including fish and seafood (EC, 2023).

2.2. Marine litter

In recent decades, marine litter, defined as any persistent, manufactured, or processed solid material discarded in marine and coastal ecosystems (UNEP, 2009), has emerged as a significant and growing threat to marine environments worldwide. Since its initial recognition in scientific literature in the 1960s, research has revealed that the vast majority of marine litter consists of plastics, whose global production has increased dramatically over the past decades (Bergmann et al., 2015). In addition, global surveys highlight their persistence and ubiquity across marine ecosystems, from pelagic to benthic environments, including isolated islands and polar regions (Barnes, 2005; Barnes et al., 2010; Bergmann & Klages, 2012).

Marine litter entering the oceans, particularly plastics, originates from a wide range of both land- and sea-based sources, including urban runoff, industrial discharges, wastewater effluents, maritime activities such as fishing and shipping, and atmospheric deposition (Browne et al., 2011; Jambeck et al., 2015). Once introduced into the marine environment, these materials undergo fragmentation due to physical, chemical, and biological processes such as photodegradation, mechanical abrasion, and hydrolysis, leading to the formation of progressively smaller particles (Andrady, 2011) **(Fig. 4)**.

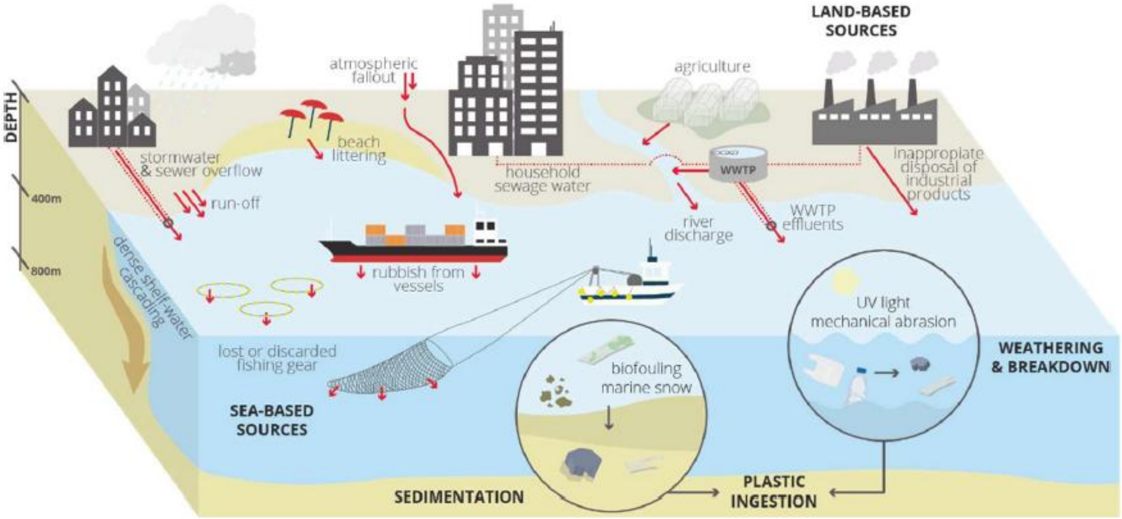


Figure 4. Main sources and pathways of plastic and microplastic litter into the environment. Source: Carreras-Colom, 2021.

Particles smaller than 5 mm in size are considered microplastics (MPs) (GESAMP, 2015). These can be classified as primary MPs, which are intentionally manufactured at small sizes for industrial or commercial purposes, and secondary MPs, which result from the environmental degradation of larger plastic debris (Hartmann et al., 2019). In addition, particles smaller than 1000 nm are referred to as nanoplastics (NPs), which also arouse scientific interest in the recent years (Brandts, 2022; Gigault et al., 2018; Hartmann et al., 2019). Among the various types of microlitter (fibres, fragments, films and pellets), fibres are among the most abundant and widespread (Rodríguez-Romeu, 2022). These include not only synthetic fibres with a petrochemical origin such as polyester or nylon, but also non-synthetic ones, including artificial cellulose (*e.g.*, rayon, viscose) and natural fibres (*e.g.,* cotton, wool) (Barrows et al., 2018; Rodríguez-Romeu, 2022). In fact, non-synthetic fibres constitute the majority of fibres found in the oceans, with cellulose accounting for approximately 80% of the total (Suaria et al., 2020).

The ingestion of different anthropogenic items (AIs) by marine organisms has been widely documented across a broad range of taxa and environments (Concato et al., 2023; Markic et al., 2020; Marmara et al., 2023). Once ingested, these particles can potentially cause both physical and physiological effects. Reported impacts include inflammation, oxidative stress, disruptions to the immune and antioxidant systems, and alterations in the absorption of nutrients, among others (Espinosa et al., 2017; Wright et al., 2013). In addition, plastics can act as vectors for hazardous chemicals and other pollutants present in the environment (*e.g.,* persistent organic pollutants, heavy metals), potentially enhancing the bioavailability and toxicity of these compounds within organisms (Avio et al., 2017; Brennecke et al., 2016; Rochman et al., 2013; Teuten et al., 2009). However, most of these findings are based on laboratory experiments, often under controlled conditions and at concentrations exceeding those typically found in the environment. Consequently, establishing clear cause-effect relationships in wild populations remains challenging. Field studies have shown high prevalence of fibre ingestion in commercially and ecologically important species, but consistent correlations with health condition or physiological biomarkers are limited (Carreras-Colom et al., 2020, 2022; Muns-Pujadas et al., 2023; Rodríguez-Romeu et al., 2020, 2022). This underlines the need for further long-term and ecological studies to better understand the effects of AIs ingestion in wild populations.

2.3. Biomonitor and bioindicator species

Biomonitors and bioindicators are biological processes, organisms, or communities that provide quantitative or qualitative information about the quality of the environment or about complex biological processes (Holt & Miller, 2010). They are valuable tools for detecting changes in the environment and assessing their potential impacts (Markert et al., 2003). These environmental changes may result from natural stressors, such as droughts, heatwaves, or late spring frosts, but most bioindicator research has focused on anthropogenic disturbances, including pollution, habitat fragmentation, and land-use change (Holt & Miller, 2010). For instance, biomonitors have been widely employed to assess the accumulation of heavy metals, and more recently, to detect the presence and patterns of plastic pollutants in aquatic ecosystems (Luoma & Rainbow, 2011; Savoca et al., 2025).

No single species can serve as a universal indicator for all types of environmental stress or disturbance. The selection of suitable bioindicator species, or groups of species, must be adapted to the specific environmental context, considering the local ecological conditions, the species present, and the nature of the disturbances affecting the area (Holt & Miller, 2010). Some of the criteria for selecting effective bioindicators are summarized in **Table 1**.

Elasmobranchs are increasingly recognized as valuable bioindicators of marine pollution due to their ecological, physiological, and biochemical traits (Alves et al., 2022). For example, their fat rich livers make them susceptible to the accumulation of lipophilic substances like persistent organic pollutants (POPs) (Carrier et al., 2010). Their high trophic position and long lifespan also makes them particularly prone to the bioaccumulation and biomagnification of environmental contaminants such as heavy metals and POPs. In addition to their ecological relevance, elasmobranchs are globally distributed and often accessible through commercial and recreational fisheries, facilitating their acquisition (Alves et al., 2022).

Table 1. Selection criteria for effective bioindicators. Modified from Gerhardt (2002) and Holt & Miller (2010).

Category	Criteria
Distribution	Widely distributed, cosmopolitan, useful for international comparisons
Ecology	High abundance and widespread in a certain type of environment
	Restricted mobility, site specificity
	Well-known ecology, physiology and life history
	Ecologically relevant position in the ecosystem (*e.g.,* keystone species)
Representativeness	Their response should mirror that of other species or of broader ecosystem health
Practicability	Easy to sample, sort, store
	Easily recognizable by non-specialists
	Robust during handling, easy to measure
	Easily culturable in the laboratory
	Cost-effective
Social importance	Economically important as a resource
	Public interest in or awareness of the species
Sensitivity (qualitative indicator)	Sensitive to specific pollutants or other stresses
Sentinel capacity (quantitative indicator)	Accumulate and concentrate pollutants above those of the surroundings
	Not affected by the pollutant

Catsharks, in particular, can be suitable for monitoring local environmental conditions, since they are non-migratory species known to exhibit philopatric behaviour (Ebert & Stehmann, 2013; Sims et al., 2001, 2005). In addition, their benthic-dwelling nature makes them particularly exposed to pollutants, as sediments constitute a major sink for contaminants such as POPs and heavy metals. (Oros, 2025; Solé et al., 2013). Similarly, different kinds of AIs, including MPs and fibres, accumulate in sediments, increasing the likelihood of ingestion by benthic species compared to pelagic ones (Neves et al., 2015; Sandgaard et al., 2023). Opportunistic predators like catsharks are particularly exposed to AIs ingestion and they have already been proposed as sentinel species for monitoring their presence on the seafloor (Sbrana et al., 2022; Valente et al., 2019).

Parasites, which are particularly diverse in elasmobranchs, often exist in equilibrium with their hosts, having co-evolved over millions of years (Caira and Healy, 2004; Hoberg & Klassen, 2002; Råberg, 2014). Thus, parasites are also powerful bioindicators, being able to provide valuable information on their host species (Timi, 2025). They have been used for many decades as indicators of fish population stocks, reflecting host phylogenetic relationships (Locke et al., 2013;

MacKenzie & Abaunza, 1998), and as indicators of complex food web interactions and migration patterns (Alarcos & Timi, 2013; Dallarés et al., 2017; Williams et al., 1992).

In addition, since its emergence 30 years ago, the field of environmental parasitology has developed to explore the potential of parasites not only as ecological indicators of their hosts, but also as indicators of environmental changes and disturbances (Lafferty, 1997; Sures et al., 2025). Since pollution and other stressors may directly or indirectly alter parasite communities, parasites can act as effect indicators of changes in ecosystems. For example, changes in the composition of certain parasite species sensitive to pollutants (*e.g.,* monogeneans) can reflect changes in the environment (Gilbert & Avenant-Oldewage, 2021). Additionally, certain parasites, such as acanthocephalans and cestodes, serve as effective accumulation indicators, capable of concentrating heavy metals and organic contaminants to levels far exceeding those found in host tissues or the surrounding environment (Sures et al., 2017). These parasites can reveal both the presence and bioavailability of contaminants, even in low-contamination conditions, such as those found in remote areas like Antarctica, where other bioindicators may fail, thus serving as early-warning indicators (Morris et al., 2016; Sures & Reimann, 2003).

Catsharks, being benthic species with usually a diverse diet, tend to harbour rich parasite faunas, which offer the opportunity to serve as useful indicators of environmental variability or trophic interactions (Carrassón et al., 1992; Klimpel, 2006, 2009; Van der Heever et al., 2020).

3. The Mediterranean Sea: A region under siege

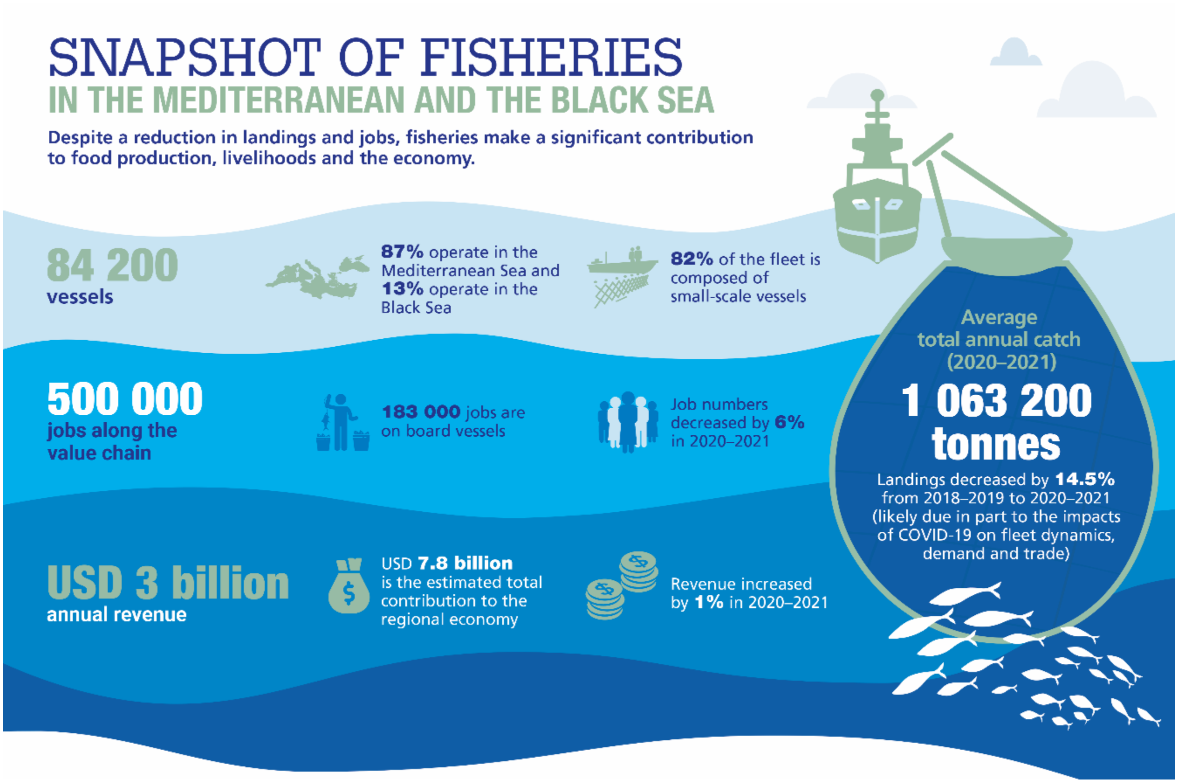
The Mediterranean Sea is a semi-enclosed marine basin located between Europe, Asia, and Africa, characterised by distinctive geological, hydrographic, physical, and ecological features (Goffredo & Dubinsky, 2014). It is considered one of the most biodiverse regions in the world, supporting more than 17,000 marine species. Of these, around 20–30% are endemic, underlining the Mediterranean's importance as a biodiversity hotspot (Coll et al., 2010). Similarly, the Mediterranean's favourable natural conditions prompted the settlement and development of human populations, being the cradle of several ancient civilizations (Abulafia, 2011). Today, Mediterranean countries are home to approximately 570 million people (Doignon et al., 2023) and stand out as the top global tourist destination, receiving more than 400 million international arrivals in 2019 (Plan Bleu, 2022). Among those living within 200 km of the coastline, nearly half reside within just 50 km, and about 30% live less than 10 km from the sea, highlighting the strong coastalisation of the Mediterranean population (Doignon et al., 2023).

The region faces several anthropogenic threats, including habitat loss and fragmentation, the introduction of alien and invasive species, and various forms of pollution (Hassoun et al., 2025). For instance, plastic accumulation is particularly significant. The Mediterranean Sea is considered a “plastic trap”, with an estimated 229,000 tonnes entering annually, resulting in plastic concentrations comparable to those found in major oceanic gyres (Boucher & Billard, 2020; Cózar et al., 2015; WWF, 2018). Similarly, the input of heavy metals through atmospheric deposition is extremely high compared to the open ocean which, together with other factors, results in elevated concentrations of certain heavy metals in the water column, sediments, and biota (Middag et al., 2022). In addition, the Mediterranean Sea is recognized as one of the world's most climate-sensitive regions, warming at least 20% faster than the global average, and facing increasingly severe droughts and ocean acidification (Ali et al., 2022; Palmiéri et al., 2015).

These projected severe environmental conditions, combined with overfishing and other anthropogenic pressures, pose serious threats to Mediterranean ecosystems and their wildlife, ultimately impacting communities relying on marine resources and compromising food security (Chatzimentor et al., 2023; Cramer et al., 2018; Hassoun et al., 2025).

The exploitation of marine resources, especially fishing resources, has long been a key economic and cultural activity in the region. In the mid-1980, total annual landings in the Mediterranean and Black Sea peaked in approximately two million tonnes. However, landings have gradually declined over the decades, with current annual production estimated at around one million tonnes, generating revenues of about USD three billion (*ca.* 2,5 million €) **(Fig. 5)** (FAO, 2023).

Unlike other major fishing areas, characterised by large mono-specific stocks, Mediterranean fisheries target a wide range of benthic and pelagic species, including various fish, molluscs, and crustaceans. The sector remains largely dominated by small-scale vessels, which account for 82% of the fishing fleet and provide over 60% of fisheries employment in the region (FAO, 2023; UNEP/MAP & Plan Bleu, 2020). Despite the predominance of small-scale fisheries, fishing pressure remains high, with 58% of assessed commercial stocks in the Mediterranean being exploited beyond biologically sustainable limits (FAO, 2023).


SNAPSHOT OF FISHERIES
IN THE MEDITERRANEAN AND THE BLACK SEA

Despite a reduction in landings and jobs, fisheries make a significant contribution to food production, livelihoods and the economy.

84 200 vessels

87% operate in the Mediterranean Sea and **13%** operate in the Black Sea

82% of the fleet is composed of small-scale vessels

500 000 jobs along the value chain

183 000 jobs are on board vessels

Job numbers decreased by **6%** in 2020–2021

USD 3 billion annual revenue

USD **7.8 billion** is the estimated total contribution to the regional economy

Revenue increased by **1%** in 2020–2021

Average total annual catch (2020–2021)

1 063 200 tonnes

Landings decreased by **14.5%** from 2018–2019 to 2020–2021 (likely due in part to the impacts of COVID-19 on fleet dynamics, demand and trade)

Figure 5. Overview of the Fisheries sector in the Mediterranean and Black Sea in 2023. Source: FAO, 2023.

3.1. The Balearic Sea: the study area

The Balearic Sea is located in the north-western Mediterranean, delimited by the Balearic Islands and the Spanish mainland coast, from Cape Sant Sebastià in the North to Cape Sant Antoni in the South, thus including most of the Catalan coast and part of the Valencian coast (IHO, 1953) **(Fig. 6)**. The Balearic Sea attains a maximum depth of about 2,600 m (Amblas et al., 2006).

The Western Mediterranean basin generally exhibits lower temperatures and salinity, and higher nutrient concentrations compared to the ultra-oligotrophic Eastern Basin, primarily due to the influence of Atlantic inflow and more active vertical mixing processes (Goffredo & Dubinsky, 2014; Schroeder & Chiggiato, 2022). Biodiversity also shows distinct spatial patterns, with a general decline in species richness from the northwest to the southeast, a trend that extends as well to elasmobranch species (Coll et al., 2010). In this sense, the Balearic Sea is within the most productive areas of the Mediterranean Sea (Bosc et al., 2004) and displays high levels of biodiversity. This little basin is itself heterogeneous: a complex system of submarine canyons crosses the mainland slope **(Fig. 6)**, enhancing the transport of organic matter to the deep sea, while these orographic elements are missing in the insular margin.

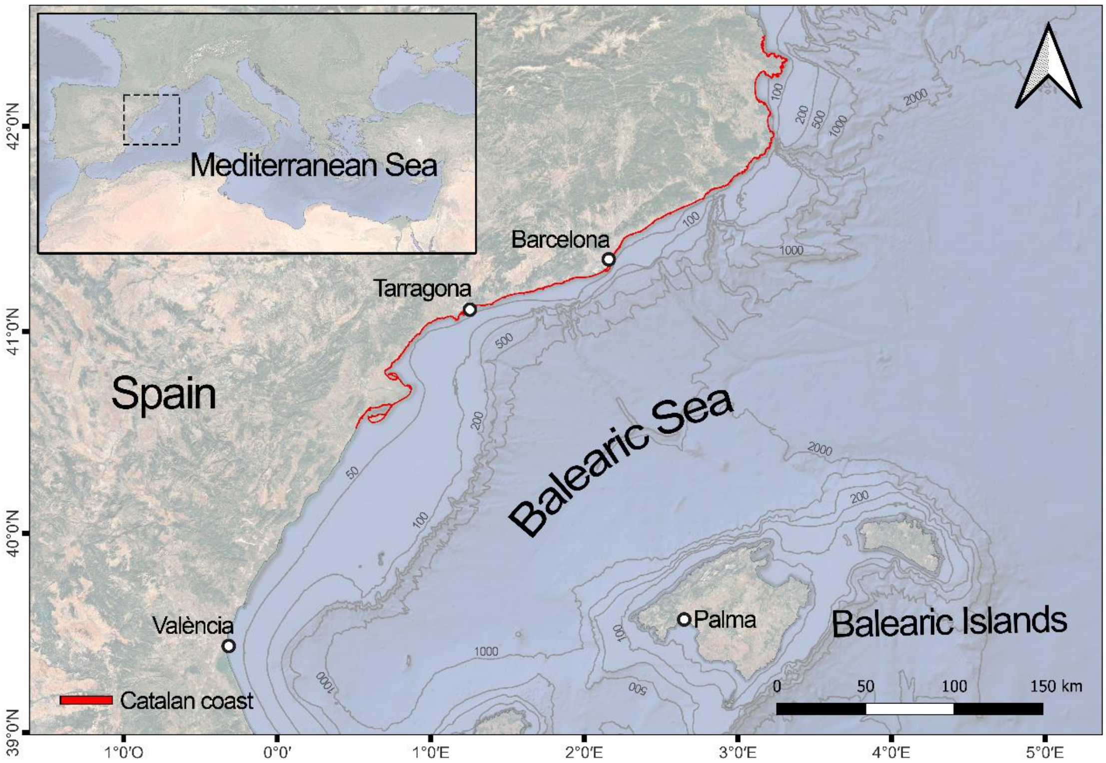


Figure 6. Map of the Balearic Sea. Background imagery from Google satellite. Depth contours retrieved from EMODnet Bathymetry Consortium (2022). Map created using QGIS 3.34.

As a result, the mainland slope supports greater biomass, faunal abundance and biodiversity and more complex food webs compared to the insular slope (Cartes et al., 2009; Durán et al., 2014; Fanelli et al., 2013).

Despite its ecological importance, the Catalan coast is subject to significant anthropogenic pressures due to intensive marine and coastal activities (Generalitat de Catalunya, 2021). Approximately 43% of Catalonia's population resides in coastal municipalities, resulting in a highly urbanized coastline (Generalitat de Catalunya, 2021). In addition, Catalonia is a major tourist destination, receiving up to 32 million visitors in 2024, 58% of whom stayed in coastal areas (Direcció General de Turisme, 2024). Maritime transport, with over 400 million tonnes of goods moved in 2023, along with intensive recreational boating, further increases the anthropogenic pressure of the Catalan littoral (Generalitat de Catalunya, 2021; Idescat, 2023).

The fishing fleet along the Catalan coast currently consists of 528 vessels, primarily deploying small-scale artisanal gear and bottom longlines (n = 280), as well as bottom trawlers (n = 192) (ICATMAR, 2025). In 2024, total landings in this region reached 18,887.84 tonnes, accounting for less than 2% of the total reported capture in the Mediterranean and Black Seas. These landings generated approximately 87 million euros in revenue, of which 60% came from bottom trawling. Despite fishing has traditionally been an important economic activity in Catalonia, recent analyses indicate a declining trend in both landings and revenue, likely associated with the reduction of the fishing fleet since the beginning of the century (ICATMAR, 2025).

In the region, approximately 27% of the trawling catch is discarded. Among the discarded fraction, two catshark species are significantly represented: the small-spotted catshark and the blackmouth catshark (ICATMAR, 2024).

3.2 Representative catsharks from the Mediterranean Sea: *Scyliorhinus canicula* and *Galeus melastomus*

The small-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758) (Carcharhiniformes: Scyliorhinidae) **(Fig. 7)**, is a small (usually less than 60 cm in total length) benthic elasmobranch widely distributed throughout the northeastern Atlantic Ocean and the Mediterranean Sea (Ebert et al., 2021). It is the most abundant catshark species on European continental shelves, typically inhabiting sandy and muddy bottoms, from nearshore areas down to 800 m depth but reaching its maximum abundance at around 100 m depth (Ebert et al., 2021; Ellis & Shackley, 1997). It is considered an opportunistic and generalist species, primarily feeding on benthic invertebrates such as crustaceans, polychaetes, gastropods, cephalopods, and small teleosts, displaying trophic variability according to body size and bathymetric distribution (Valls et al., 2011). *Scyliorhinus canicula* is oviparous, producing egg cases in pairs (single oviparity) with long tendrils used for attachment. It shows a continuous reproductive cycle and displays early maturity, fast growth and short generation times compared to other elasmobranchs (Ebert et al., 2021; Ramírez-Amaro et al., 2020). An increasing population trend has been reported for this species, which is currently assessed as Least Concern by the IUCN Red List of Threatened Species (Finucci et al., 2021a). Fishing pressure is the only documented threat to this species, theoretically counteracted by the high survival rate of released individuals (Rodríguez-Cabello et al., 2005).

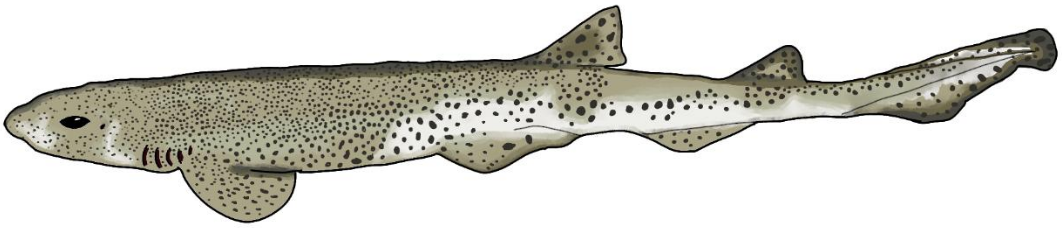


Figure 7. Illustration of *Scyliorhinus canicula* (Linnaeus, 1758). Author: Andrea Higueruelo

The blackmouth catshark, *Galeus melastomus* Rafinesque, 1810 (Carcharhiniformes: Pentanchidae) **(Fig. 8)**, is a demersal elasmobranch, slightly bigger than *S. canicula* (usually less than 70 cm in total length) with a similar geographical distribution (Costa et al., 2005; Ebert et al., 2021). While *S. canicula* is the most abundant catshark in the continental shelves, *G. melastomus* is the most representative catshark in the upper and lower slopes, being mainly found between 200 and 500 m depth, occasionally between 55 m and 2000 m (Ebert et al., 2021). It is also considered an opportunistic generalist predator, with a relatively low trophic position (Valls et al., 2011). Its mode of reproduction is oviparity, with females producing up to 13 egg cases per litter (multiple oviparity), which lack tendrils (Costa et al., 2005). It is also assessed as Least Concern by the IUCN, with stable or increasing trends across its area of distribution (Finucci et al., 2021b).

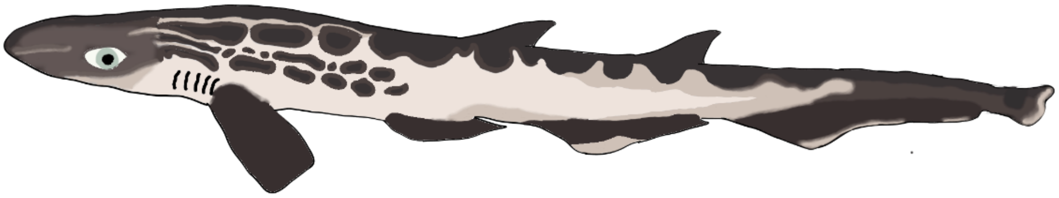


Figure 8. Illustration of *Galeus melastomus* Rafinesque, 1810. Author : Andrea Higueruelo

These two species can occasionally be landed after skinned, though they have low commercial value and are usually discarded (Carbonell et al., 2003). In the Catalan coast, *S. canicula* is in fact the most discarded species by bottom trawling in terms of biomass, representing 22% of total discards and up to 65% of upper slope discards. In turn, *G. melastomus* is the most discarded species in the lower slope, representing 33% of total discards at this depth (ICATMAR, 2024).

4. Icelandic waters: a comparatively unexplored area

Iceland is situated between Greenland in the West and the Faroe Islands in the East, surrounded by the North Atlantic Ocean and the Arctic Ocean **(Fig 9)**. With an extension of 103,000 km^2, it is one of the youngest islands in the world, characterised by a subpolar oceanic climate and high volcanic activity (Fraedrich & Heidari, 2019).

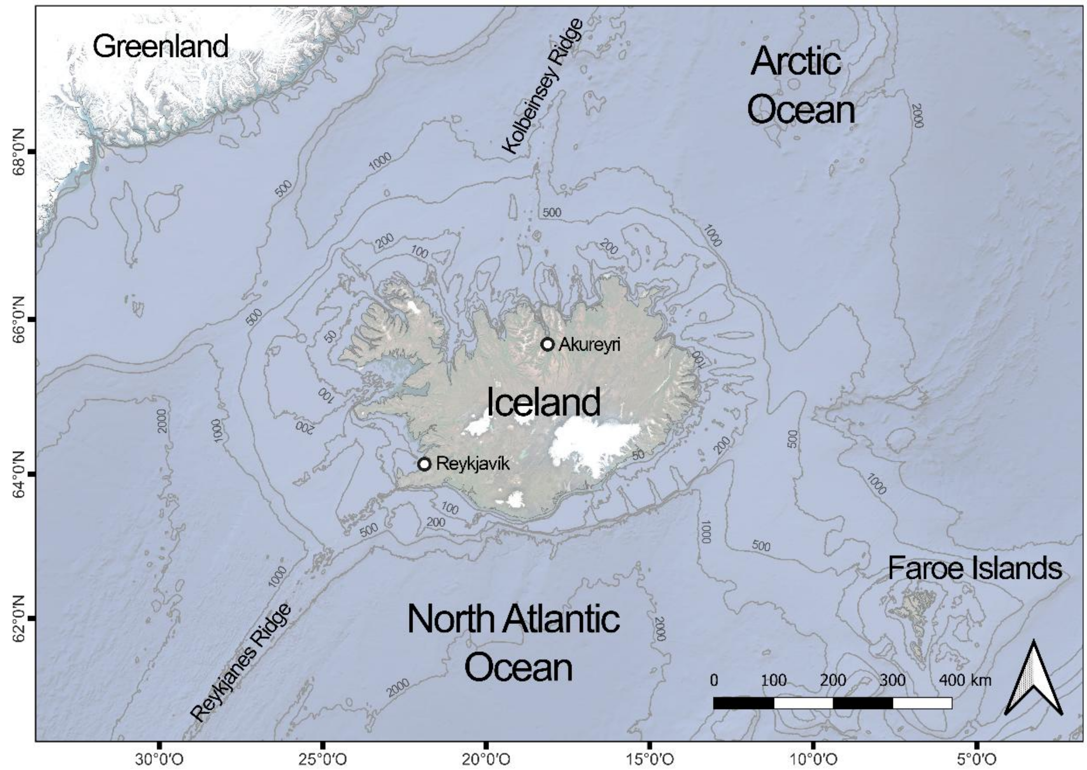


Figure 9. Map of Iceland and surrounding waters. Background imagery from Google satellite Depth contours retrieved from EMODnet Bathymetry Consortium (2022). Map created using QGIS 3.34.

The submarine ridges of the region, such as the Reykjanes Ridge and Kolbeinsey Ridge, influence the oceanic circulation and the distribution of water masses in the area (Jónsson & Valdimarsson, 2005). As a result, Iceland lies at the convergence of contrasting oceanic currents, where relatively warm, saline Atlantic waters meet very cold, less saline polar waters (Astthorsson et al., 2007; Logemann et al., 2013). The distinct oceanographic features around Iceland make it a nutrient-rich and fertile region, supporting highly productive marine ecosystems, particularly in the southwest (MFRI, 2021).

The modern marine fauna of Iceland is composed of boreal, subarctic-boreal species and, in less proportion, arctic species (Símonarson et al., 2021). Species richness in European Atlantic waters exceeds that of the Mediterranean (Costello et al., 2010). Nevertheless, in contrast to the Mediterranean Sea, where extensive research has been conducted for decades (particularly in the northern area) and where some of the earliest deep-sea investigations took place, marine biodiversity studies around Iceland have been comparatively limited (Costello et al., 2010; Símonarson et al., 2021). Prior to 1992, knowledge of Icelandic benthic fauna was largely restricted to depths shallower than 400 m. It was not until the BIOICE project (1992–2004) that comprehensive sampling within the Icelandic Economic Zone (IEZ), extending to depths of 3000 m, revealed several hundred species new to the Icelandic fauna, including at least 29 species new to science (Símonarson et al., 2021). Research on Iceland's fish fauna began in the early twentieth century. As of 2013, 360 species had been recorded, including 39 cartilaginous and 311 bony fishes (Hlíðberg et al., 2013). Parasitological research in Iceland began in the 20th century, with records of Trematoda, Cestoda, Nematoda, and Copepoda in marine fishes and other hosts (Símonarson et al., 2021). Yet, comprehensive studies of parasite communities are scarce, and is largely absent for chondrichthyans. A significant number of species likely remain undescribed, particularly within the Platyhelminthes, which are globally recognised as one of the taxa with the poorest state of knowledge (Costello et al., 2010).

Since the first settlement in the late 9th century, fishing has been a vital activity in Iceland and continues to be one of its most important economic sectors today. In 2023, its fleet was formed by 1,535 fishing vessels including 39 trawlers, with an annual catch totalling 1,374,823 tonnes, exceeding therefore the total landings of the Mediterranean and Black Seas on the same year (Statistics Iceland, 2025a). Moving out from fishing, industrial activity is very limited and the country is sparsely populated, with only 389,444 inhabitants and a population density of 3.78 inhabitants/km² (Statistics Iceland, 2025b). Consequently, anthropogenic pressure on marine ecosystems is relatively low, especially when contrasted with the Balearic Sea.

4.1. Representative catsharks from Icelandic waters

Around Iceland, about 20 demersal species of cartilaginous fish are found, of which around 17 species (including dogfishes, skates, and rays) typically occur at depths greater than 400 m (Jakobsdóttir et al., 2023). Among them, three pentanchids are freqüent, namely the white ghost catshark (*Apristurus aphyodes* Nakaya & Stehmann, 1998), the Iceland catshark [*Apristurus laurussonii* (Saemundsson, 1922)] and the mouse catshark [*Galeus murinus* (Collett, 1904)] **(Fig. 10)**.

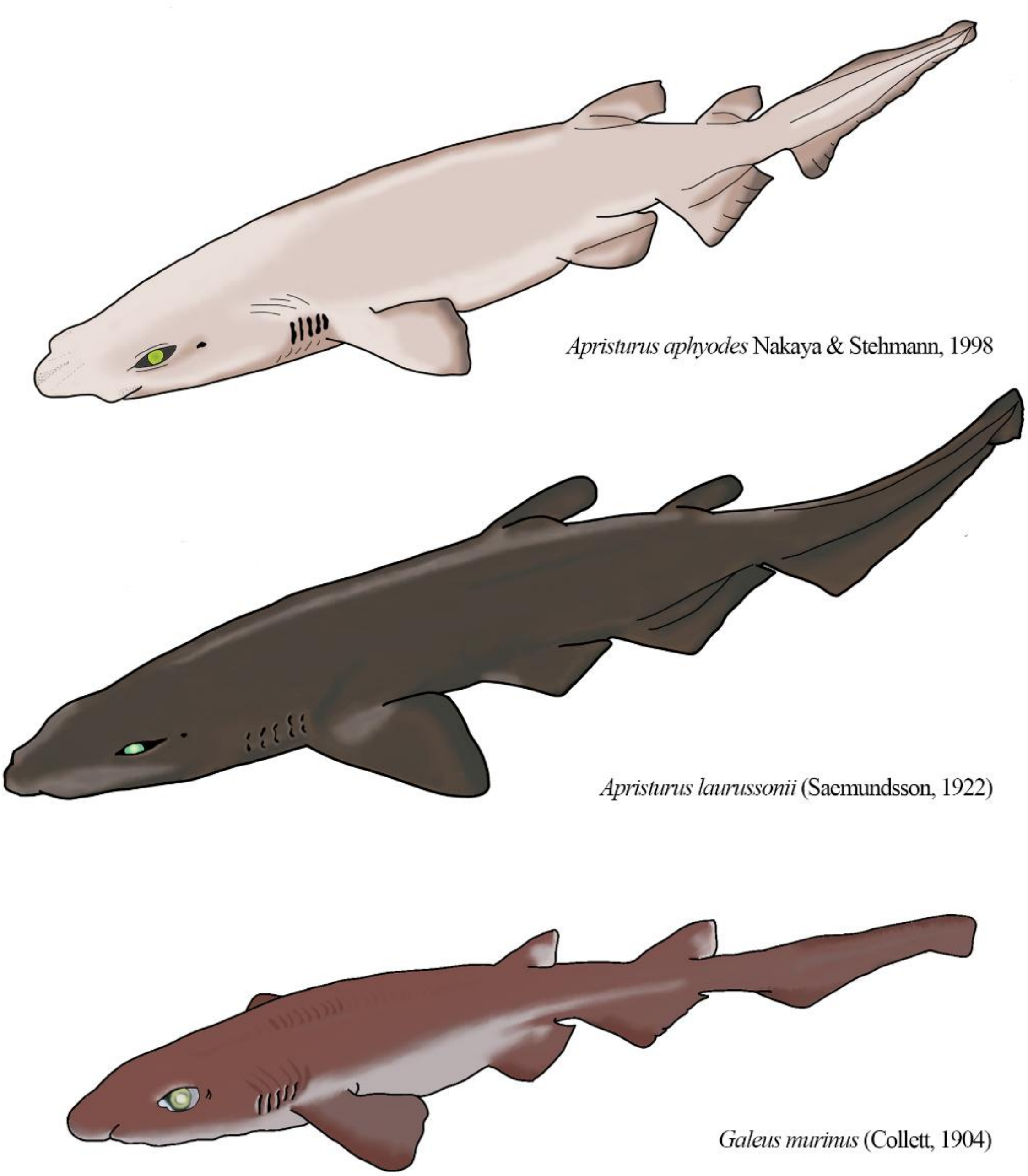


Figure 10. Illustration of main deepwater catsharks (Carcarhiniformes: Pentanchidae) found in Icelandic waters. Author: Andrea Higueruelo

These bottom-dwelling catsharks are relatively small (*i.e.*, less than 80 cm in total length) and are found across a wide depth range (300–2,060 m) along the continental slopes of the Atlantic Ocean. While *A. laurussonii* exhibits the broadest distribution, occurring in both the Central and North Atlantic, *A. aphyodes* and *G. murinus* are restricted to the North-East Atlantic Ocean (Ebert et al., 2021; Sólmundsson et al., 2025). The three species have single oviparity as a mode of reproduction. Their egg cases range from approximately 4 to 7 cm in length and 1.5 to 3 in width, with only *A. laurussonii* having very long and tightly coiled tendrils (Iglésias et al., 2002). In Iceland waters, they feed primarily on mesopelagic fish (myctophids and pearlside) and crustaceans (Sólmundsson et al., 2025). All three species are currently assessed as Least Concern by the IUCN. Although they inhabit continental slopes and may be caught as bycatch in deepwater trawling operations, none show evidence of significant population declines (Finucci & Armstrong, 2024, Kulka et al., 2020, Rigby et al., 2024). Nonetheless, their biology is still poorly understood, with few studies available on key aspects such as diet, behaviour, reproduction, or parasite infections.

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OBJECTIVES

Considering the importance of both marine ecosystems' sustainability and human safe consumption of seafood, the **main objective** of this thesis was to expand current knowledge on the biology, ecology, health status, and parasite communities of catsharks, while also assessing potential risks to consumers of species of this group. In addition, the study aimed to evaluate the suitability of catsharks as biomonitors of marine pollution and to explore the use of their parasite assemblages as ecological indicators. Given its frequent occurrence in fisheries of the Mediterranean Sea and Atlantic Ocean and specific ecological traits, particular emphasis was placed on the small-spotted catshark (*Scyliorhinus canicula*) from off the Catalan coast (north-western Mediterranean) as a model species.

In order to fulfil these general goals, the following specific objectives were stablished:

Objective I (Chapter 1)

To enhance understanding of the sexual development and annual reproductive cycle of *Sycliorhynus canicula* in the Catalan coast (NW Mediterranean) to endorse scientific-based management actions for its exploitation if required.

- To study the development and reproductive cycle of *S. canicula* in the NW Mediterranean along the year.
- To investigate temporal and geographic trends of reproductive parameters across the distribution areas of of *S. canicula.*

Objective II (Chapter 2)

To holistically assess the current health status of *S. canicula* and potential risks to human consumption in the NW Mediterranean by evaluating ingestion of anthropogenic items, trace metal concentrations, condition indices, histopathology and parasite infections. Samples of *S. canicula* were obtained from three areas off the Catalan coast for comparison purposes.

- To quantify and characterise anthropogenic items ingestion, including microplastics and cellulose.
- To examine the diversity and composition of parasite assemblages and determine different parasitological indices.

- To analyse the concentrations of trace metals in the liver and muscle.
- To evaluate its health status considering the abovementioned variables, including condition indices and liver histopathology in relation to environmental and ecological factors of the different areas studied.
- To discuss the potential implications for human consumption of the results obtained.

Objective III (Chapter 3)

To investigate the influence of biological factors (e.g., fish size and sex) and habitat characteristics to the accumulation of mercury (Hg), lead (Pb), and cadmium (Cd) in the muscle tissue of marine species with different ecological roles, and to assess the potential of catsharks as bioindicator species.

- To quantify muscle concentrations of Hg, Pb and Cd in ten ecologically distinct commercial marine species from off the Catalan coast, with special emphasis on the catsharks *S. canicula* and *Galeus melastomus.*
- To examine the potential influence of biological factors (i.e. fish size, sex) and habitat (including depth) on their accumulation patterns in muscle.
- To assess geographic variation in Hg, Pb, and Cd concentrations in *S. canicula* across three Catalan and three Spanish Atlantic regions, further conduct a literature review of Hg concentrations across its distribution range, and ultimately evaluate the suitability of *S. canicula* as a biomonitor for trace metals.
- To evaluate the potential risks of the ten selected species to human consumption and provide consumption guidelines.

Objective IV (Chapters 4 and 4.1)

To characterise the parasite communities of Icelandic deep-water catsharks considering biological and ecological factors and to compare their infection patterns with those of Mediterranean catsharks.

- To fully characterise the parasite community of the most frequent deep-sea catsharks of Icelandic waters, named *Apristurus aphyodes*, *Apristurus laurussonii* and *Galeus murinus.*
- To evaluate differences on the parasite assemblages of these three species considering different environmental and biological factors

- To compare the parasitological indices and infection patterns of Icelandic deep-water catsharks with those from Mediterranean counterparts.
- To discuss the potential use of parasites of demersal sharks as indicators of host ecology.
- To highlight novel parasite diversity found in deep-sea catsharks, and to potentially describe new species of parasites that may arise during the characterisation of parasite assemblages of Icelandic deep-water catsharks.

CHAPTER 1

Novel insights into the reproductive strategy of the small-spotted catshark, *Scyliorhinus canicula* (L.) in the North West Mediterranean: a year-long in-depth study

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Abstract

The small-spotted catshark (*Scyliorhinus canicula*) is one of the most captured elasmobranchs off the Mediterranean Sea. Characterisation of its sexual maturation, reproductive features, and the role of the liver in reproduction is detailed based on individuals captured off the Catalan coast (Balearic Sea, Northwest Mediterranean) between February 2022 and February 2023. Five and three reproductive stages for females and males, respectively, are described macroscopically and supported by microscopic description and changes in body size and somatic indices. The significant reduction of the hepatosomatic index when maturation is reached confirms the important role of the liver as an energy provider during reproduction, linked to lipid expenditure for vitellogenesis, spermatogenesis or other energy-consuming activities related to reproduction. Liver colour variability was associated with reproduction rather than nutritional status, as suggested by differences observed across reproductive stages and sex, along with the relatively stable values of the Le Cren condition index. The presence of mature-laying females and mature males and the similar mean values of gonadosomatic index throughout the year confirmed a continuous reproductive period. A decreasing trend in the length at 50% maturity (L50) compared to previous estimates is observed in the Balearic Sea, but could also be occurring in other areas. This is further discussed in the present study, as several hypotheses arise (*e.g.*, a species' response to fishing pressure, ocean warming).

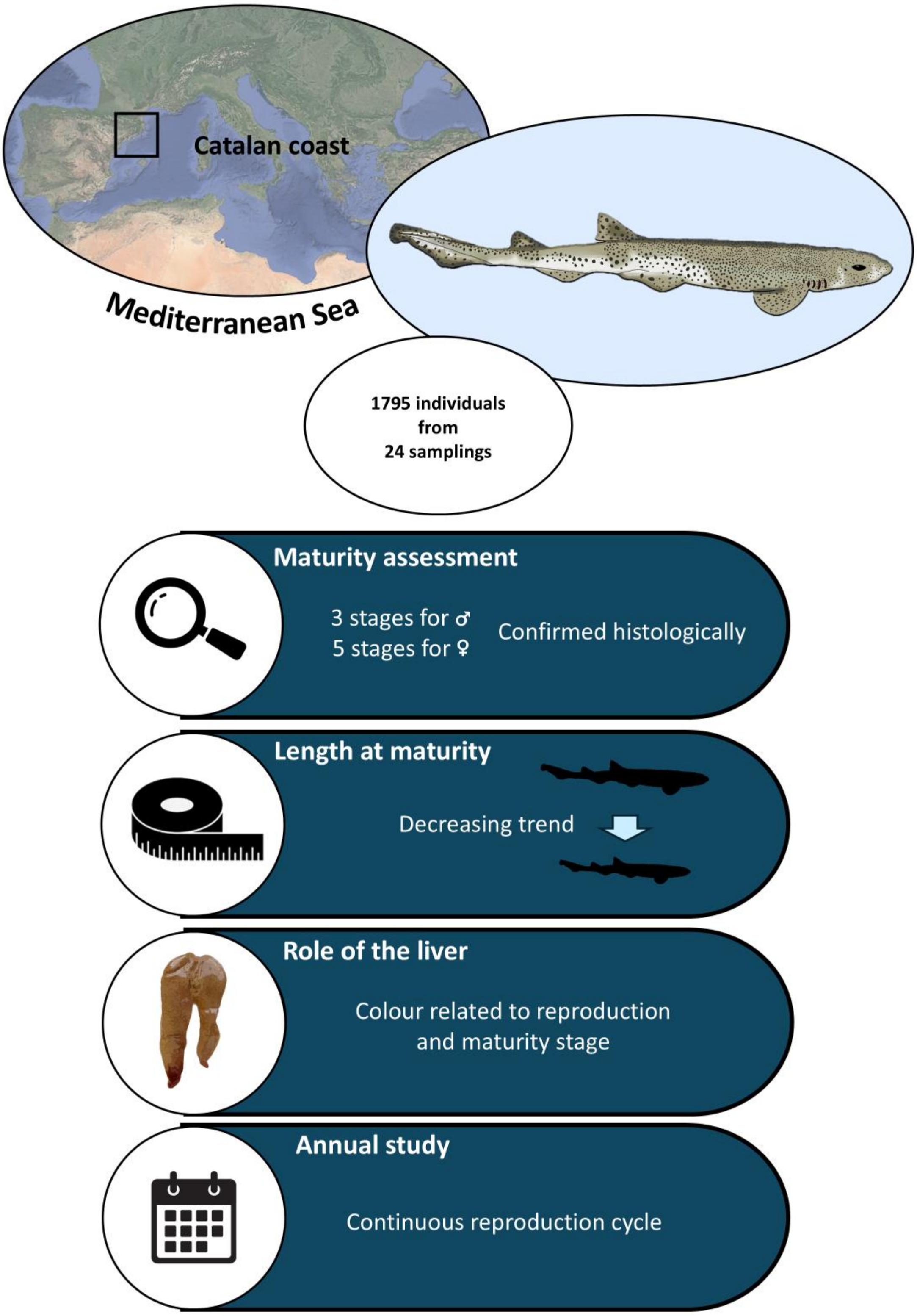
Keywords

Elasmobranchs, Gonads, Histology, Liver, Reproduction, Sexual maturity

Highlights

- *S. canicula* exhibits a continuous reproductive cycle
- Sperm was present in the oviducal glands of 70% of the mature females analysed
- The liver plays a key role in reproduction as energy provider
- The length at 50% maturity (L50) from *S. canicula* in the Balearic sea was 37.9 and 37.2 cm TL for females and males, respectively
- Available data suggests a decreasing trend in L50 across the species' distribution range

Graphical abstract



1795 individuals
from
24 samplings

Maturity assessment

3 stages for ♂
5 stages for ♀

Confirmed histologically

Length at maturity

Decreasing trend

Role of the liver

Colour related to reproduction
and maturity stage

Annual study

Continuous reproduction cycle

1. Introduction

Chondrichthyans (*i.e.,* sharks, rays, and chimaeras) represent one of the most ancient extant vertebrate lineages, having persisted for approximately 440 million years, in part due to their specialised life strategy perfected during hundreds of millions of years (Ebert et al., 2021). However, this strategy, characterised by internal fertilisation, slow growth, and low reproductive rates (Carrier et al., 2004), now exposes them as particularly vulnerable in the present context of increasing fishing pressure, habitat degradation, and climate change (Dulvy et al., 2021). According to a recent report, over one-third of chondrichthyans are threatened by overfishing, urging management measures to prevent further extinctions and preserve the ecosystem functions they provide as top predators (Dulvy et al., 2021). In the Mediterranean Sea, sustained fishing pressure from the mid-1950s to the early 1980s (Punzón et al., 2020) resulted in a marked decline in chondrichthyan landings from 1970 onwards, suggesting an overexploited status (Ramírez-Amaro et al., 2020). In fact, the Mediterranean Sea has been identified as a hotspot of extinction risk for chondrichthyans by the International Union for Conservation of Nature (IUCN), mainly due to overfishing and bycatch, with more than half of the assessed species considered threatened (Dulvy et al*.,* 2016).

The small-spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758) (Carcharhiniformes: Scyliorhinidae), represents an exception to the general trend observed in chondrichthyans in this area, being the most abundant species of catshark in European inshore waters (Ellis and Shackley, 1997) and currently assessed as Least Concern by the IUCN Red List of Threatened Species (Finucci et al., 2021). This small, bottom-dwelling elasmobranch is distributed from the sub-littoral and continental shelf to the upper slope, and in some areas is targeted for consumption (Capapé et al., 2008a), while in other parts, including the North West (NW) Mediterranean Sea, it is one of the most common bycatch species, thus being mainly discarded (ICATMAR, 2023). *Scyliorhinus canicula* has shown increasing population trends in the North East Atlantic Ocean and Mediterranean Sea over the past 30 years (Finucci et al., 2021). The only threat acknowledged for this species is fishing pressure, theoretically counteracted by the high survival rate of released individuals (Rodríguez-Cabello et al. 2005) compared to other by-catch species. In addition, their life history traits (*e.g.*, early maturity, short generation time, fast growth, continuous reproductive cycles) make them more resilient than other elasmobranchs (Ramírez-Amaro et al., 2020). However, some studies have reported a decline in *S. canicula* landings, suggesting that local population decreases may be occurring in heavily exploited areas, such as the northern Adriatic Sea (Barausse et al., 2014) and off the Tunisian coast (Capapé et al., 2014). A more detailed assessment on some particularities of its life cycle might be crucial to understand the reproductive strategies of this shark.

Previous studies conducted across the Mediterranean Sea and the Atlantic Ocean have explored various aspects of this species' reproductive strategy, including size and age at sexual maturity, annual egg capsule production, monthly variations of somatic indices, and sexual dimorphism (Kousteni and Megalofonou, 2019, and references therein). Nonetheless, in relation to the assessment of sexual maturity, most of these studies were exclusively grounded on macroscopic traits, and only two of them used histological observations (Lupi, 2008; Kousteni and Megalofonou, 2019). Histology is a valuable tool for reproductive studies, and a lack of knowledge may lead to uncertain maturity assessments and overlook key processes relevant to reproductive biology, such as gonadal alterations, diseases, sperm storage in females, or oocyte formation (Blazer, 2002). Furthermore, the histological analysis of the liver may be a useful complement to the study of elasmobranchs' reproductive biology. Some authors have linked the depletion or absence of lipid stores in elasmobranchs' liver to poor nutritional condition and emaciation (Garner, 2013; Stedman and Garner, 2018). However, liver lipidic content is related to the mobilisation of lipid reserves for reproduction in teleosts (Lal and Singh, 1987), and in elasmobranchs the pattern may be similar, as observed in *S. canicula* (particularly in females, *e.g.*, for the synthesis of egg yolk) (Valls et al., 2016; Higueruelo et al., 2024). Therefore, it is important to further explore the role of the liver in the reproduction of elasmobranchs.

Studies on the reproductive biology of target species are vital for effective fisheries management and conservation measures. For example, an ecologically meaningful minimum landing size (MLS) should be at least equal to the length at 50% maturity (L50, the mean length at which 50% of the individuals of the studied population reach sexual maturity), to ensure that small, immature individuals are not caught (Stergiou et al., 2009). In addition, different selective pressures, like fishing and biotic and abiotic factors (*e.g.*, temperature, food availability) are known to affect a species' morphological traits, growth, size structure and maturity across time and space (Stevens et al., 2000; Heino and Godø, 2002). For instance, Finotto et al. (2015) found differences in the L50 among *S. canicula* populations in the northern Adriatic Sea and the Strait of Sicily, while Ramírez-Amaro et al. (2020) recently detected a decreasing trend in the L50 over time in the Western Mediterranean, which could be related to an evolutionary response to overfishing. Therefore, assessing growth and maturity patterns over time and across different geographic regions is necessary towards the determination of an accurate and region-specific MLS, paramount for the management and maintenance of the populations under study.

The present study aims to understand and increase knowledge about the development and reproductive cycle of *S. canicula* to endorse scientific-based management actions (*e.g.*, MLS or temporal ban) if required for its exploitation. To achieve this goal the following specific objectives have been determined: i) to redefine and redescribe the reproductive stages based on macroscopic and microscopic observations of reproductive organs, and analyse its relation with length and somatic indices, to provide a readily understandable maturity scale, ii) to infer the length at maturity (L50) in the Balearic Sea (NW Mediterranean) for further comparisons along time and across areas of distribution, iii) to determine the potential relationship of liver colour with reproductive stages, and iv) to explore the temporal trends of somatic indices throughout a year in relation to the reproductive cycle of the species.

2.Materials and methods

2.1. Study area and sample collection

Specimens of *S. canicula* were obtained from discards brought by commercial fishing trawlers off the Catalan coast (Balearic Sea, NW Mediterranean Sea) on sandy-muddy and detritic bottoms of the continental shelf (50–200 m depth) and the upper slope (200–500 m depth). Between February 2022 and February 2023, samplings were conducted twice per month, resulting in 24 sampling events **(Figure S1)**. Specimens were kept in ice or cold water (onboard) and once in the laboratory were dissected immediately or stored in the freezer (-20ºC) awaiting dissection. Samples intended for histological analysis followed a distinct protocol (see below).

2.2. Processing and dissection of specimens

Initially, sex was determined externally based on the presence or absence of claspers. Claspers were measured from their attachment to the anal fin to the tip and the calcification level was also categorised as flexible (not calcified, soft), mid-calcified (base of the clasper starting calcification but tip still flexible), and calcified (entire clasper rigid and stiff).

For each individual, total length (TL; cm) was measured to the nearest millimetre, from the distal part of the snout to the end of the caudal fin, and total weight (TW; g) was measured to the nearest 0.01 g. After the removal of all abdominal organs, eviscerated weight (EW; g) was recorded. Liver was also weighed (LW; g). The reproductive system was processed as follows: for females, the ovary weight (GW; g) and the number of capsules were recorded. In some cases, especially in samples from July, the ovaries were damaged and could not be weighed;

consequently, these individuals were excluded from specific statistical analyses. For males, testes were weighted (GW; g).

2.3. Macroscopic determination of gonadal maturity stages and liver condition

The maturity stages of female and male *S. canicula* were determined based on the reproductive system's macroscopic features. For females, maturity stages were assessed by the appearance of the ovary, oviducal gland (based on colour and shape) and uterus, and the presence of egg cases. For males, maturity stages were inferred by the size and level of clasper calcification, as well as weight and overall appearance of testes and sperm ducts (epididymis, vas deferens and seminal vesicle). Liver condition of 734 specimens was assessed from thawed specimens based on external colouring (from yellowish to blackish) and presence or absence of groove marks. Previously, a verification was conducted comparing liver coloration in the same fresh samples (before freezing) and thawed samples, and it was found that the preservation procedures did not affect the colour of the liver.

2.4. Histological procedures

During the first four sampling, in February and March 2022, fish were collected still alive from fishing trawlers, stored in boxes full of seawater and brought to the laboratory. Once there, a subsample of 101 specimens of different sizes and from both sexes (F: n = 51; M: n = 50) were selected for histological assessment. Just after being anaesthetised and sacrificed with an overdose of 2-phenoxyethanol and spinal severance, a portion of each ovary and oviducal gland or testes and sperm ducts were fixed in 10% buffered formalin and processed by routine histology techniques (Roberts et al., 2012). Briefly, they were embedded in paraffin, sectioned at 4 µm and stained with Haematoxylin and Eosin (H&E) for histological examination under light microscopy.

Additionally, in the last sampling, February 2023, 18 livers with different colourations and from both sexes were selected (F: n = 9; M: n = 9) from fresh samples. The distal part of a liver lobe was immediately fixed in 10% buffered formalin and processed according to the same protocol (H&E) to further explore the link of colour with lipid content. In six cases, an additional stain with Periodic acid–Schiff (PAS) was performed to discard the presence of glycogen.

2.5. Data analysis

Based on the sampled data, several somatic indices were calculated: Le Cren's relative body condition index ($Kn = EW/(\alpha \times TL^{\beta})$) (Le Cren, 1951), where Kn is the relative body condition, α and β are the slope and the intercept of the weight-length relationship representing the entire dataset of sampled fish; gonadosomatic index (GSI = (GW/EW) x 100), and hepatosomatic index (HSI = (LW/EW) x 100) (Capapé et al., 2008a).

All variables were tested for normality and homoscedasticity using Shapiro-Wilk and Levene tests, respectively. Data distribution was plotted for visual assessment. When necessary, variables were log or square root transformed to comply with normality. Non-parametric tests were used when normality and/or homoscedasticity were not achieved.

Wilcoxon test (W) (Zar, 1999) was used to detect differences between sexes in adults for fish size and somatic indices. Hereafter, analyses were conducted grouped by sex. Correlations between somatic indices and body size were tested using non-parametric Spearman's correlation test (SC) (Zar, 1999). To test differences in somatic indices and body size among stages of maturity and liver colour categories (LC), Generalised Linear Models (GLM) with gamma distribution were used. To assess monthly variation in somatic indices, GLMs with a gamma distribution were also used. Maturity stage was included as a covariate for the Kn, while separate models were fitted for each stage in the case of GSI and HSI. To estimate which pair of categories contributed to the overall significance, estimate marginal means (EMMs) tests were computed, and pairwise comparisons were performed using a Bonferroni adjustment. Chi-square tests (χ^2) (Zar, 1999) with Bonferroni-adjusted post hoc comparisons were used to assess associations between LC and both sexual maturity stages and sex. The same approach was applied to evaluate differences in maturity stages across months.

Length at maturity ogives were assessed for each sex by using a logistic regression of the proportion of mature sharks depending on the TL. The variables were fitted to a logit function with the formula: $P = 1/(1+\exp(-\beta_0 + \beta_1 * X))$, where P is the probability of an individual being mature at X length. β_0 is the intercept, and β_1 is the slope, both estimated parameters. The length at which 50 or 95% of the individuals were sexually mature (L50 and L95, respectively) are inferred based on this formula (Roa et al., 1999). The logit regression was based on GLM. The package "sizeMat" was used for this analysis.

Data analysis was performed using RStudio software (version: 4.3.0). Statistical significance was set at 0.05. To improve readability, all statistical results are shown in **Table S1**.

3. Results

3.1. General biometric description

A total of 1795 specimens were collected, with monthly samples ranging from 104 to 220 (females: 50 - 170; males: 37-98), depending on the natural variability of *S. canicula* catches. Individuals ranged from 13.7 to 52.0 cm TL (mean = 39.8 ± 4.5 cm) **(Table S2)**. Significant differences between sexes in mature individuals were observed, being the total length higher in males and all somatic indices (Kn, GSI, and HIS) higher in females **(Table S1, S2)**. A significant positive correlation between GSI and TL was observed for both sexes, while no correlations with HSI and Kn were found.

3.2. Reproductive system description and maturation

3.2.1. Macroscopic and microscopic reproductive system description

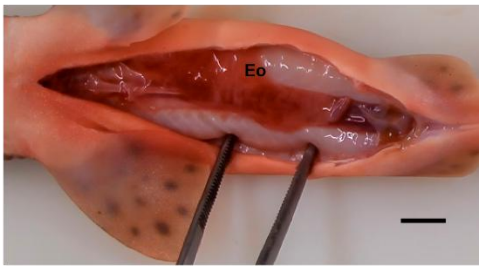
Five and three maturity stages (St), modified from Ivory et al. (2004) and Kousteni and Megalofonou (2019), were established for females and males, respectively, following the terminology in Jones et al. (2005) and Parsons and Grier (1992).

Figure 1 describes and illustrates the macroscopic reproductive stages in *S. canicula* females: one immature stage (Stage 1), two developing stages, early-developing (Stage 2a) and late-developing (Stage 2b), and a mature adult stage (Stage 3), which includes a sub-stage for egg-laying females (Stage 3*). The histology sections are presented in **Figure 2.** During the development of the ovary, four types of follicles are present, from immature to mature, capable of being fertilised **(Figure 2a-e)**. Primordial follicles are predominant in the immature stage (St 1), characterised by a follicular epithelium composed of a single layer of squamous cells **(Figure 2a)**. Primordial follicles transform into primary follicles, which are also present in St 1, and are characterised by a prominent zona pellucida and a cuboidal follicular epithelium below the external thecal layer **(Figure 2b)**. The epigonal organ is predominant in the ovary in this stage of reproduction, and the oviducal gland is undeveloped, without tubules, but with some lamellae surrounding the lumen **(Figure 2f)**. In early-developing females (St 2a), primordial, primary and previtellogenic follicles can be distinguished in the ovary. Previtellogenic follicles, are characterised by a zona pellucida and a columnar follicular epithelium, which is larger than previous stage in contrast to the zona pellucida and also surrounded by the thecal layer. In this stage, yolk starts to develop in small yolk platelets **(Figure 2c)**. In late-developing females (St 2b), the first vitellogenic follicles appear, with yolk platelets in the oocyte cytoplasm, and a

Stage 1. Immature

Ovary hardly discernible from the epigonal organ (Eo), small, whitish or reddish in colour, follicles undistinguishable to the naked eye.

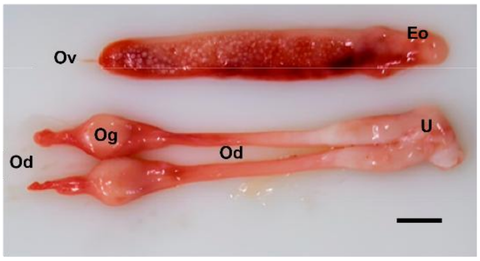
Oviducal gland undeveloped, translucid, boundary with threadlike oviducts not clearly defined; uterus narrow.



Stage 2a. Early developing

Ovary (Ov) larger than in previous stage, containing small, yet visible, semitransparent or whitish follicles.

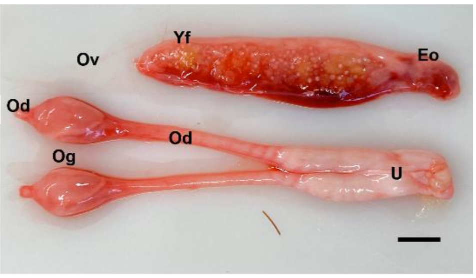
Oviducal gland (Og) whitish, small but well differentiated from the oviducts (Od). Uterus (U) beginning to differentiate from the oviducts, thicker than in previous stage.



Stage 2b. Late developing

Ovary full of small whitish follicles, but small yellow follicles (Yf) (< 5 mm) also observed.

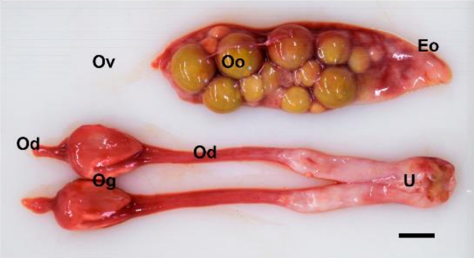
Oviducal gland and uterus generally larger than in previous stage.



Stage 3. Mature (adult)

Ovary developed: semi-transparent and yellow small follicles (< 5 mm), but also completely developed yellow oocytes (> 5 mm) rich in yolk (Oo) present, normally between 8 – 16 in number.

Oviducal gland fully developed, large, white, sometimes reddish in colour, usually hardened. Uterus completely formed but empty.



3* (egg-laying) 1 or 2 egg capsules (Ca) completely formed or under development in the posterior zone of the oviduct.

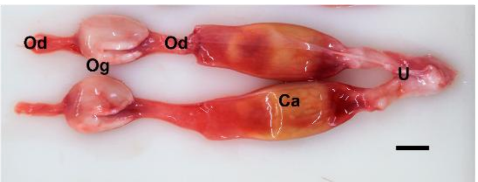
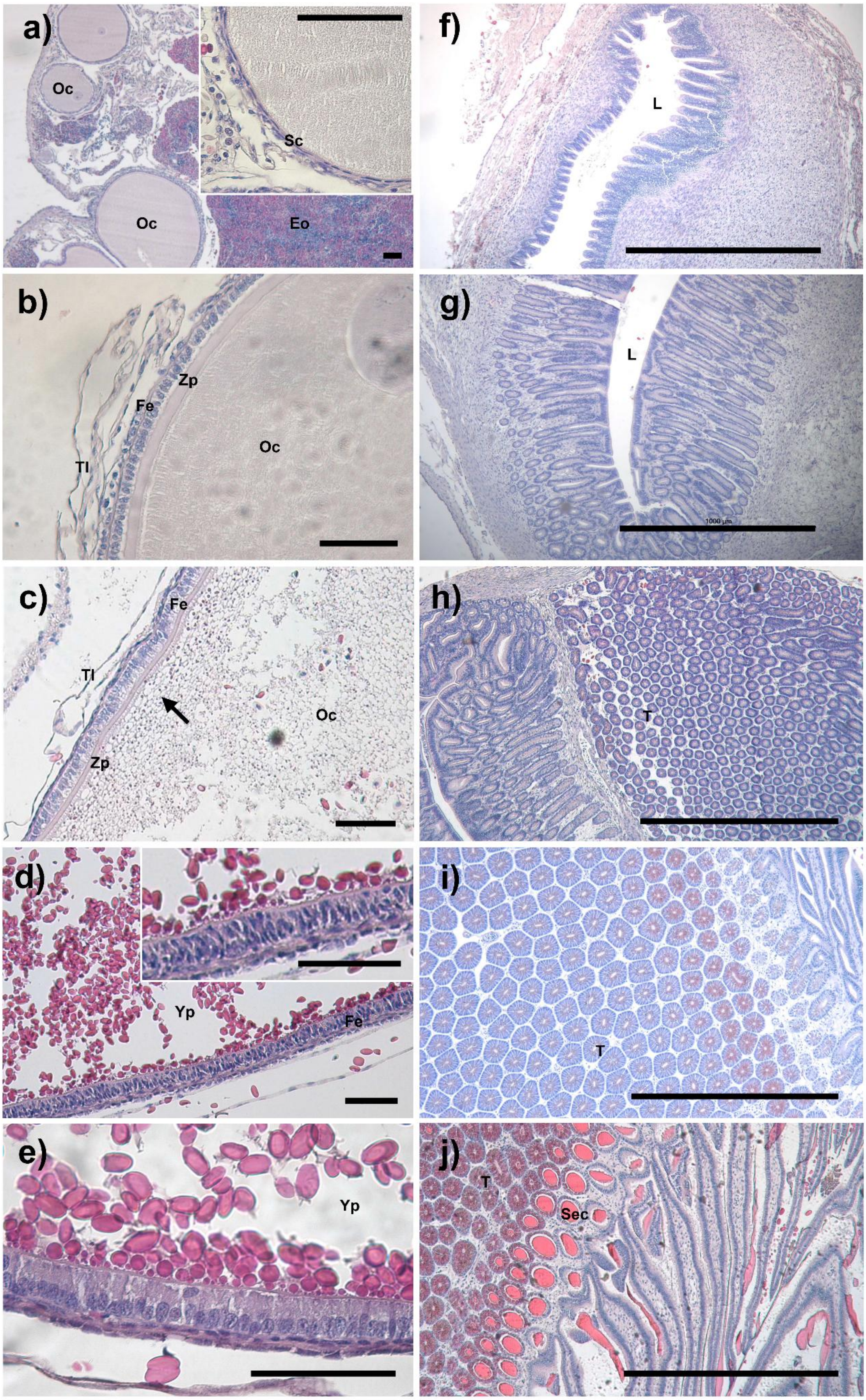


Figure 1. Proposed scale of maturity stages for female *Scyliorhinus canicula*. For each stage, the macroscopic features are described with each corresponding picture. Scale bar: 1 cm.


a)
Oc
Sc
Oc
Eo
b)
Zp
Fe
Oc
Tl
c)
Fe
Tl
Oc
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Yp
Fe
e)
Yp
f)
L
g)
L
1000 µm
h)
T
i)
T
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Sec

Figure 2. Transverse sections of *Scyliorhinus canicula female reproductive organs* (H & E stain). a) Immature ovary with primordial oocytes and the epigonal organ. Detail of a primordial follicle. b) Fragment of a primary follicle where the different layers can be observed. c) Fragment of a previtellogenic follicle where the different layers can be observed. See small yolk platelets at the periphery (arrow). d) Fragment of a vitellogenic follicle with a pseudostratified follicular epithelium. e) Detail of a vitellogenic follicle with a columnar follicular epithelium. f) Undeveloped oviducal gland with no visible gland tubules. g) and h) Different stages of a developing oviducal gland. i) Mature oviducal gland, fully developed. j) Presence of secretions in the tubules and lumen of a mature oviducal gland. Scale bar: a) – e) = 100 μm, f) – j) 1000 μm. Eo: epigonal organ, Fe: follicular epithelium, L: lumen, Oo: oocyte, Sec: secretions, Sc: squamous cells, T: tubules, Tl: thecal layer, Yf: yellow follicle, Yp: yolk platelets, Zp: zona pellucida.

pseudostratified follicular epithelium **(Figure 2d)**. During developmental stages, the oviducal gland has larger lamellae and already contains some differentiated gland tubules filling the space between the lumen and the peripheral zone **(Figure 2g, h)**. Finally, in mature females (St 3), vitellogenic follicles are predominant and variable in size, with large mature oocytes normally having a thinner columnar follicular epithelium **(Figure 2e)**. When fully developed, the oviducal gland is completely composed of gland tubules **(Figure 2i)**. In egg-laying females (St 3*), the oviducal gland is full of secretions in the tubules **(Figure 2j)** and sometimes also in the uterus.

Sperm was present in the oviducal glands of 70% of the mature females analysed. Sperm was observed throughout the transverse sections of the oviducal gland, from the lumen **(Figure 3a)** to the peripheral tubules, usually forming bundles **(Figure 3b)**.

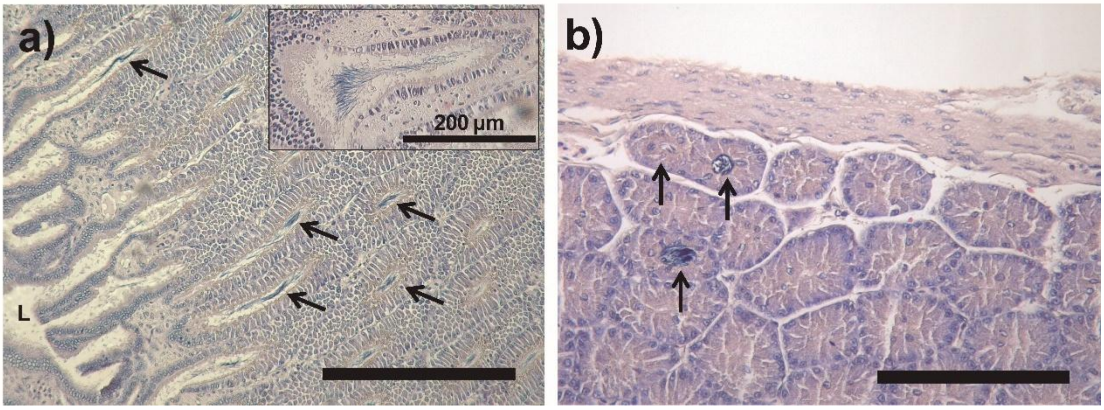
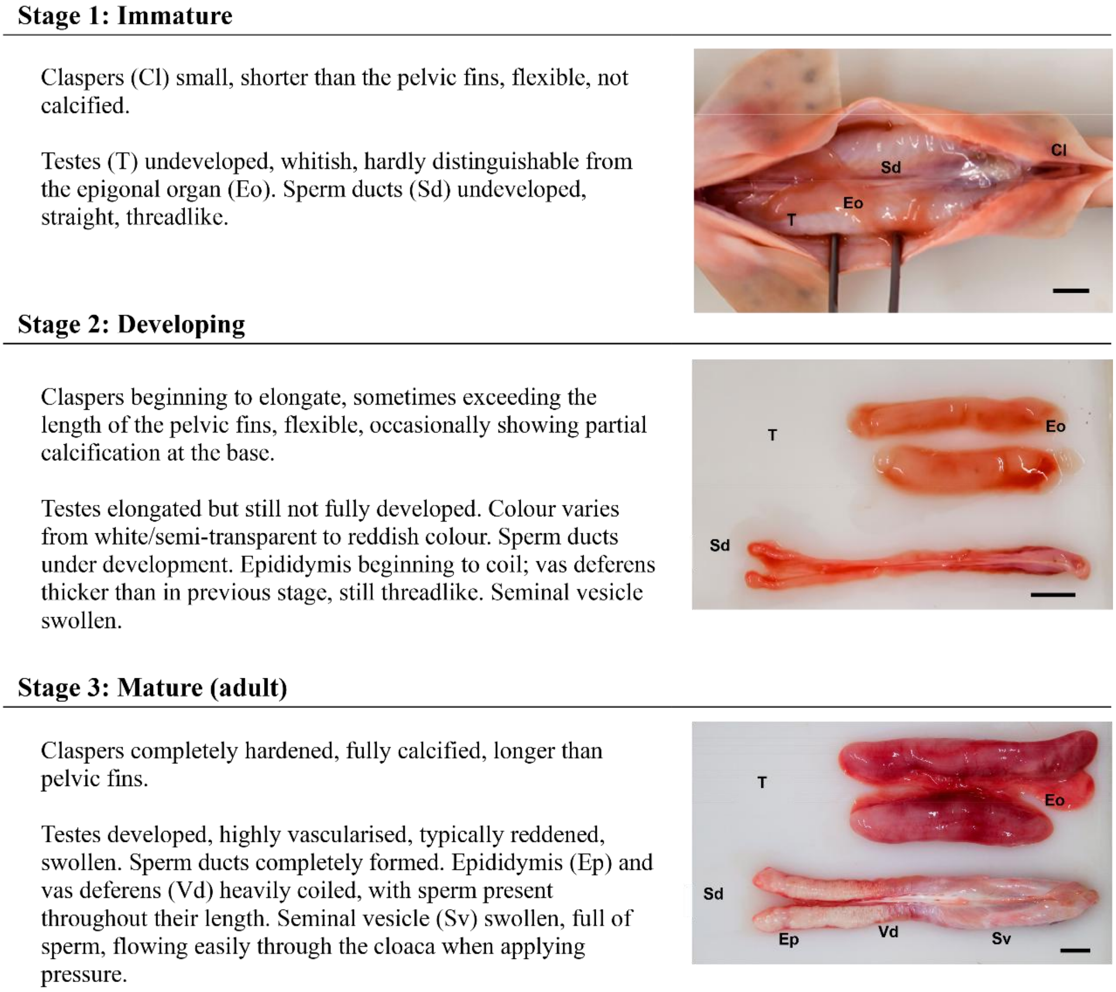


Figure 3. Presence of sperm in transverse sections of *Scyliorhinus canicula* oviducal gland. a) Sperm (arrows) observed close to the lumen of the oviducal gland in a mature female b) Bundles of sperm (arrows) in peripheral tubules of the oviducal gland in a mature female. Scale bar: a = 400 μm, b) = 200 μm. L: lumen.

For males, an immature stage (immature; St 1), a developing stage (developing, St 2) and a mature stage (adult; St 3) were described. The macroscopic description and pictures of the three maturity stages for males are shown in **Figure 4**. Regarding claspers, 97.83% of immature



Stage 1: Immature

Claspers (Cl) small, shorter than the pelvic fins, flexible, not calcified.

Testes (T) undeveloped, whitish, hardly distinguishable from the epigonal organ (Eo). Sperm ducts (Sd) undeveloped, straight, threadlike.

Stage 2: Developing

Claspers beginning to elongate, sometimes exceeding the length of the pelvic fins, flexible, occasionally showing partial calcification at the base.

Testes elongated but still not fully developed. Colour varies from white/semi-transparent to reddish colour. Sperm ducts under development. Epididymis beginning to coil; vas deferens thicker than in previous stage, still threadlike. Seminal vesicle swollen.

Stage 3: Mature (adult)

Claspers completely hardened, fully calcified, longer than pelvic fins.

Testes developed, highly vascularised, typically reddened, swollen. Sperm ducts completely formed. Epididymis (Ep) and vas deferens (Vd) heavily coiled, with sperm present throughout their length. Seminal vesicle (Sv) swollen, full of sperm, flowing easily through the cloaca when applying pressure.

Figure 4. Proposed scale of maturity stages for male *Scyliorhinus canicula.* For each stage, the macroscopic features are described with each corresponding picture. Scale bar: 1 cm.

individuals displayed soft claspers; 85.90% of developing individuals showed soft or medium calcified claspers, and 97.98% of mature individuals showed completely calcified claspers.

The microscopic differences between maturity stages are based on the presence and proportion of different spermatocysts (units of the testes that mature and produce the spermatozoa) stages (Parsons and Grier, 1992). In immature individuals (St 1), the epigonal organ is predominant in the gonad, which contains spermatocysts of stages I, II, III, and occasionally stage IV **(Figure 5a)**. In the developing stage (St 2), the testes contain all spermatocysts stages (I – VII), with predominance of stage IV **(Figure 5b)**. Finally, in mature stage (St 3), testes contain spermatocysts of all stages, with predominance of stages V – VII (spermatids, immature, and mature spermatozoa) **(Figure 5c)**. The epididymis consists of a convoluted tubule with a large lumen containing disorganized spermatozoa **(Figure 5d)** and lined by a ciliated columnar epithelium, varying from pseudostratified to simple **(Figure 5e)**. The Leydig gland (a modified

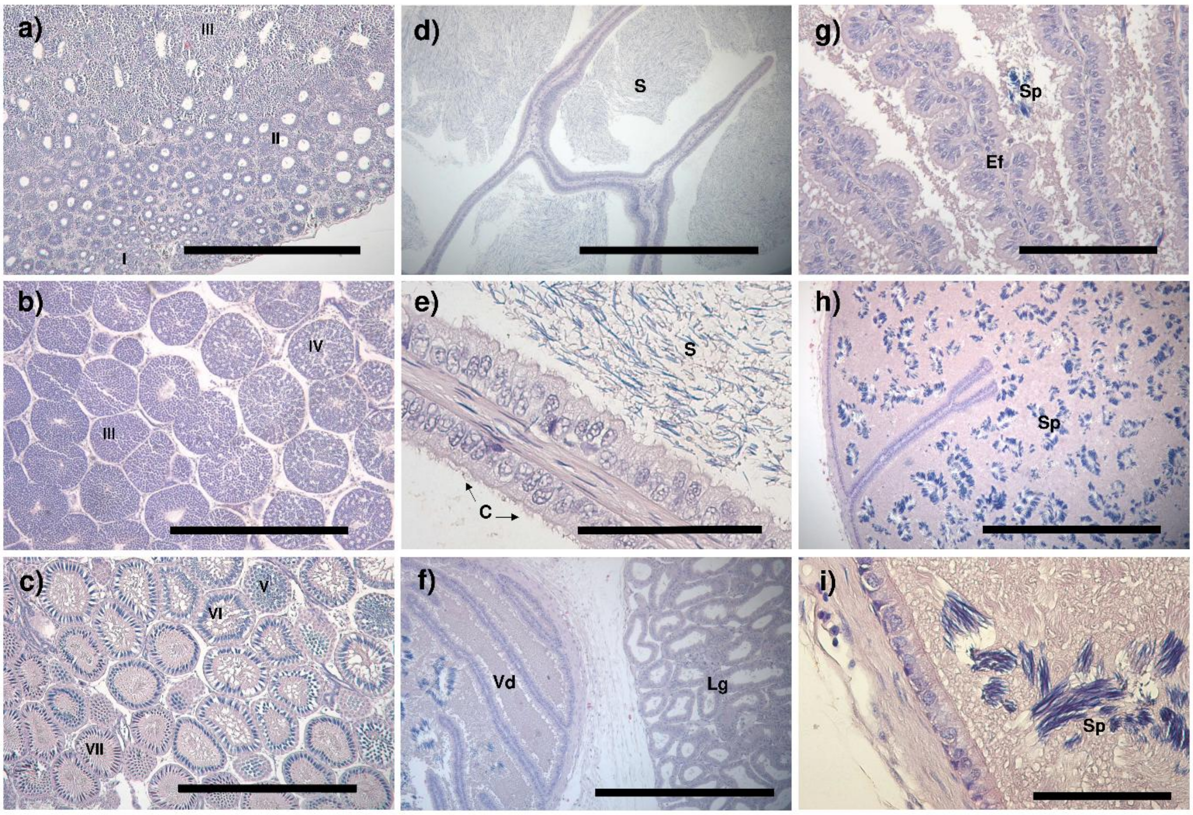


Figure 5. Transverse sections of *Scyliorhinus canicula* male reproductive organs (H & E stain). a) Testis, primary stages of spermatogenesis. Spermatocysts containing: gonocytes (I), spermatogonia (II) and primary spermatocytes (III). b) Testis, developing stage with primary spermatocytes (III) and secondary spermatocysts (IV) c) Testis, mature stage. Spermatocysts containing spermatids (V), immature spermatozoans (VI) and mature spermatozoans (VII). d) Epididymis. e) High magnification of epididymis epithelium. f) Vas deferens and Leydig gland. g) High magnification of vas deferens epithelial folds. h) Seminal vesicle. i) High magnification of the seminal vesicle containing spermatozeugmata. Scale bar: a), b), c), d), f), h) = 1000 µm; e) i) = 100 µm; g) 200 µm. C: cilia, Ef: epithelial folds, Lg: Leydig gland, S: spermatozoa, Sp: spermatozeugmata, Vd: vas deferens.

anterior mesonephros) is adjacent to the vas deferens by connective tissue **(Figure 5f)**. The vas deferens, which has also a pseudostratified columnar epithelium with cilia, has numerous luminal epithelial folds containing some spermatozeugmata (unencapsulated masses of naked sperm) within the lumen **(Figure 5g)**. Finally, the seminal vesicle comprises a pair of ducts lined by simple cuboidal epithelium, with a broad lumen densely packed with spermatozeugmata **(Figure 5h-i)**.

Among all the examined fish, a maturing individual measuring 39.6 cm was macroscopically identified as intersexual, possessing short, soft claspers (0.9 cm and 0.4 cm) along with internal female reproductive organs.

3.2.2. Variation of somatic indices with maturation

Differences in TL, Kn, and somatic indices among the different maturity stages for females and males are shown in **Figure 6**, and statistical results are available in **Table S1**.

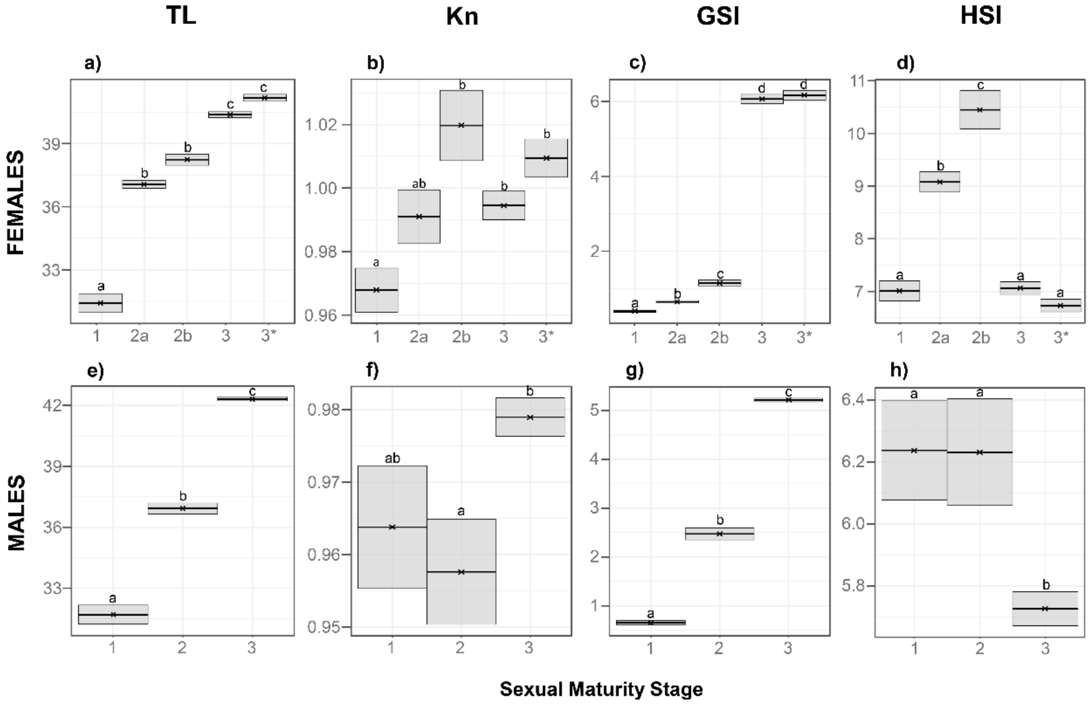


Figure 6. Graphical representation of TL, Kn and somatic indices values grouped by maturity stages and sex in *Scyliorhinus canicula*. GSI: gonadosomatic index, HSI: hepatosomatic index, Kn: Le Cren condition index, TL: total length. Different letters indicate significant differences among reproductive stages. Each box represents the mean and the standard error of the data.

TL increased significantly across all stages for both sexes except for the transition from St 2a - 2b and St 3 - 3* in females. The Kn exhibited higher values in St 2b, 3 and 3* in comparison with St 1 individuals in females, and a significant increase between St 2 - 3 in males. However, values close to one were observed across all stages for both sexes. A significant increase in the GSI for all pairs of consecutive stages of maturation was observed in both sexes. Regarding the HSI, a significant increase was detected in females from the immature to developing stages, followed by a significant decrease in adult stages. HSI in males was similar in immature and developing stages but decreased significantly in mature individuals.

3.2.3. Length at maturity

The smallest sexually mature female and male in the present study measured 34.5 and 33.8 cm TL, respectively. The L50 was similar in both sexes (37.9 cm and 37.2 cm TL for females and males, respectively), whereas the L95 was slightly higher in females than in males (41.8 cm and

40.3 cm TL, respectively) **(Figure 7)**. Model parameters of this analysis can be found in **Table S3**.

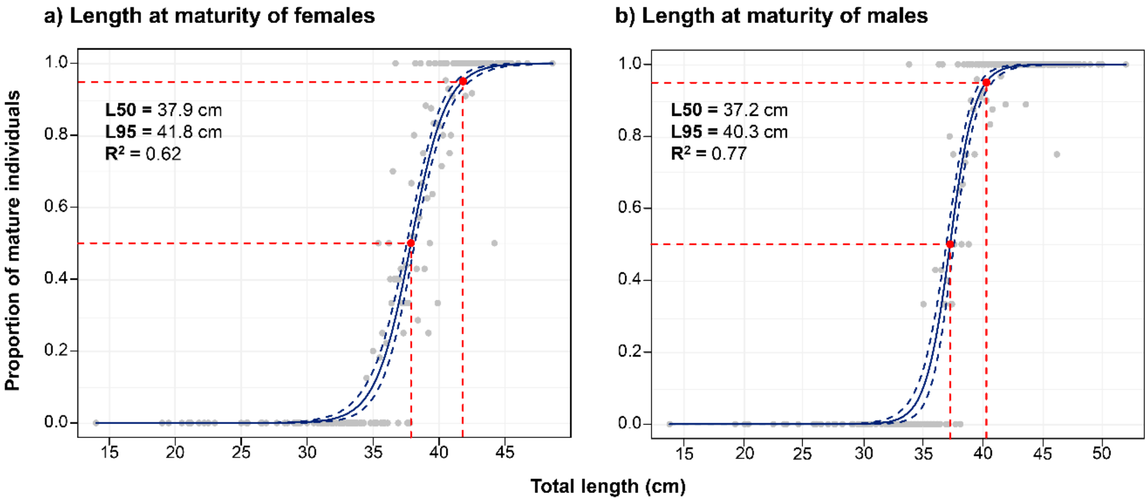


Figure 7. Graphical representation of the length at maturity (proportion of mature individuals and total length) of *Scyliorhinus canicula* a) females and b) males. Lengths at 50% and 95% of maturity are indicated by red dots.

3.3. Liver variability and relation with sexual maturity

3.3.1. Liver colour and lipidic content

Based on liver colour observations and external appearance, a liver colour scoring index was created, comprising four distinct categories (LC1 to LC4). Liver Colour 1 (LC1) was attributed to livers with a light and uniform colouration (yellowish, sometimes pale brown or greyish) **(Figure 8a)**. Liver Colour 2 (LC2) corresponded to light brown or greyish livers, mostly lacking yellow tones, and usually with stretch marks **(Figure 8b)**. Liver Colour 3 (LC3) consisted of a medium dark brown colour, never yellow **(Fig, 8c)**. These livers could also exhibit a pattern of stretch marks or a plain surface. Finally, Liver Colour 4 (LC4) corresponded to dark brown or even blackish colour, with sometimes stretch marks but usually plain surface **(Figure 8d)**.

Histologically, all livers presented the basal structure of the hepatic parenchyma, with polyhedral hepatocytes, lined with sinusoids. Although the hepatocytes' membranes were sometimes irregularly shaped, the central round nuclei could always be observed inside. Diffuse hepatocytes macro and micro vacuolation, differing in number, was found in most of the livers. This vacuolization is normally attributed to glycogen granules or lipid droplets. Since PAS-stained

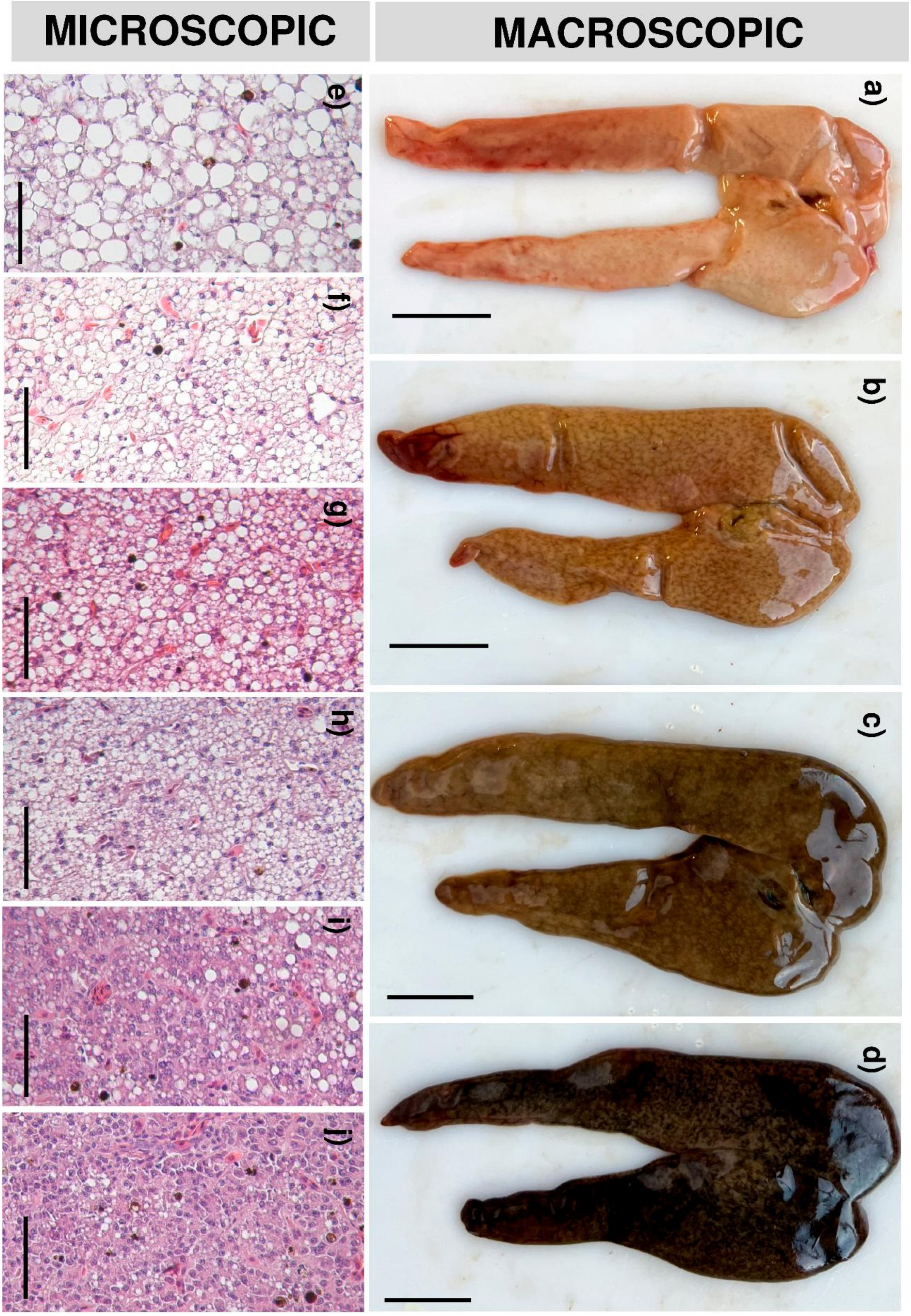


Figure 8. Liver categorization. Macroscopic liver categories of *Scyliorhinus canicula* based on colour: a) LC1, b) LC2, c) LC3 and d) LC4. Microscopic liver categories based on the quantity and size of lipid droplets: e), f) Type I (high lipidic content), g), h) Type II (medium lipidic content), and i), j) Type III (low lipidic content). Scale bar: a) – d) = 2 cm, e) – j) 100 μm.

sections were negative, the presence of glycogen was discarded, and these empty vacuole-like structures were attributed to areas previously occupied by lipid droplets that were removed during tissue processing. According to the quantity and size of these lipid droplets, three different types of liver were identified microscopically: Type I: high lipidic content, with most hepatocytes containing lipid droplets of different sizes, usually aggregated and forming bigger droplets larger than the nucleus of the cell, nucleus displaced to the periphery and cytoplasm difficult to discern **(Figure 8e-f)**. Type II: medium lipidic content, liver with approximately half of the parenchyma occupied by lipid droplets, which had the same or larger size than the hepatocyte nucleus **(Figure 8g-h)**. Most of the hepatocytes' nuclei were displaced by lipid droplets towards the periphery. Type III: low lipidic content, lipid droplets occupying less than half of the parenchyma **(Figure 8i)** or almost absent **(Figure 8j)**. Parenchyma dominated by typically shaped hepatocytes with centred round nuclei.

The high lipidic content category (Type I) always corresponded to lighter liver colour categories (3 associated with LC1 and 2 with LC2), and the lower lipidic content category (Type III) corresponded entirely to darker liver colour categories (2 associated with LC3 and 3 with LC4). The medium lipidic content category (Type II) was mainly represented by intermediate stages, LC2 and LC3 (5 of 7 Type II).

3.3.2. Condition indices, maturity, and liver colour

For both sexes, GSI from individuals with LC1 was much lower compared to the other categories **(Figure 9a, e)**, while there was a consecutive decrease of the HSI with increasing darkness of the organ for both sexes **(Figure 9b, f)**. The Kn showed little variation regardless of liver colour (mean values for each stage between 0.99 and 1.01 in females, and 0.98 and 1.00 in males) **(Figure 9c, g)**. Significant differences were observed when comparing the proportions of each liver colour among the maturity stages for both sexes **(Figure 9d, h; Table S1)**. LC1 females significantly outnumbered females of all other liver colours in immature and developing stages (97.0%, 93.5%, and 93.1% for St 1, St 2a, and St 2b, respectively) and were proportionally fewer in adult stages (36.3% and 14.0% in St 3 and St 3*, respectively). Females with LC2 and LC3 were predominant within adult females (15.4% and 29.7% in St 3 and 7.0% and 46.5% in St 3*, respectively) and LC4 was found only in females from St 2b, 3 and, 3*, corresponding to 3.4%, 18.7%, and 32.6% within each stage **(Figure 9d)**. Similarly, in males, LC1 was found in a much higher proportion in St 1 and 2 (97.2% and 95.5%, respectively), LC2 and LC3 was mainly found in St 3 individuals with percentages of 23% and 29.1% and LC4 was exclusively found in St 3 males but in a low proportion (1%). In addition, a significant association was found between liver colour and sex in mature individuals, with post hoc tests revealing that LC1 was more frequent in males, while LC4 was more frequent in females **(Table S1)**.

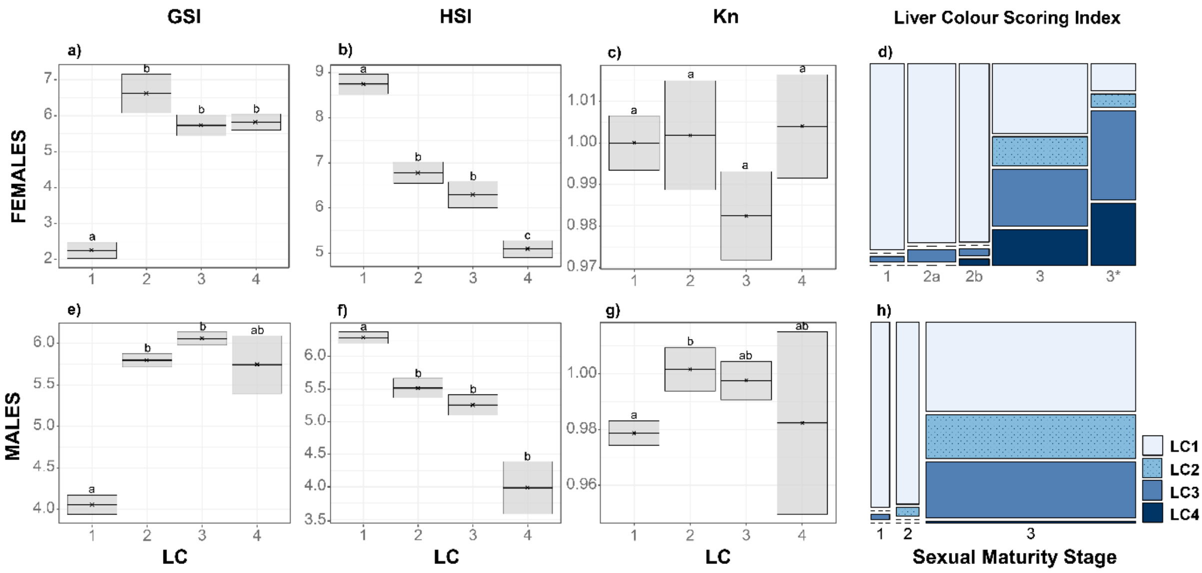


Figure 9. Variation in somatic indices by liver colour (LC) (a–c, e–g) and LC category proportions by maturity stage (d, h) for each sex in *Scyliorhinus canicula*. GSI: gonadosomatic index, HSI: hepatosomatic index, Kn: Le Cren condition index. Different letters indicate significant differences among LC categories. Each box represents the mean and the standard error of the data. The width of the columns in d) and h) represents de proportion of individuals in each sexual maturity stage.

3.4. Temporal variation and trends

Specimens of all maturity stages were found throughout the year for both sexes, but some significant differences among them were sometimes detected **(Figure S2a-b)**. In May, the proportion of St 3 females was higher, in October, St 2a and 2b were significantly more represented, and, in November, St 1 females were proportionally more abundant. In males, St 3 dominated in all months, although in April, there was a higher proportion of St 1 specimens compared to the other months **(Table S1)**.

Kn in females showed lower levels at the end of winter and the beginning of spring (February and March) and higher levels during summer (June, August, September) except for July **(Figure S2c)**. GSI in each female stage remained quite uniform throughout the year, except for the decrease in June, after the high values of May in St 3 females **(Figure S3)**. Egg-laying females (St 3*) were found throughout the year, with monthly percentages between 22.6 and 53.6%. In males, Kn was significantly lower during February, March and July **(Figure S2d)**. Mature males had a significantly higher GSI in October and November compared to June and July **(Figure S4)**.

No statistically significant pattern was detected in HSI; however, a tendency toward lower values between December and April was observed in both sexes **(Fig S3 and S4)**.

4. Discussion

A straightforward macroscopical characterisation of sexual maturity stages, together with histological description and biological indices variations, offers a reliable, comprehensive and replicable way to compare reproduction stages of *S. canicula* in different geographical areas. This, alongside the determination of length at maturity and the characterisation of annual trends, provides essential information for clear recommendations, which are crucial for planning stock management measures.

4.1. Description and assessment of sexual maturity stages

Based on the new findings of the present study and the revision of maturity scales proposed for other oviparous elasmobranchs (Serra-Pereira et al., 2011), some changes in the sexual maturity scale of *S. canicula* are implemented from those proposed by other authors (Ivory et al., 2004; Kousteni and Megalofonou, 2019). Mainly, in females, the developing stage has been divided into two different stages, as macroscopic and microscopic differences have been observed. The variation in somatic indices between these two stages further corroborates the scale proposed herein. A resting or regressing stage, typically described in other elasmobranch species (Barone et al., 2007; Serra-Pereira et al., 2011; MEDITS, 2017), was not detected and therefore should not be considered for *S. canicula* inhabiting the Catalan coast.

The high proportion of females found with sperm present in the oviducal glands (which in fact may be underestimated, as histological sections only allow the examination of a limited portion of the organ) contrasts with the findings of Kousteni et al. (2019), who did not detect sperm in any of the specimens examined. While the presence of sperm in certain regions of the oviducal gland has been associated with sperm storage, particularly in viviparous species, there is currently no clear consensus in oviparous species. Dutilloy and Dunn (2020) suggested that sperm storage is likely ubiquitous across all chondrichthyans, and Marongiu et al. (2021) reported that the oviparous *Galeus melastomus* is capable of long-term sperm retention. In contrast, Jordan et al. (2021) stated that the presence of sperm in the oviducal gland of *S. canicula* could be incidental occurrence. However, the fact that *S. canicula* was able to lay fertilised eggs up to 214 days after isolation from males (Ellis and Shackley, 1997), along with evidence of multiple paternity in this species (Griffiths et al., 2012), supports the possibility of sperm storage. Further research is needed in this aspect, given its importance in the reproductive biology of chondrichthyans.

In the case of males, previous studies divided the adult stage into “mature stage” and, when sperm flowed after applying pressure on the cloaca, “running stage” (Ivory et al., 2004; Kousteni et al*.,* 2010). In the present study, sperm was consistently observed in the seminal vesicle of adult individuals proving that adult males are always able to mate, and therefore only one adult stage should be considered for the Catalan coast population. All three macroscopic stages assessed corresponded to a distinct microscopic stage, confirming the histological classification by Kousteni et al. (2019). In addition, the difference in total length and in somatic indices at each stage supports the maturity scale proposed. Certain authors defended that maturity assessments based on clasper calcification alone were imprecise (Pratt, 1979; Conrath, 2004). As observed in this study, the relation of clasper calcification with gonad maturity stages demonstrates it is an effective, easy and fast way of distinguishing immature and maturing individuals from adult ones without the need for dissection. The monitorisation of mature males (simply through the external observation of claspers) of *S. canicula* and potentially other vulnerable elasmobranchs (Awruch et al., 2008), may be useful for management and conservation purposes (*e.g.*, implementation of protected areas, MLS).

Regarding the intersexual individual found, it is an unusual abnormality that can occasionally occur and that has already been reported in *S. canicula* (see Ellis and Shackley, 1997 and references therein).

4.2. Differences in body size and maturity

Unlike other species of elasmobranchs, in which size dimorphism between males and females is skewed towards the latter, the present study showed that adult males of *S. canicula* are significantly larger than females. Different studies have reported the same size dimorphism in this species (Ivory et al., 2004; Capapé et al., 2008a, 2008b; Finotto et al., 2015; Kousteni and Megalofonou, 2019). In viviparous and oviparous species with seasonal spawning, females tend to be bigger and have a larger body cavity as a selective response to fit more offspring or develop more eggs (Carrier et al., 2004). In the case of *S. canicula,* however, being an oviparous elasmobranch that lays eggs throughout the year, this selective pressure is not expected to occur. In this case, an investment towards continuous egg development and ovulation is made by females, leading to slower growth after reaching maturation (Leloup and Olivereau, 1951). Ivory et al. (2004) estimated a slower growth rate for males than females during the immature and developing stages of maturation, but since females’ investment towards reproductive-related tasks starts when reaching maturity and male contribution to gametogenesis is presumed to be less energy-consuming, female growth is reduced after maturation. Thus, males could allocate more lipid reserves towards growth, reaching larger maximum sizes than females. In addition, male sharks display various reproductive behaviours that may act as a selective pressure towards a larger body size, such as male-to-male competition, increased "patrolling" and aggressive behaviours to ensure mating (Rangel et al. 2021). Harassment and aggression towards females have also been described in *S. canicula* (Ellis and Shackley, 1995; Sims et al., 2001).

Size differences are maintained among populations across the Mediterranean Sea and the Atlantic Ocean, with bigger sizes attained in the latter (Ebert et al., 2021). These differences among populations extend, accordingly, to the size at maturity **(Table 1)**. As a general pattern, animals from higher latitudes or colder regions attain larger body sizes (Bergmann, 1847), and this has also been documented in other elasmobranchs (Colonello et al., 2007). Therefore, individuals from the Mediterranean Sea, as a semi-enclosed basin with higher water temperatures than the Atlantic Ocean (Schroeder and Chiggiato, 2022), are expected to be shorter and attain maturity at smaller sizes. In addition, *S. canicula* forms multiple stocks with genetic differences within its distributional range (Gubili et al., 2014), which can also explain size differences in the maximum and maturation sizes.

More recently, it has been suggested that an observed decreasing trend in size at maturity across time for *S. canicula* could be a response to fishing pressure (Ramírez-Amaro et al., 2020). Ramírez-Amaro et al. (2020) analysed its size at maturity between 2000 and 2015 in the Western Mediterranean Sea, and detected a significant decrease in the L50, with a reduction of 3.4 and 2.5 cm TL in females and males, respectively. Seven years later, our data suggests another reduction in the same area of 2.2 and 1.5 cm, respectively, being the smallest L50 ever reported for this species **(Table 1)**. Moreover, this trend seems to be also happening in the Atlantic populations, with a reduction not only in the L50 but also in the maximum lengths attained across time **(Table 1)**. This underscores the importance of implementing region-specific and regularly updated MLS. In the case of *S. canicula*, MLS would be the most suitable management measure, as a seasonal ban would not be effective given that the species reproduces year-round. However, this species is not under great fishing pressure since it is mainly discarded with high survival rates, accounting for nearly two thirds (64%) of the discarded biomass in the upper slope (Rodríguez-Cabello et al., 2005; ICATMAR, 2023).

Table 1. Maximum size and size at sexual maturity reported for *Scyliorhinus canicula* from different areas and different time periods. TL$_{50}$: length at which 50 of the individuals are sexually mature; TL$_{100}$: length at which 100% of the individuals are sexually mature (* indicates TL$_{95}$: length at which 95% of individuals are sexually mature); M: males; F: females; n: number of individuals samples.

Area of study	Sampling year	n	Maximum size (cm)		TL first maturity (cm)		TL$_{50}$ (cm)		TL$_{100}$ (cm)		Reference
			M	F	M	F	M	F	M	F	
Atlantic Ocean											
Off Plymouth (English channel)	1920-21		> 70			54	57 - 60				Ford, 1921
Concarneau (Atlantic)			72		60		66		68		Fauré-Frémiet, 1942
Off Roscoff (English channel)			66		52		54		60		
Off Roscoff (English channel)		60	68		52				60		Leloup and Oliverau, 1951
Bristol Channel	1992 - 94	972	66.2	67	49	52	52	55	55	65	Ellis and Shackley, 1997
Cantabrian Sea	1994 - 95	740				49.7		54.2		59.1	Rodríguez - Cabello *et al.* 1998
West Coast of Ireland	1997 - 98	560			53	52	57.5	58.1	61	64	Henderson and Casey, 2001
Celtic and Irish Sea	1999 - 00	745	71	70	49	52	53.5	57	62	69	Ivory *et al.*, 2004
North Portuguese coast	2013 - 14	476	64.4	59.5	48.9	48.2	53.2	50.6	59.2	54.7	Cardoso, 2014
South Portuguese coast	2015 - 18	148	57.1	58			42.6	44.5			Moreira *et al.*, 2022
Mediterranean Sea											
Off Banyuls		360	48.5	52	38.5		40		44.5		Leloup and Oliverau, 1951
Gulf of Tunis			58	56			40	40 - 45			Capapé, 1977
Gulf of Lyon	1988 - 89	985	55	51			44	41 - 47			Capapé *et al.*, 1991
Gulf of Lyon	2000 - 06	896	55	51	43	410			44	45	Capapé *et al.*, 2008
Tuscany coast (North Thyrrenian Sea)	2007	1345	50.5	49	39	39.5	40.2	40.5	49	46.5	Lupi, 2008
Aegan Sea	2005 - 07	325	48.8	46.7	37.1	36.4	39.6	39.9			Kousteni *et al.*, 2010
Northern Tunisian coast	2009 - 10	1124	52.1	53.1	40	34.6			48	47.1	Capapé *et al.*, 2014
Hellenic waters (Aegan, Ionian Sea and Corinthian Gulf)	2005 - 12	2253	51.7	49.2	35	34.9	38.2	39.7	41.5*	44.3*	Kousteni and Megalofonou, 2019
Northern Adriatic	2012 - 13	249	50.5	49	37	39	40.1	41			Finotto *et al.*, 2015
Strait of Sicily	2012 - 13	236	49.5	48	33.5	34.5	38.5	36.8			
Catalan and Valencia coast	2015	1073				36.5	38.7	40			Ramírez-Amaro *et al.*, 2020
Balearic Sea	2015	1556				36	38.3	39.5			
Alboran Sea	2015	615				37	39.7	40.6			
Catalan coast	2022 - 23	1795	52	48.6	33.8	34.5	37.2	37.8	40.3*	41.8*	Present study

Recent studies pointed out that higher sea temperatures are also causing a decrease in length at maturation in other fish species (Shapiro et al., 2019; Niu et al., 2023). In the Mediterranean Sea, surface water is warming approximately 20% faster than the global mean (Lionello and Scarascia, 2018), but in water masses below 400 m, where this species is also found, warming is not so considerable (Bethoux et al., 1990). This fact deserves more attention and studies to elucidate if this is happening to other elasmobranch species and if it is due to fishing pressure or other changes in environmental conditions (*i.e.* ocean warming, acidification, salinity) or even changes in food supplies.

4.3. Liver mobilization of lipids

The liver is known to be dimorphic between sexes in elasmobranchs, and several studies focusing on *S. canicula* have observed larger livers in females (Craik, 1978; Capapé et al., 2008a, 2008b, 2014), as in the present study (higher HSI levels recorded in adult females). The liver of elasmobranchs is also known for its lipidic energy storage capacity (Craik, 1978) in the form of lipid droplets, and associated with the production of vitellogenin in females (Winner, 1990), as in teleosts (Hara et al., 2016).

To the best of our knowledge, this is the first study to present a gradient of colour from an elasmobranch liver, with four different categories ranging from light to dark colour, correlating macroscopic liver colour (LC) to lipid content and elucidating the relationship of LC with somatic indices. Stedman and Garner (2018) stated that a dark brown liver in elasmobranchs is related to a poor nutritional condition. However, in the present study, the Le Cren condition index (Kn) was not lower in individuals with darker livers. Generally, the darkening of the organ is related to a decrease in the HSI and the lipid content, a relation that is even more evident in females. These results are supported by studies that relate the high lipid concentration in livers with light-colour appearance (Rossouw, 1987). Therefore, the significant increase in darker livers when maturity is reached is mainly explained by the lipid expenditure in reproduction, especially in females, due to the high demand of energy to produce egg yolk and egg capsules. This hypothesis is also supported by an inverse correlation between GSI and HSI during maturation, as other authors also suggested (Craik, 1978). The observed drop of HSI levels between late-developing and mature females, together with the increase of GSI values between the same stages of maturation, would correspond to the production of yolk by the oocytes with lipid precursors from the liver. Other studies also observed lipid mobilisation from the liver to the ovary in *S. canicula* (Valls et al., 2016) and variation of lipid-related parameters linked to maturity stages (Garcia-Garrido et al., 1990; Valls et al., 2016). In males, a lower but significant drop in the HSI levels between the developing and mature stages may indicate an increase in lipid expenditure for spermatogenesis (Garcia-Garrido et al., 1990) and other reproductive-related behaviours when reaching maturity (Rangel et al., 2021).

4.4. Temporal trends

Regarding the reproductive period, the absence of resting females and the presence of egg-laying females throughout the year, as well as of mature males and high, relatively stable mean values of GSI for both sexes, confirm a continuous reproductive period with egg-laying occurring throughout the year. This is in accordance with other authors, who reported egg-capsules all year in *S. canicula* from NW Mediterranean (Capapé et al., 1991), in the Tunisian coast (Capapé et al., 2014) and in British waters (Ford, 1921). However, other authors reported a gap in egg laying between August and October in British waters (Craik, 1978; Ellis and Shackley, 1997) and in September in NW Mediterranean (Capapé et al., 2008a), potentially due to the few mature specimens obtained during that month (n=16).

Controversy intensifies when addressing reproductive peaks. Different studies detected a minimum of reproduction from August to October in the Atlantic Ocean (Ford, 1921; Craik, 1978; Ellis and Shackley, 1997). Contrary, a reproduction peak was found from April to August in the Gulf of Lyon (Capapé et al., 2008a, 2008b). These studies report minimums and peaks mainly based on proportions of females bearing eggs, but in the present study, there is no clear peak or minimum in reproduction based on this criterion. The fact that females are able to lay eggs throughout the year can further support the lack of peaks.

The temporal variation in females Kn followed a trend that can be explained by the accessibility to food, being lower during late winter and leading to a decrease in the general condition of the individuals in this season. During summer months, when conditions are more favourable, this factor rises (Craik, 1978). The lower mean value of females Kn in July has no relation with the other somatic indices analysed. Other studies have not reported a similar phenomenon, which leads us to attribute this value to a stochastic event. In general, Kn patterns in females are similar to those of HSI, as the energy reserves are stored in the liver. In mature females (St 3 and 3*), HSI and GSI are generally stable throughout the year, a phenomenon that could support the constant vitellogenesis in mature females, keeping mature oocytes always in the gonads for reproduction (Craik, 1978). In mature males, similar trends are observed regarding Kn, HSI, and GSI. The significantly higher HSI levels from May to November, in comparison to levels between December and April (except during July), are explained by the lipid deposition during periods of food abundance and usage during a period of food scarcity (Craik, 1978).

5. Conclusions

An in-depth one year-round reproductive study of *S. canicula* was carried out in the Balearic Sea, leading to the proposal of five and three maturity stages for females and males, respectively, based on macroscopical and microscopical observation of gonads. Based on these results, a continuous reproductive cycle is confirmed, as well as the common presence of sperm in the oviducal gland of females, potentially linked to sperm storage capacity. Moreover, a relation between macroscopic liver colour and lipid content in the same organ is established and associated with the mobilisation of lipids to the gonad during reproduction. Lastly, a decreasing trend with time on the length at maturation is reported for this species globally and in the Mediterranean Sea. In this sense, a comprehensive gathering data on other species to see if this is a general trend among elasmobranchs should be endorsed. This should be of special interest in the case of commercial species, for which the length at maturity is of paramount importance for fishing management measures.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

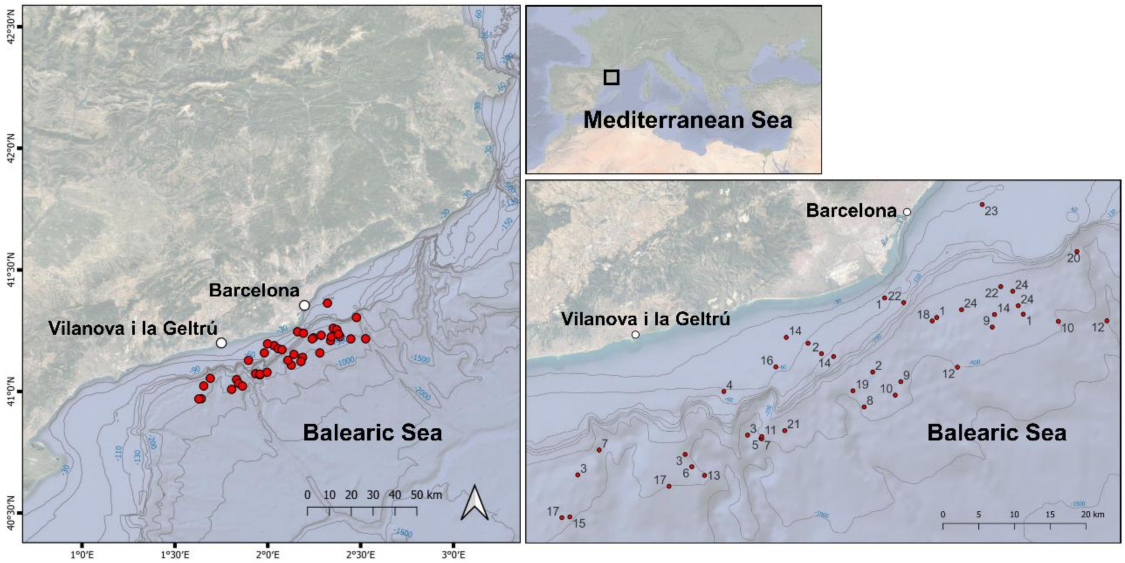

Figure S1. Map of the Mediterranean and Balearic Sea indicating the sampling sites of the hauls during which the 1795 specimens of *Scyliorhinus canicula* were collected. Red dots indicate different sampling sites and numbers indicate different sampling days. White dots indicate the location of the two landing ports.

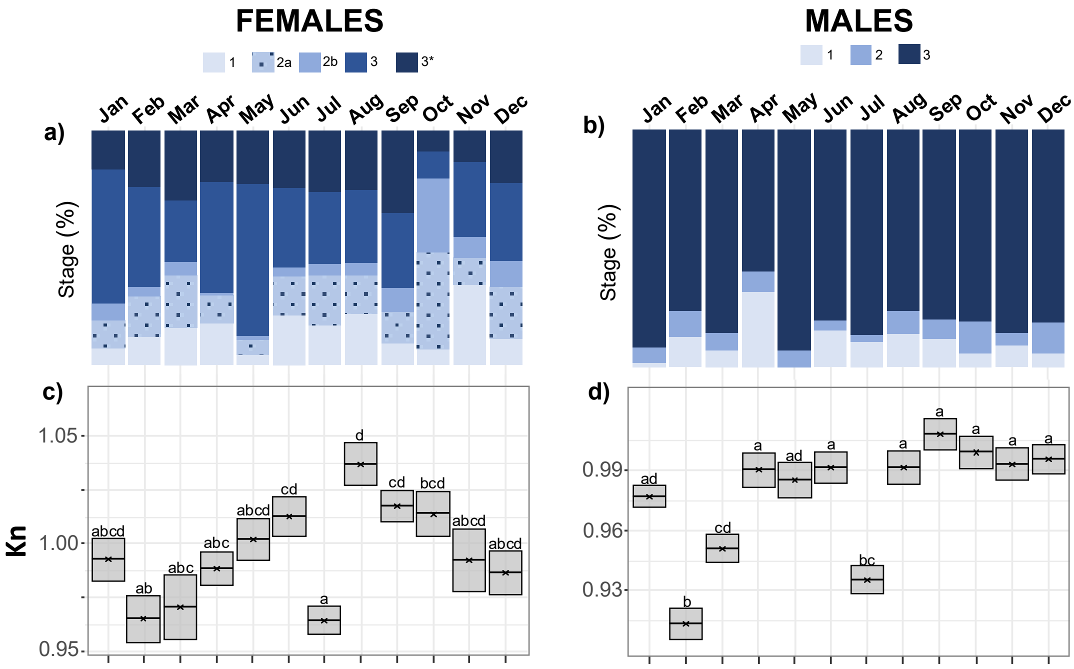

Figure S2. Proportion of each sexual maturity stage per month in a) females a) and males b) of *Scyliorhinus canicula*. Variation in Kn (Le Cren condition index) (c, d) among months. Different letters indicate significant differences among months. Each box represents the mean and the standard error of the data.

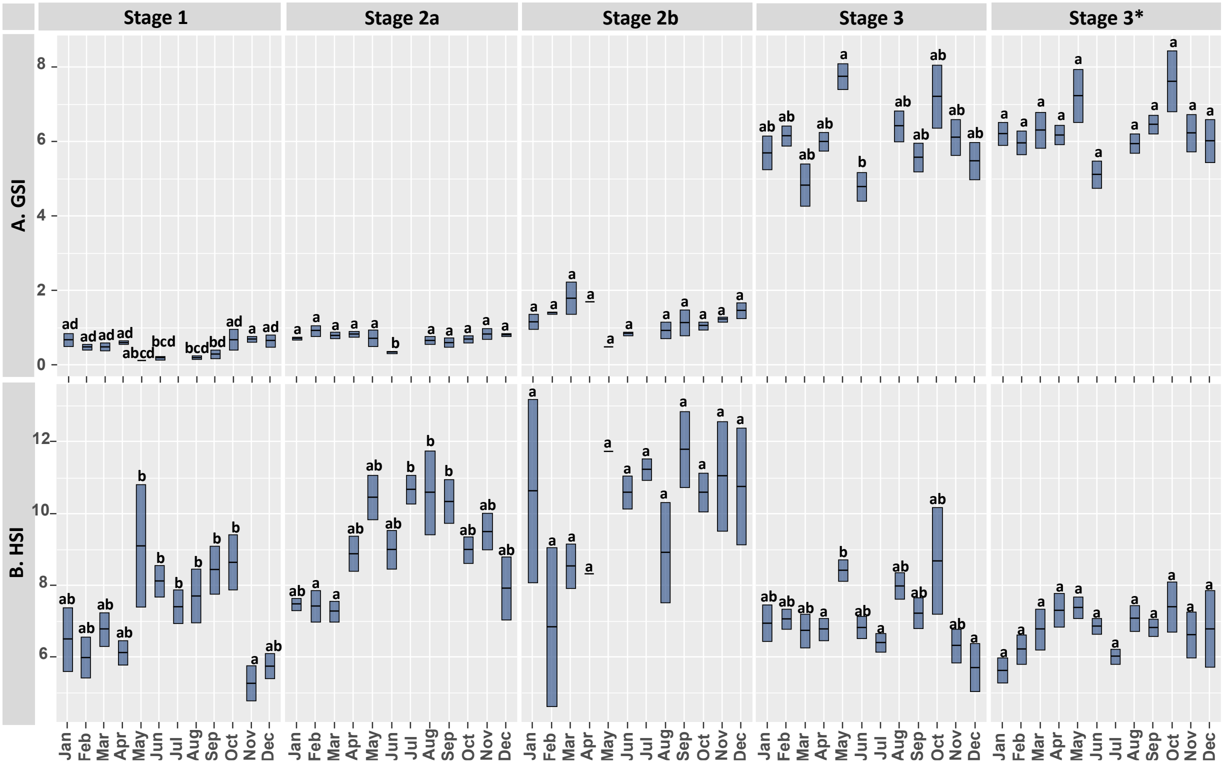

Figure S3. Significant differences among months in gonadosomatic (GSI, **A**) and hepatosomatic (HSI, **B**) indices for each maturity stages in females of *Scyliorhinus canicula*. Different letters indicate significant differences among months within the stage. Each box represents the mean and the standard error of the data.

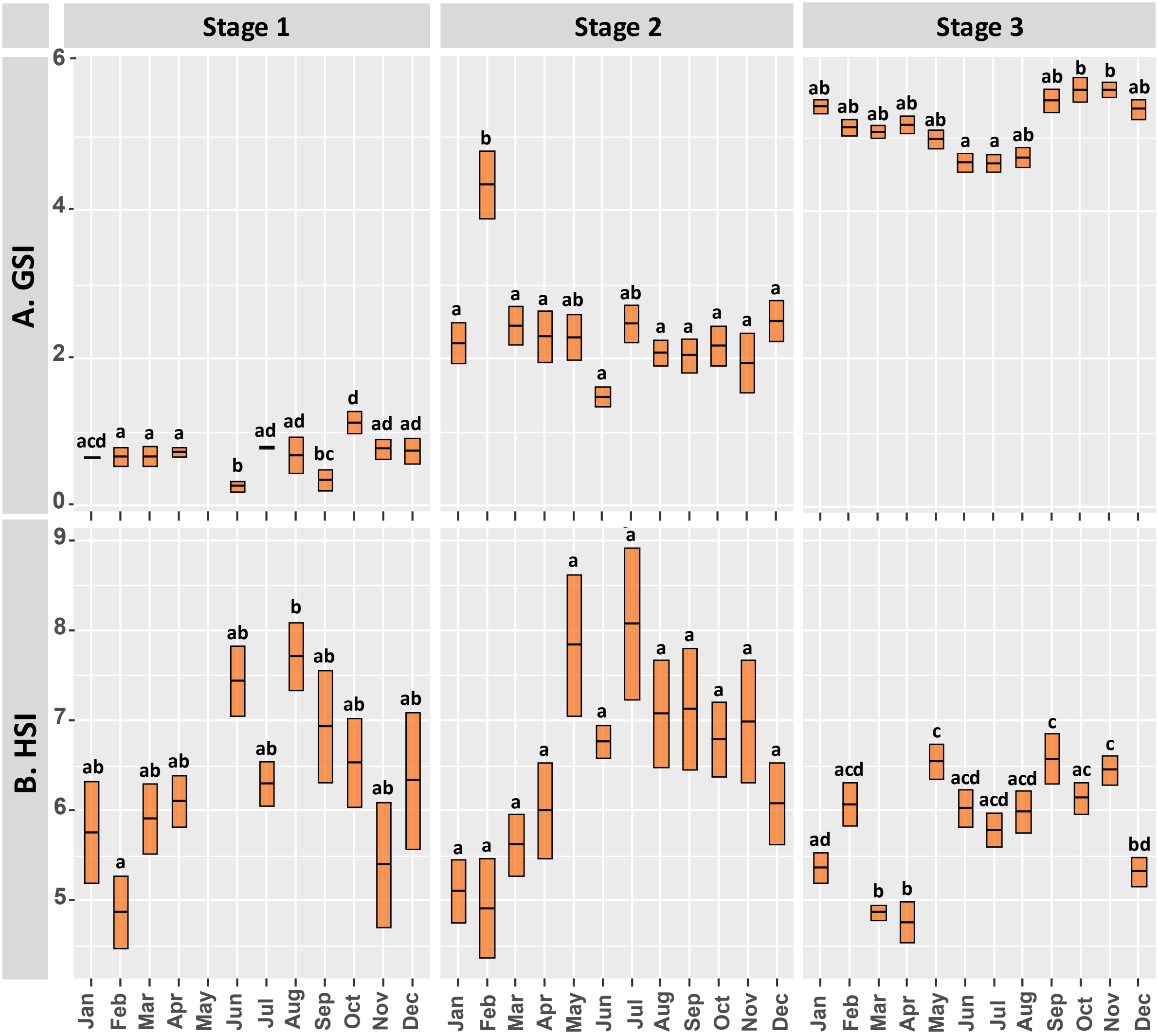

Figure S4. Significant differences among months in gonadosomatic (GSI, **A**) and hepatosomatic (HSI, **B**) indices for each maturity stages in males of *Scyliorhinus canicula*. Different letters indicate significant differences among months within the stage. Each box represents the mean and the standard error of the data.

Table S1. Significant estatistical results of all tests. Only results with a p-value < 0.05 are reported.

	Test	Estatistic	p - value
General differences Males – Females (Table S2)			
Sex – GSI (mature)	Wilcoxon rank sum test	W = 212666	< 0.001
Sex – HSI (mature)	Wilcoxon rank sum test	W = 265412	< 0.001
Sex – Kn (mature)	Wilcoxon rank sum test	W = 222342	< 0.001
Sex - TL (mature)	Wilcoxon rank sum test	W = 123696	< 0.001
Correlations			
GSI - TL (Males)	Spearman's rank correlation	rho = 0.56	< 0.001
GSI - TL (Females)	spearman's rank correlation	rho = 0.65	< 0.001
Differences of somatic indices with maturation – Females (Figure 6)			
TL: St1 - St2a	GLM, post-hoc	t-ratio = 16.24	< 0.001
TL: St1 - St2b	GLM, post-hoc	t-ratio = 15.53	< 0.001
TL: St1 - St3	GLM, post-hoc	t-ratio = 27.12	< 0.001
TL: St1 - St3*	GLM, post-hoc	t-ratio = 27. 65	< 0.001
TL: St2a - St3	GLM, post-hoc	t-ratio = 10.08	< 0.001
TL: St2a - St3*	GLM, post-hoc	t-ratio = 11.51	< 0.001
TL: St2b - St3	GLM, post-hoc	t-ratio = 4.54	< 0.001
TL: St2b - St3*	GLM, post-hoc	t-ratio = 5.91	< 0.001
Kn: St1 - St2b	GLM, post-hoc	t-ratio = 3.92	0.001
Kn: St1 - St3	GLM, post-hoc	t-ratio = 3.01	0.03
Kn: St1 - St3*	GLM, post-hoc	t-ratio = 4.34	< 0.001
GSI: St1 - St2a	GLM, post-hoc	t-ratio = 7.35	< 0.001
GSI: St1 - St2b	GLM, post-hoc	t-ratio = 12.64	< 0.001
GSI: St1 - St3	GLM, post-hoc	t-ratio = 19.98	< 0.001
GSI: St1 - St3*	GLM, post-hoc	t-ratio = 19.99	< 0.001
GSI: St2a - St2b	GLM, post-hoc	t-ratio = 8.01	< 0.001
GSI: St2a- St3	GLM, post-hoc	t-ratio = 22.08	< 0.001
GSI: St2a- St3*	GLM, post-hoc	t-ratio = 22.09	< 0.001
GSI: St2b - St3	GLM, post-hoc	t-ratio = 12.40	< 0.001
GSI: St2b - St3*	GLM, post-hoc	t-ratio = 12.43	< 0.001
HSI: St1 - St2a	GLM, post-hoc	t-ratio = 7.41	< 0.001
HSI: St1 - St2b	GLM, post-hoc	t-ratio = 9.42	< 0.001
HSI: St2a - St2b	GLM, post-hoc	t-ratio = 3.32	0.009
HSI: St2a - St3	GLM, post-hoc	t-ratio = -9.16	< 0.001
HSI: St2a - St3*	GLM, post-hoc	t-ratio = -9.79	< 0.001
HSI: St2b - St3	GLM, post-hoc	t-ratio = -11.01	< 0.001
HSI: St2b - St3*	GLM, post-hoc	t-ratio = -11.54	< 0.001
Differences of somatic indices with maturation – Males (Figure 6)			
TL: St1 - St2	GLM, post-hoc	t-ratio = 13.94	< 0.001
TL: St1 - St3	GLM, post-hoc	t-ratio = 32.83	< 0.001
TL: St2 - St3	GLM, post-hoc	t-ratio = 15.13	< 0.001
Kn: St2 - St3	GLM, post-hoc	t-ratio = 2.41	0.049
GSI: St1 - St2	GLM, post-hoc	t-ratio = 23.21	< 0.001
GSI: St1 - St3	GLM, post-hoc	t-ratio = 28.63	< 0.001
GSI: St2 - St3	GLM, post-hoc	t-ratio = 16.48	< 0.001
HSI: St1 - St3	GLM, post-hoc	t-ratio = -3.04	0.007
HSI: St2 - St3	GLM, post-hoc	t-ratio = -2.77	0.017
Condition indices and Liver colour – Females (Figure 9)			
GSI: LC1 - LC2	GLM, post-hoc	t-ratio = 6.20	< 0.001
GSI: LC1 - LC3	GLM, post-hoc	t-ratio = 6.55	< 0.001
GSI: LC1 - LC4	GLM, post-hoc	t-ratio = 6.17	< 0.001
HSI: LC1 - LC2	GLM, post-hoc	t-ratio = -3.11	0.013
HSI: LC1 - LC3	GLM, post-hoc	t-ratio = -6.45	< 0.001
HSI: LC1 - LC4	GLM, post-hoc	t-ratio = -7.92	< 0.001
HSI: LC2- LC4	GLM, post-hoc	t-ratio = -3.42	0.004
HSI: LC3- LC4	GLM, post-hoc	t-ratio = -3.17	0.010

Condition indices and Liver colour – Males (Figure 9)

GSI: LC1 - LC2	GLM, post-hoc	t-ratio = 8.96	< 0.001
GSI: LC1 - LC3	GLM, post-hoc	t-ratio = 10.70	< 0.001
HSI: LC1 - LC2	GLM, post-hoc	t-ratio = -3.99	< 0.001
HSI: LC1 - LC3	GLM, post-hoc	t-ratio = -5.87	< 0.001
HSI: LC1 - LC4	GLM, post-hoc	t-ratio = -2.75	0.038

Proportion of Liver colour by Sex in Mature individuals

LC1: F	Chi-squared, post-hoc	Residuals = - 3.62	0.002
LC4: F	Chi-squared, post-hoc	Residuals = 9.02	< 0.001

Proportions of Liver colour among stages – Females (Figure 9)

LC1: St1	Chi-squared, post-hoc	Residuals = 4.85	< 0.001
LC1: St2	Chi-squared, post-hoc	Residuals = 5.38	< 0.001
LC1: St3	Chi-squared, post-hoc	Residuals = 4.06	< 0.001
LC1: St4a	Chi-squared, post-hoc	Residuals = -5.39	< 0.001
LC1: St4b	Chi-squared, post-hoc	Residuals = -6.50	< 0.001
LC2: St4a	Chi-squared, post-hoc	Residuals = 3.95	0.002
LC3: St4b	Chi-squared, post-hoc	Residuals = 4.41	< 0.001
LC4: St4b	Chi-squared, post-hoc	Residuals = 4.13	< 0.001

Proportions of Liver colour among stages – Males (Figure 9)

LC1: St1	Chi-squared, post-hoc	Residuals = 5.29	< 0.001
LC1: St2	Chi-squared, post-hoc	Residuals = 5.65	< 0.001
LC1: St3	Chi-squared, post-hoc	Residuals = -8.11	< 0.001
LC2: St1	Chi-squared, post-hoc	Residuals = -3.08	0.025
LC2: St3	Chi-squared, post-hoc	Residuals = 4.21	< 0.001
LC3: St1	Chi-squared, post-hoc	Residuals = -3.15	0.019
LC3: St2	Chi-squared, post-hoc	Residuals = -3.97	< 0.001
LC3: St3	Chi-squared, post-hoc	Residuals = 5.30	< 0.001

Temporal variation and trends (Figure S2)

Proportion of female stages per month (Figure S2)

November: St1	Chi-squared, post-hoc	Residuals = 3.59	0.02
October: St2	Chi-squared, post-hoc	Residuals = 4.33	< 0.001
September: St3	Chi-squared, post-hoc	Residuals = 1.25	< 0.001
October: St3	Chi-squared, post-hoc	Residuals = 6.64	< 0.001
May: St4a	Chi-squared, post-hoc	Residuals = 4.12	0.002
September: St4a	Chi-squared, post-hoc	Residuals = -1.04	0.018
October: St4a	Chi-squared, post-hoc	Residuals = -3.61	< 0.001

Proportion of male stages per month (Figure S2)

April: St1	Chi-squared, post-hoc	Residuals = 6.54	< 0.001
April: St3	Chi-squared, post-hoc	Residuals = -5.11	< 0.001

Kn in females (Figure S2)

February - June	GLM, post-hoc	t-ratio = 3.91	< 0.001
February - August	GLM, post-hoc	t-ratio = 5.11	< 0.001
February - September	GLM, post-hoc	t-ratio = 4.20	< 0.001
March - August	GLM, post-hoc	t-ratio = 4.25	< 0.001
April - August	GLM, post-hoc	t-ratio = 3.54	0.027
June - July	GLM, post-hoc	t-ratio = -4.10	0.003
July - August	GLM, post-hoc	t-ratio = 5.29	< 0.001
July - September	GLM, post-hoc	t-ratio = 4.39	< 0.001
July - October	GLM, post-hoc	t-ratio = 3.41	0.046

Kn in males (Figure S2)

January - February	GLM, post-hoc	t-ratio= -6.02	< 0.001
January - July	GLM, post-hoc	t-ratio= -3.68	0.016
February - March	GLM, post-hoc	t-ratio= 3.91	0.006
February - April	GLM, post-hoc	t-ratio= 6.62	< 0.001
February - May	GLM, post-hoc	t-ratio= 5.81	< 0.001
February - June	GLM, post-hoc	t-ratio= 6.69	< 0.001
February - August	GLM, post-hoc	t-ratio= 6.09	< 0.001
February - September	GLM, post-hoc	t-ratio= 7.70	< 0.001
February - October	GLM, post-hoc	t-ratio= 7.58	< 0.001
February - November	GLM, post-hoc	t-ratio= 7.597	< 0.001
February - December	GLM, post-hoc	t-ratio= 7.60	< 0.001
March - April	GLM, post-hoc	t-ratio= 3.86	0.008
March - June	GLM, post-hoc	t-ratio= 3.95	0.006

March - August	GLM, post-hoc	t-ratio= 3.48	0.035
March - September	GLM, post-hoc	t-ratio= 5.21	< 0.001
March - October	GLM, post-hoc	t-ratio= 4.93	< 0.001
March - November	GLM, post-hoc	t-ratio= 4.80	< 0.001
March - December	GLM, post-hoc	t-ratio= 4.87	< 0.001
April - July	GLM, post-hoc	t-ratio= -4.44	< 0.001
May - July	GLM, post-hoc	t-ratio= -3.80	0.010
June - July	GLM, post-hoc	t-ratio= -4.52	< 0.001
July - August	GLM, post-hoc	t-ratio= 4.14	0.003
July - September	GLM, post-hoc	t-ratio= 5.57	< 0.001
July - October	GLM, post-hoc	t-ratio= 5.28	< 0.001
July - November	GLM, post-hoc	t-ratio= 5.12	< 0.001
July - December	GLM, post-hoc	t-ratio= 5.20	< 0.001

Table S2. Descriptive statistics. Total length (TL; cm) and somatic indices (Kn: Le Cren condition index; GSI: gonadosomatic index; HSI: hepatosomatic index) sorted by sex. Data is presented separately considering all 1795 individuals and a subset of mature 1302 individuals of *Scyliorhinus canicula* sampled in the Balearic Sea (North West Mediterranean). S.D.: standard deviation, n: number of individuals sampled. Different superscript letters indicate significant differences ($p < 0.05$) among sexes.

	FEMALES				**MALES**			
	Mean	**S.D.**	**Range**	**n**	**Mean**	**S.D.**	**Range**	**n**
TL	38.49	4.31	14.00 – 48.60	760	40.82	4.38	13.70 – 52.00	1033
Kn	1.00	0.08	0.71 – 1.55	760	0.98	0.08	0.66 – 1.34	1030
GSI	3.81	3.01	0.03 – 11.28	636	4.57	1.76	0.01 – 10.68	1016
HSI	7.57	2.32	1.32 – 16.42	757	5.82	1.61	2.24 – 13.37	1027
Mature								
TL	40.69^a	2.29	34.50 – 48.60	458	42.31^b	2.56	33.80– 52.00	844
Kn	1.00^a	0.08	0.71 – 1.29	458	0.98^b	0.08	0.66 – 1.34	841
GSI	6.10^a	1.73	0.99 – 11.28	368	5.21^b	1.05	0.70 – 10.68	838
HSI	6.93^a	1.86	1.32 – 15.24	457	5.82^b	1.61	2.24 – 13.37	838

Table S3. Parameters of the logistic regression P(*maturity*) $=1/(1+\exp(-\beta_0+\beta_1*X))$, obtained for males and females. n is the sample size, β_0 and β_1 are the estimated parameters followed by the standard error (s.e.).

	n			
Sex	**Immature**	**Mature**	**β_0 (s.e.)**	**β_1 (s.e.)**
Females	244	458	- 28.356 (0.066)	0.749 (0.002)
Males	185	844	- 35.786 (0.108)	0.961 (0.003)

CHAPTER 2

Coping with current impacts: The case of *Scyliorhinus canicula* in the NW Mediterranean Sea and implications for human consumption

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Abstract

The small-spotted catshark (*Scyliorhinus canicula*) is a bottom-dwelling elasmobranch that represents the most discarded catch in terms of biomass in the Catalan coast (NW Mediterranean). Potential impacts affecting its population and food safety implications have been assessed in three localities along the Catalan coast. Distinct indicators were integrated, such as biological data, ingested anthropogenic items (plastic and cellulose-like items), parasitological indices, trace metal concentrations and histopathology using liver as target organ. Although high ingestion rates of fibres and levels of some heavy metals, they do not seem negatively affected by any major pathology nor by the current levels of pollutants. Small-scale differences among localities and depths were found and discussed. No zoonotic parasites were found. Encysted larvae of *Grillotia adenoplusia* and, above all, the levels of Hg found in the musculature, that are well over the European Commission limits, rise concerns regarding human consumption of *S. canicula* in this region.

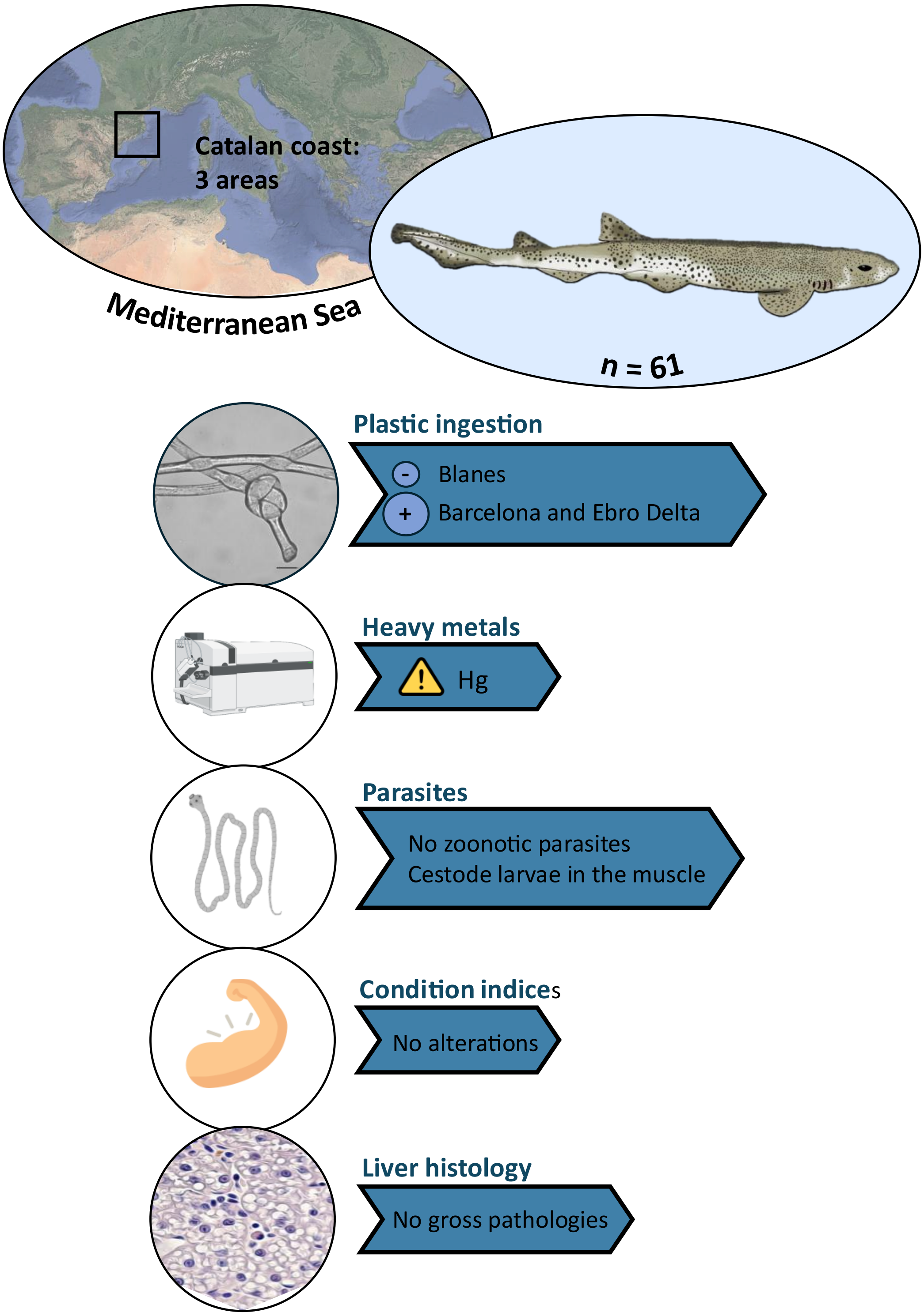
Keywords

Catsharks, Parasites, Microplastics, Histology, Trace metals, Elasmobranch

Highlights

- *S. canicula* health seems unaffected by significant pathologies or pollutant levels
- *S. canicula* ingests fibres, with a prevalence of 87 % and a mean abundance of 5.47
- Fibres seem to accumulate in the spiral valve rather than in the stomach
- 95 % of sharks exceeded Hg limits established by the EC for human consumption
- No zoonotic parasites found but G. adenoplusia in flesh diminishes its quality

Graphical abstract



1. Introduction

The small-spotted catshark *Scyliorhinus canicula* (Linnaeus, 1758) (Carcharhiniformes: Scyliorhinidae) is a bottom-dwelling elasmobranch distributed throughout the Eastern Atlantic, from Norway to West Africa, and throughout the Mediterranean Sea (Compagno, 1984). *S. canicula* has a wide bathymetric range, reaching its maximum abundance at around 100 m depth, thus being the dominant shark species in the Mediterranean continental shelf (Follesa et al., 2019; Massutí and Moranta, 2003).

As one of the most common sharks in the Mediterranean, its biology and ecology are well known. This rather small shark lives primarily in sandy and muddy substrates and reproduces through oviparity, being able to lay eggs throughout the year thanks to the females' capacity to store sperm (Capapé et al., 2014; Metten, 1939; Sánchez, 1993). It is considered a generalist species and an opportunistic mesopredator, since it feeds on a variety of invertebrates and small fish (Ford, 1921; Lyle, 1983). This species has a key role in demersal fish communities, being a link in the trophic web between small teleosts and invertebrates and larger predatory fish (Storelli et al., 2005). According to the Food and Agriculture Organization of the United Nations, this species is extensively fished, with 7613 tons landed in 2019 across Europe (FAO, 2019). However, it has a relatively low commercial value in North-Western Mediterranean waters (Carbonell et al., 2003) and in the Catalan coast is one of the most discarded species (ICATMAR, 2020). Therefore, there is a raising interest from the fishing sector to promote its consumption in the area. Although some studies suggested a population decline of *S. canicula* in heavily exploited areas (Barausse et al., 2014; Capapé et al., 2014; Cardinale and Osio, 2013), it is currently assessed as Least Concern in the IUCN Red List and estimated to have an increased population trend in the Mediterranean Sea (Finucci et al., 2021).

Sharks are at the top of the marine food chain or very close to it, acting as final receptors of environmental pollution. These characteristics, along with the species availability, wide benthic distribution, critical role in local trophic webs, and small size, make *S. canicula* an excellent bioindicator for assessing the presence of various growing pollutants, such as plastic waste or heavy metals (Alves et al., 2022; Bellas et al., 2016; Fossi et al., 2018; Reinero et al., 2022).

In the last decades, the amount of litter ending up in the oceans has grown exponentially, with an estimation of 23 million tons of plastic waste that have already entered the oceans (Borrelle et al., 2020), and the Mediterranean Sea being considered one of the greatest accumulation areas for marine litter (UNEP/MAP, 2015). This situation has led to a growing concern among the scientific community regarding the exposure of marine species, and ultimately humans, to the ingestion of marine litter (Avio et al., 2017; Canals et al., 2021 and references therein). The small-spotted catshark could be more vulnerable to this type of pollution due to its benthic feeding behaviour, considering that marine sediments are the ultimate sink for synthetic and non-synthetic anthropogenic items (AIs) (Galgani et al., 1995, 1996, 2000; Pham et al., 2014). Although several adverse effects have been attributed to the ingestion of microplastics in experimental conditions (Limonta et al., 2019; Ye et al., 2021), these effects do not seem to be clear cut in the wild (Carreras-Colom et al., 2020; Mancia et al., 2020; Mancuso et al., 2022; Muns-Pujadas et al., 2023; Rodríguez-Romeu et al., 2020). Previous studies have already reported the presence of microplastics in the small-spotted catshark with substantial differences in abundance, occurrence, size and polymer composition (Mancuso et al., 2022 and references cited therein). This variability and the expected increase in the amount of plastic debris entering the oceans (Jambeck et al., 2015), highlights the importance of monitoring debris ingestion trends over time and space, and its potential health impacts in these populations.

In addition, parasites are ubiquitous components of all ecosystems that respond to environmental changes and are also used as indicators of the effects of marine pollution on ecosystems and hosts populations (MacKenzie, 1999; Vidal-Martínez et al., 2010). Furthermore, although parasites populations are usually in balance with their host in natural conditions, parasites can on occasions negatively affect their hosts health causing different pathologies or even their death (Lafferty, 2013). The composition and dynamics of the parasite assemblages infecting the small-spotted catshark have been studied in the Atlantic Ocean (Henderson and Dunne, 1998) and, more recently, in Mediterranean waters (Bakopoulos et al., 2018; Dallarés et al., 2017a; Gangemi et al., 2019), with substantial differences reported in the prevalence and abundance of different parasites. Noteworthy, none of these studies evaluated possible parasite infections in the muscle tissue of this species with the exception of Santoro et al. (2021), who found significant infections of encysted cestode larvae in specimens from the Tyrrhenian Sea. As *S. canicula* is a species for human consumption, evaluating the presence of zoonotic parasites or other parasites from the musculature (that can have implications for food safety and flesh quality) holds high relevance.

Trace metals are also potential pollutants that may affect this species. They have a strong affinity for particulate organic matter that tends to accumulate in bottom sediments (Zwolsman et al., 1993), increasing the exposure of benthic species to them (Cresson et al., 2014). Particularly, there is a concern regarding the “Mediterranean mercury anomaly”; a set of biochemical and ecological factors that makes the Mediterranean Sea an area of high methylation potential, thus making methylmercury (a highly toxic Hg species easily magnified) available in the food chain (Cossa and Coquery, 2005; Sandheinrich and Wiener, 2011).

The effects of these pollutants also adversely affect the health of marine organisms such as teleost fish, mollusks and marine mammals, potentially causing suppressed reproductive development, immunosuppression, endocrine disruption and oxidative stress, among others (Barone et al., 2018; Blocksom et al., 2010; Desforges et al., 2018; Genchi et al., 2017; Jepson et al., 2016; B. M. Sharma et al., 2014; Streit, 1998; Tanabe et al., 1983). Although sharks are known to uptake higher quantities of mercury and other metals than other fish species through bioaccumulation and biomagnification processes, few research studies have focused on the harmful effects of metals on sharks' health (Jeffree et al., 2006; Marques et al., 2021; Tiktak et al., 2020).

There is also an increasing concern on the implication of trace metals exposure through fish consumption to human health, particularly for vulnerable groups (such as pregnant women and young children) (Bose- O'Reilly et al., 2010; Karagas et al., 2012). This exposure has been linked to cancer, liver and kidney damage, immunosuppression, reproductive defects, endocrine disruption and nervous system damage, among others (Genchi et al., 2017; Grandjean and Herz, 2011; Park and Zheng, 2012; Vračko et al., 2007; Zheng et al., 2007). For these reasons, the European Commission established safety thresholds for the intake of sea animals in terms of Hg, Pb and Cd concentrations (EC, 1881/2006).

One of the most trustworthy and appropriate methods for evaluating potential biological responses to pollutants and other impacts is through histology techniques. These are of major importance in detecting pathologies or effects of environmental contamination exposure in cells, tissues, or organs (Au, 2004; Feist et al., 2004; Stentiford et al., 2003). Among the different organs usually screened, the liver is one of the most employed in histopathology due to its leading role in metabolism, biochemical transformations, detoxification processes and lipid and glycogen storage (Bernet et al., 1999; Costa, 2018).

Therefore, the present study aims to assess the relevance of pollutants and parasites affecting the health of *S. canicula* in the north-western Mediterranean Sea. To accomplish this: i) ingested anthropogenic items, including microplastics, levels of trace metals and the parasitic community were quantified and characterized, ii) their potential health impact on *S. canicula* populations was inferred through condition indices and liver histopathology evaluation, iii) an integrated analysis addressing the relationships among the different pollutants, parasites, fish biometrics and condition indices, bathymetry and locality was carried out, and iv) the potential implications of present findings for human consumption were discussed. Ultimately, present results are compared with available data from other areas of distribution of the species, and the observed patterns are discussed.

2.Materials and methods

2.1. Study area and sample collection

A total of 61 individuals of *S. canicula* were captured on board of commercial fishing trawlers during summer 2019 in the framework of the PLASMAR project (Spanish Ministry of Science, Innovation and Universities). Three different sites (off Blanes, off Barcelona and off Ebro Delta) were chosen along the Catalan coast (NW Mediterranean) at depths ranging between 60 and 470 m **(Fig. 1, Table 1)**. Captured fish were immediately fixed in 10 % buffered formalin and stored until further analyses. Ten additional fish were collected from the deepest site (off Blanes) and ten more from the shallowest site (off Barcelona) and preserved frozen at - 20 ◦C for trace metal analyses.

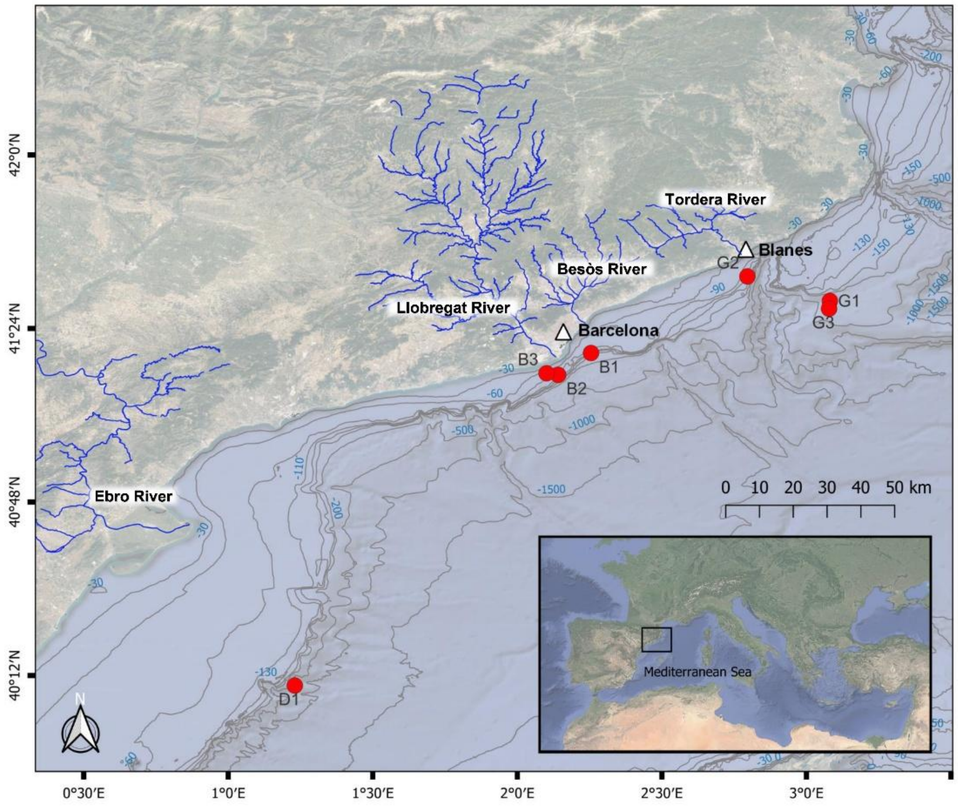


Figure 1. Map of the sampling area. Red dots indicate sampling sites and white triangles the nearby important cities. From north to south, Blanes (G1, G2 and G3), Barcelona (B1, B2 and B3) and Ebro Delta (D1).

Environmental variables such as temperature, salinity, oxygen concentration and turbidity were also recorded at 5 m above the seafloor in the same study locations using a CTD profiler (ASTD152-ALC) and are available as supplementary material **(Table S1)**.

Among the three sampling sites, that off Barcelona is the most impacted area, receiving the inputs of two important rivers, the Besós and the Llobregat (18 and 170 km long, respectively). It supports a population of 1,6 million people living in the nearby urban coastal area (Instituto Nacional de Estadística, 2023), one of the most important commercial and tourist ports of the Mediterranean coast and is further characterized by an important industrial activity (Garcia-Garin et al., 2019). The Ebro River (910 km long, 14,000 hm3/y), which receives inputs from industrial and agricultural activities, may also have an impact on Ebro Delta sampling site (Galimany et al., 2019). The sampling point near the city of Blanes is considered the least impacted, receiving the inputs of the rather small Tordera River (59 km long, Durán et al., 2014).

2.2. Dissection procedure

Once in the laboratory, all sharks (except those intended for metal analyses) were measured to the nearest mm (total length = TL) and weighed to the nearest g (total weight = TW). All relevant organs were removed (*i.e.*, liver, gonads, spleen, heart, kidney, and gills) and stored individually in 70 % ethanol. The stomach and spiral valve were stored in glass jars with filtered 70 % ethanol for further analyses. In addition, the weights of liver (LW), stomach (SW) and gonads (GW) were recorded using a precision scale to the nearest mg, as well as the eviscerated weight (EW), which was recorded to the nearest g. For the 20 specimens devoted to trace metal analyses, TL, TW, LW and sex were recorded and a portion of muscle and liver were obtained and frozen at - 20 ◦C until further treatment. These dissections were carried out without airborne contamination prevention methods since samples were not devoted to AIs analyses.

2.3. Anthropogenic items (AIs) extraction and characterization

The contents of stomach and spiral valve were carefully screened separately in order to assess the presence of AIs by visual inspection, under a binocular stereomicroscope at 5× to 40× magnification. Every AI detected was collected, mounted between glass slides in filtered distilled water, and labelled individually for further characterization. For this aim, images were obtained at 50× to 400× magnification, using a camera (Leica CTR5000) attached to a light microscope (Leica DM500 B). Measurements of total length and mean cross section (based on three random measures) were obtained using an image-processing software (ProgRes® CapturePro).

After microscopical observations, AIs were classified into different typologies according to their morphological features, such as general appearance, cross-section shape, patterns of the fibre's body, ends appearance, breakages and alterations of the fibre's body, birefringence, and colour (Robertson et al., 2017).

Polymer composition of fibres >5 mm was analysed by Fourier-Transformed Infrared Spectrometry (FTIR) to assure the correct characterization of AIs, using a Tensor 27 FTIR spectrometer (Bruker Optik GmbH, Germany) operating in Attenuated Total Reflectance (ATR) mode. Spectra was recorded as 16 scans in the spectral range of 600–4000 cm^{-1} (Servei d'Anàlisi Química, Autonomous University of Barcelona). In addition, doubtful AIs and a subsample of each typology for fibres <5 mm (a total of 23,4 % of fibres found), were analysed by micro-FTIR using a Thermo Scientific™ Nicolet™ iN10 MX Infrared Imaging Microscope, and spectra were recorded as four scans in the spectral range of 800–4000 cm^{-1} (CCitUB, University of Barcelona). Resulting spectra were treated (baseline corrections, peak normalization, and selection of characteristic band) with Spectragryph 1.2.11 (Menges, 2022) and compared with reference spectra (Primpke et al., 2018), applying a correlation matrix. Correlation values over 70 % were accepted for reliable identification. Those ranging between 60 and 70 % were also accepted when their spectra and fibre appearance matched visually with the reference (Muns-Pujadas et al., 2023).

2.4. Parasitological assessment

All individuals were inspected macroscopically for ectoparasites before dissection. Gills and internal organs (including stomach and spiral valve (after AIs screening), liver, spleen, gonads, heart, kidney and brain) were checked for ecto- and endoparasites using a stereomicroscope. Finally, the musculature between the pectoral and caudal fin was cut into thin slices and thoroughly inspected in search for possible encysted endoparasites.

All metazoan parasites found were counted, identified to the lowest possible taxonomic level, and stored in 70 % ethanol. Parasite identification was based on dichotomic keys and specialized bibliography (mainly the monographs Kabata, 1979; Moravec, 1994, 2001 and Palm, 2004). For an accurate identification of taxa, platyhelminth parasites were stained with iron acetocarmine, dehydrated through a graded ethanol series, cleared in clove oil and examined as permanent mounts in Canada balsam (Georgiev et al., 1986). Tentacles of trypanorhynch cestodes were examined as temporary mounts in pure glycerine for oncotaxis analysis. Nematodes were cleared in glycerine and also examined as temporary preparations.

2.5. Trace metals quantification

Muscle and liver portions from the 20 specimens devoted to trace metal analyses were freeze-dried, ground to a fine powder and homogenized. These portions were weighed before and after lyophilization to determine the percentage of water on each sample.

Digestion was carried out in 300 mg of homogenized sample using concentrated HNO_3 (PlasmaPURE, SCP Sciences) on Teflon reactors in a microwave digestion system (MARS6, CEM) using the procedure described in Besada et al. (2014). Because some results were near of the limit of quantification (LOQ) for some metals, analyses were repeated using a hotplate digestion method consisting of an addition of 3 mL of concentrated HNO_3 (PlasmaPURE, SCP Sciences) and 200 µL of 30 % H_2O_2 (Suprapur, Merck) per 100 mg of dried sample in subsequent steps and heating at 90 °C during 1 h (Sánchez-Marín et al., 2023). Since, for this method limit of detection (LOD) and limit of quantification (LOQ) were lower, all metals could be quantified in all samples. Results obtained by both digestion methods were very similar, so final results are given as the average of both methodological replicates when possible.

Quantification of nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) from the muscle and liver was performed using inductively coupled plasma mass spectrometry (Agilent 8900 ICP-MS) as described in Sánchez-Marín et al. (2023). Total mercury (Hg) was determined in the solid samples by pyrolysis atomic absorption spectrometry with gold amalgamation (employing an AMA254 Advanced Mercury Analyzer (LECO Instruments), as described in Belmonte et al. (2021). Obtained results are expressed in milligrams per kilogram of wet weight ($mg \cdot kg^{-1}$ ww). Detection limits were 0.003, 0.0005, 0.005, 0.0003, 0.0003, 0.0008 and 0.0008 µg/g dry weight (µg/g dw) for Ni, Cu, Zn, As, Cd, Pb and Hg, respectively. Hg content is reported as total mercury (THg), thus including methylmercury (MeHg), which makes up to 70–100 % of total mercury in elasmobranchs (Storelli et al., 2022; Tiktak et al., 2020 and references therein).

2.6. Liver histology

After the initial results, samples from fish containing the highest values of trace metals (*i.e.,* those off Blanes) were analysed in search of possible histopathological alterations. Thus, a portion of liver of those individuals was embedded in paraffin, sectioned at 4 µm and stained with Haematoxylin and Eosin.

All resulting histological sections were completely screened under a light microscope for the detection of histopathological alterations according Bernet et al. (1999) and Feist et al. (2004).

Liver samples were classified into three categories according to the quantity and size of lipid droplets: a) liver with low lipidic deposition **(Fig. 2A)**; b) liver with intermediate lipidic deposition **(Fig. 2B)**; and c) liver with high lipid deposition **(Fig. 2C)**. The presence of pigmented macrophages in liver was also assessed through a semiquantitative analysis. For this purpose, three fields of view were randomly selected from each section at 200× magnification and examined microscopically.

Moreover, when alterations were detected, a morphological evaluation of each of them was performed, and their prevalence (*i.e.,* percentage of fish affected by a specific alteration) was calculated.

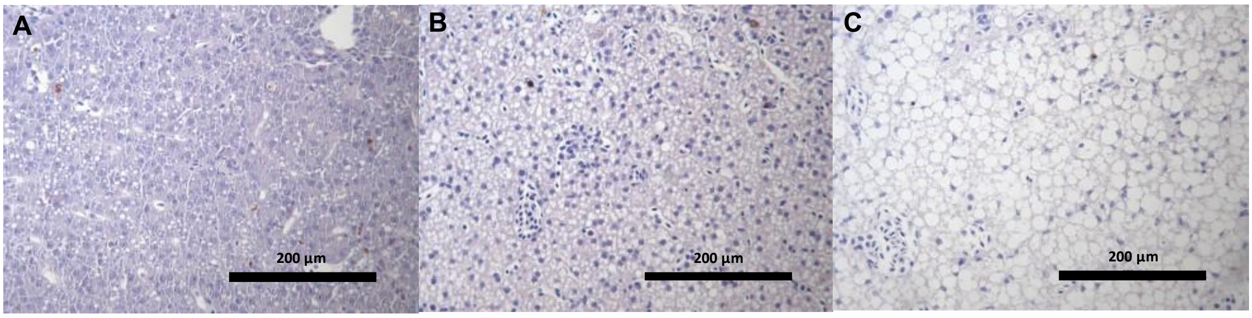


Figure 2. Categories of liver lipid deposition of *Scyliorhinus canicula* (20×). A) Low lipidic deposition, B) Medium lipidic deposition, C) High lipidic deposition.

2.7. Quality assurance/quality control (QA/QC)

To prevent airborne contamination that may bias results of anthropogenic items, all dissections were carried out in a laminar flow cabinet. All used tools were rinsed twice with filtered distilled water, and nitrile gloves and cotton lab coat were always worn. Distilled water and 70 % ethanol used during dissections were previously filtered, using a metal sieve with a 50 µm mesh size.

Moreover, AI's visual inspection was done under an isolation device adapted from that proposed by Torre et al. (2016). All laboratory material was rinsed twice with filtered water before use. Procedural controls, consisting of Petri dishes containing a few ml of filtered distilled water, were placed inside and outside the isolation device during screenings to assess possible background airborne contamination. As in previous studies (Carreras-Colom et al., 2018, 2020; Muns-Pujadas et al., 2023; Rodríguez-Romeu et al., 2020, 2022), contamination found in the inside controls was much lower than in outside controls, and all fibres found in both types of controls were clean and on the water surface (indicating deposition from the air). Therefore, fibres displaying these same characteristics were excluded from gut contents analyses and no correction factor was applied to the final values of AIs found.

In the case of trace metal analyses, results obtained were validated using procedural blanks and certified reference material DORM-2 (dogfish muscle from the National Research Council of Canada). Recovery of metal concentrations in certified material ranged from 88 to 114 % for all metals analysed. Quality assurance is also endorsed by participation in QUASIMEME (Quality Assurance of Information in Marine Environmental Monitoring in Europe) with satisfactory *Z*-scores for each metal reported.

2.8. Data analysis

Basic calculations were performed as follows: fish general condition was assessed through the gonadosomatic index (GSI = (GW/EW) x 100), the hepatosomatic index (HSI = (LW/EW) × 100) and Le Cren's relative body condition index ($Kn = EW / (\alpha \times TL\beta)$), where α and β are the slope and the intercept of the weight-length relationship, representing the entire dataset of sampled fish (Le Cren, 1951). Feeding intensity was estimated through the stomach fullness index (FULL = (CW/EW) × 100), using the total stomach content weight (CW), which was recorded after AIs screening.

Prevalence of AIs (% AIs; percentage of fish containing AIs with respect to the total number of fish analysed), mean abundance (nAI = number of AIs/total number of individuals), mean intensity (IAI = number of AIs/individuals with ingested AIs), mean fibre load (TLAI = sum of the lengths of the fibres ingested/total number of individuals) and percentage of AIs found in the spiral valve (with respect to the total number of Ais ingested) were calculated for each locality. Fibre lengths were classified into four size clusters by partitioning around medoids (PAM) applying the k-medoids algorithm on a matrix of dissimilarity, using the Manhattan distances to calculate dissimilarities between observations.

Parasite prevalence (P), mean abundance (MA) (with the 95 % confidence interval) and species richness (S) were calculated following Bush et al. (1997) for each locality. Moreover, infracommunity parasite descriptors such as mean species richness (MSR), total mean abundance (TMA), Berger-Parker's dominance index (BPdom; Berger and Parker, 1970) and mean diversity index (H) were also obtained. The mean diversity index was estimated by Brillouin's index and calculated with PRIMER 6 software (Anderson et al., 2008). Parasite taxa with a prevalence >10 % in at least one locality were considered non-accidental and are henceforth called common.

After carrying out these basic calculations, the following statistical tests and analyses were performed. Fish biometric data (TW and TL), condition indices (HSI, Kn and FULL), AIs ingested (nAI, AIs from the stomach and AIs from the spiral valve) as well as variables related to parasites and trace metals were tested for normality and homoscedasticity using the Shapiro-Wilk test and Levene's test, respectively. GSI was tested separately by gender. Data distribution was also plotted for visual assessment. When necessary, variables were log or square root transformed to comply with normality and homoscedasticity requirements. There was a single individual from Ebro Delta that had ingested 59 fibres and was excluded from some statistical analysis.

Differences among localities on biometric data, condition indices, abundance of AIs ingestion, AIs ingestion between organs and parasitological data were tested using ANOVA, Wilcoxon or Kruskal-Wallis tests, for parametric and non-parametric data, or with Generalized Linear Models when interactions were detected. Post-hoc pairwise comparisons were carried out using TukeyHSD and Dunn's multiple comparison tests. Pearson's Chi-squared Test was used to test differences of prevalence of AIs ingestion, fibre size and polymer composition among localities. When significant differences were detected, pairwise tests were performed to identify the differences between categories with the function "pairwiseNominalIndependence". Differences from samples related to trace metal concentrations among sampling areas (only Blanes and Barcelona) were tested with *t*-test or Wilcoxon test, when normality was not satisfied.

In order to detect any potential associations among biometric and condition indices, depth where the fish were caught, AIs related factors, parasite related variables and lipid deposition and macrophage abundance, correlations were explored with Spearman's or Pearson's correlation test (when normality was not satisfied). For those variables obtained from the three localities, a correlation matrix was built using the "corrplot" R package (Wei and Simko, 2017). Possible correlations with TL and LW of individuals devoted to trace metal analyses were also tested with Spearman's or Pearson's correlation tests.

Finally, some multifactorial analyses were done. The ordination of parasite infracommunities according to sampling site was visualized with a non-metric multidimensional scaling (nMDS) based on a Bray- Curtis dissimilarity matrix calculated from log-transformed species abundance data. Furthermore, a permutation analysis of variance (PERMANOVA) (Adonis2 function) and subsequent pairwise tests was performed under unrestricted permutation of raw data and 999 permutations to test for significant differences among parasite assemblages from the three sampling sites, and the Indicator Value Index (IndVal) (Dufrêne and Legendre, 1997) was used to determine which species were more representative of each assemblage.

Lastly, different variables and the locality were structured into quantitative and qualitative group sets and assessed by a Multiple Factor Analysis (MFA). For this purpose, we used the variables obtained for individuals of the three sampling areas, those being biometric and condition indices, depth where the fish were caught, AIs related variables and parasite related variables. This multivariate analysis allows to visualize and differentiate groups of samples according to the different factors and its discriminating importance (Escofier and Pagès, 1994).

All data analyses were performed with R version 4.2.3. Correlations were considered significant when the coefficient (R) was higher than 0.65. Statistical significance was set at 0.05.

3. Results

3.1. Biometric data and condition indices

Biometric data and condition indices of specimens caught in the three different localities are shown on **Table 1**. Total length of the examined sharks ranged from 26.8 to 45.2 cm. Significant differences among locations were only observed for TL and the fullness index (ANOVA, F = 5.82 and 7.04, p = 0.005 & 0.002), both being lower in Blanes.

Table 1. Biometric data, condition indices and anthropogenic items (AIs) Ingestion data for *Scyliorhinus canicula* on each of the three sampling sites and total mean values for the Catalan Coast. Mean values and standard deviation (SD, in brackets) for total length (TL), hepatosomatic index (HSI), gonadosomatic index (GSI), stomach fullness (Fullness), Le Cren relative condition index (Kn), AIs mean abundance and intensity and sum of total AIs lengths (TLAI). Significant differences among localities are expressed by different superscript letters. "*" values calculated without an outlier.

Locality	**Blanes**	**Barcelona**	**Ebro Delta**	**Catalan Coast**
Depth (m)	111 - 473	61 - 88	229	**61 – 473**
n	19	22	20	**61**
Biometric data and condition indices				
TL (cm)	33.91 (5.05) ^{a}	37.88 (3.33) ^{b}	36.85 (2.84) ^{b}	**36.30 (4.11)**
HSI	6.17 (2.43)	6.91 (1.60)	7.35 (2.23)	**6.82 (2.11)**
GSI Males	2.17 (2.24)	3.92 (1.55)	3.38 (1.63)	**3.26 (1.86)**
GSI Females	4.14 (7.20)	8.71 (7.92)	4.91 (4.99)	**5.92 (6.93)**
Fullness	1.95 (1.45) ^{a}	5.12 (4.47) ^{b}	3.42 (1.79) ^{b}	**3.58 (3.26)**
Kn	1.01 (0.07)	1.03 (0.07)	0.98 (0.11)	**1.01 (0.09)**
AIs Ingestion data				
Mean abundance (n/ind)*	2.00 (1.90) ^{a}	5.68 (4.08) ^{b}	7.21 (5.83) ^{b}	**5.00 (4.69)**
Mean intensity (n/ind)*	2.92 (1.61) ^{a}	6.25 (3.82) ^{b}	7.21 (5.83) ^{b}	**5.77 (4.58)**
TLAI (mm/ind)*	7.74 (11.81) ^{a}	38.40 (42.95) ^{b}	20.97 (21.15) ^{ab}	**23.17 (31.63)**
Prevalence (%)	68.42 ^{a}	90.90 ^{ab}	100.00 ^{b}	**86.89**
AIs from the Spiral valve (%)	68.42	71.20	72.96	**71.87**

3.2. AIs ingestion and characterization

A total of 359 AIs were found in the digestive tracts of all examined sharks. All items found were fibres with neither fragments nor films. Mean values for AIs ingestion variables for each sampling area are shown in **Table 1**. The abundance of AIs was significantly higher in the spiral valve (where 72 % of all fibres were found) than in stomach (Wilcoxon test, W = 1236.5, $p < 0.001$), but no differences were found in terms of prevalence or size among organs.

Regarding the geographical comparison, a significantly lower prevalence was found in fish off Blanes compared to samples off Ebro Delta (Chi-square, $\chi^2 = 9.02$, p = 0.01). Fish off Blanes also presented the lowest abundance and intensity of AIs ingestion (ANOVA, F = 9.88 and 4.38, $p < 0.001$). Finally, TLAI (mm of fibre ingested per individual) was also lower in fish off Blanes compared to those of the other localities (K–W, $\chi^2 = 13.03$, p = 0.001) **(Table 1)**.

Fibres were classified into four different size classes according to the portioning around medoids algorithm used: small (< 3.5 mm), medium (3.5–13 mm), large (13–50 mm) and extra-large (> 50 mm). The predominant size class was small (76.82 %), followed by medium (17.60 %), and less abundant size categories were large (5.03 %) and extra-large (0.56 %) **(Fig. 3A)**. Fish sampled off Barcelona had ingested significantly larger fibres than those off Ebro Delta (Chi-square, χ^2 = 28.72, $p < 0.001$; **Fig. 3A**).

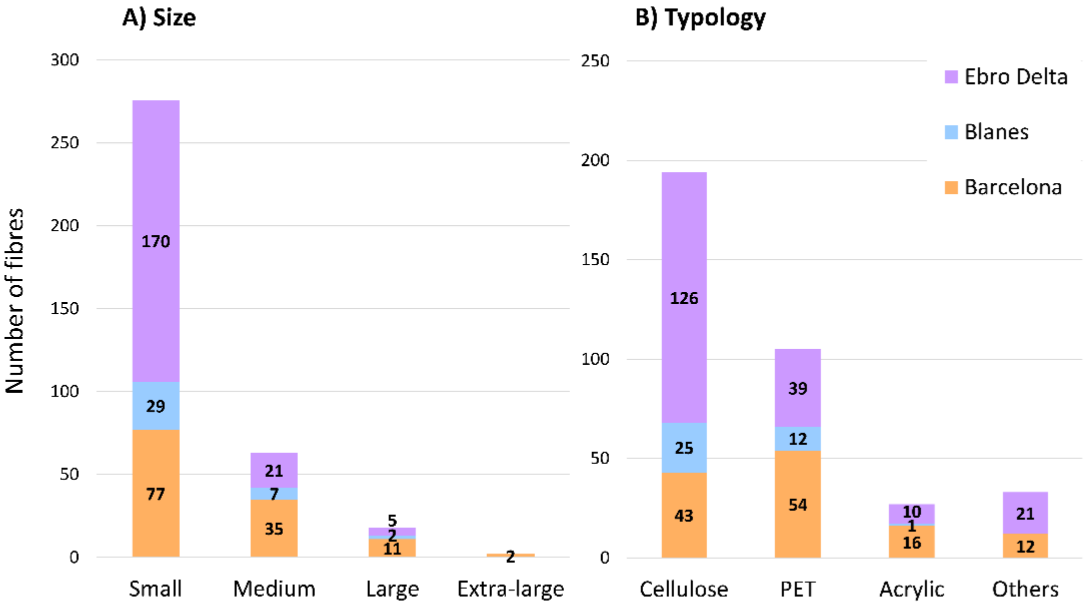


Figure 3. Differences in Size (A) and Typology (polymeric composition) (B) of fibres found in the digestive tracts of *Scyliorhinus canicula* captured off three localities of the Catalan Coast. The category "Others" includes unknown composition, PP (polypropylene), PA (polyamide) and wool.

Visual and spectroscopic AIs identification resulted in six different categories of fibres. The most predominant typology found was cellulose (54.04 % of the total), followed by polyethylene terephthalate (PET, 29.25 %), polyacrylonitrile (acrylic, 7.52 %), polyamide (PA, 2.23 %), polypropylene (PP, 0.56 %) and dyed wool (0.28 %) **(Figs. 3 and 4)**. The low quality of the spectra obtained of the remaining 22 fibres (6.13 % of the total) did not allow a reliable polymeric identification. Therefore, those fibres were classified as unknown.

Composition of fibres found in specimens from off Barcelona was also different compared to that of fibres from specimens from off Blanes and Ebro Delta (Chi-square, $\chi^2 = 51.03$, $p < 0.001$), with synthetic fibres being predominant in Barcelona, in contrast to the other two areas, where cellulose was the dominant typology **(Fig. 3B)**. Ingested fibres made from cellulose were significantly shorter than those made from PET and acrylic (K–W, $\chi^2 = 40.84$, $p < 0.001$).

Some AIs factors were positively correlated among them ($R_s > 0.65$). Total abundance of AIs was correlated with abundance of synthetic AIs, sum of fibres length and AIs abundance in the spiral valve ($R_s = 0.78$, 0.80 and 0.89, $p < 0.05$). The AIs abundance in the spiral valve was also correlated with abundance of synthetic AIs and sum of fibres length ($R_s = 0.66$ and 0.70, $p < 0.05$). No other correlations with the other variables explored were found ($R_s < 0.65$ or $p > 0.05$) **(Fig. S1)**.

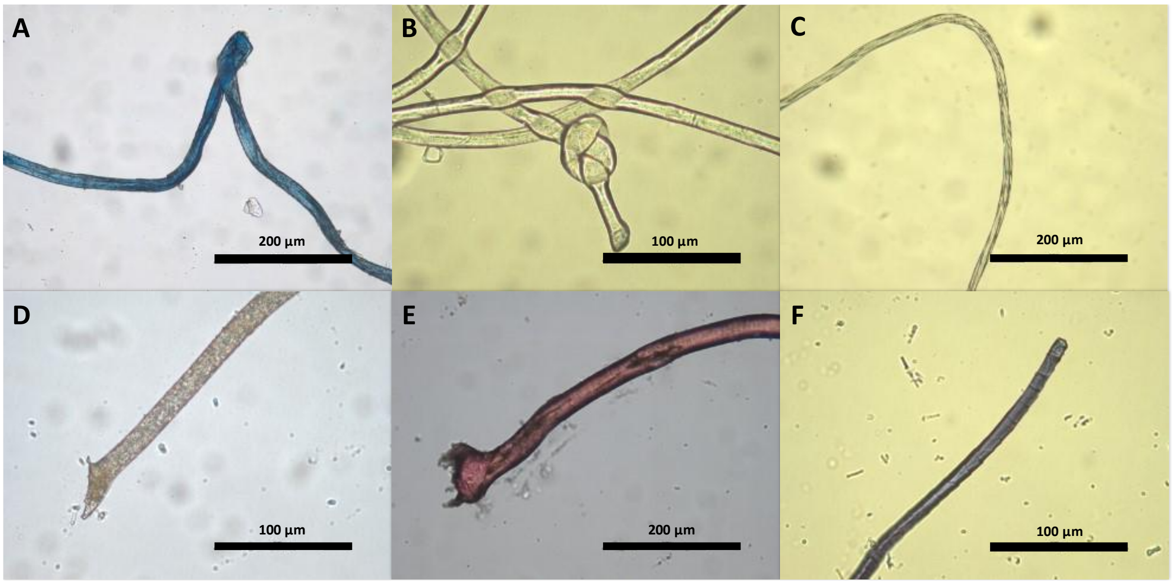


Figure 4. Representative images of the six different polymeric categories of fibres found in the digestive tracts of *Scyliorhinus canicula.* A) Cellulose, B) PET (polyethylene terephthalate), C) Acrylic (polyacrylonitrile), D) PA (polyamide), E) PP (polypropylene), F) Wool.

3.3. Parasitological assessment

All fish were infected by at least one parasite. A total of 3366 parasites belonging to 12 different taxa were identified: five nematodes, one monogenean, two cestodes, one copepod and three isopods. Of these taxa, five are reported for the first time in *S. canicula* **(Table 2)**. Five taxa were frequently found (prevalence >10 % at least in one locality) and thus considered as "common": the nematodes *Proleptus obtusus* (Dujardin, 1845) and *Piscicapillaria baylisi* (Moravec, 1987), the monogenean *Hexabothrium appendiculatum* (Kuhn, 1829), the copepod *Lernaeopoda galei* (Krøyer, 1837) and the larval cestode *Grillotia adenoplusia* (Pinter, 1903). None of these common parasites were zoonotic.

Total mean abundance was 55.18 parasites/fish, but sharks from off Delta displayed significantly lower abundances than those of the other two localities (ANOVA, F = 22.09, p < 0.001) **(Table 2)**. Total mean parasite richness was 2.36 with no significant differences detected among localities. Brillouin's diversity and Berger-parker's dominance indices also showed differences among localities, with fish collected off Barcelona displaying lower parasite diversity and higher dominance (K–W, χ^2 = 7.77 and 9.50, p = 0.02 and 0.009, respectively). Only one significant difference was found between sexes, with males having a higher abundance of *L. galei* (Wilcoxon test, W = 315, p = 0.003).

The most representative parasite in sharks from off Blanes and Barcelona was *P. obtusus*, whereas in those from off Ebro Delta was *G. adenoplusia*, in the larval stage, encysted in the tail musculature (Indicator Value Index = 0.41, 0.48 and 0.47, respectively). The correlation matrix revealed no clear relationships among fish condition indices, parasite abundances, parasitological descriptors and AIs related variables (All $R_s < 0.65$ or $p > 0.05$). The only correlation found was between the Berger-Parker's dominance index (B Pdom) and the mean diversity index (H) (Rs = - 0.98, p < 0.001), as expected, since a higher dominance of a specific parasite implies a lower diversity **(Fig. S1)**.

Multivariate analyses revealed significant differences among localities for the composition and structure of the parasite community (PERMANOVA, pseudo-F = 48.91, p = 0.001) with sharks off Ebro Delta displaying the highest variability and being more differentiated than those off Blanes (pseudo-F = 90.48, p = 0.001) and Barcelona (pseudo-F = 90.48, p = 0.001), that were in turn more similar (pseudo-F = 4.30, p = 0.006). This could be clearly observed in the non-metric multidimensional scaling (nMDS) ordination plot **(Fig. S2)**. The Bray-Curtis similarity index ranged between 41 % and 77 % among areas and between 69 % and 81 % within areas.

Table 2. Prevalence (P%), mean abundance (MA) with 95% confidence interval (in brackets) of the parasite taxa recovered from *Scyliorhinus canicula* captured on three localities off the Catalan coast, as well as Total Richness, Mean Species Richness, Total Mean Abundance, Brillouin Diversity Index and Berger-Parker Dominance Index. Abbreviations for sites of infection within hosts: B, buccal cavity; C, cloaca; G, gills; I, intestine (spiral valve); M, muscle; S, stomach. Different subscript letters indicate significant differences ($p < 0.05$) among localities. "*" Indicates new host record.

Parasite taxa		Site of infection	**Blanes**		**Barcelona**		**Ebro Delta**	
			P%	MA (95% CI)	P%	MA (95% CI)	P%	MA (95% CI)
NEMATODA								
	Anisakis type I	S	0	0	0	0	5	0.05
	Hysterothylacium fabri *	I	0	0	0	0	5	0.05
	Piscicapillaria baylisi	I	5.26	0.05	9.09	0.09	30	0.45 (0.06 - 0.84)
	Proleptus obtusus	I, S	100 ^{a}	46.58 (33.18 - 59.97) ^{a}	100 ^{a}	81.68 (59.64 - 103.72) ^{a}	55 ^{b}	2.30 (0.98 - 3.62) ^{b}
	Nematode larvae	I, S	5.26	0.05	9.09	0.09	0	0
PLATYHELMINTHES								
Monogenea	*Hexabothrium appendiculatum*	G	5.26	0.05	13.63	0.18	5	0.05
Cestoda	*Grillotia adenoplusia* *	M	94.74	8.16 (5.30 - 11.01) ^{a}	90.91	2.95 (2.13 - 3.78) ^{b}	100	18.80 (12.17 - 25.43) ^{c}
	Nybelinia lingualis	I	0	0	4.55	0.05	0	0
ARTHROPODA								
Copepoda	*Lernaeopoda galei*	C	26.32	0.42 (0.02 - 0.82)	13.63	0.18	15	0.15
Isopoda	Cymothoidae gen. sp. *	S	0	0	0	0	5	0.05
	Gnathia sp. *	B	0	0	4.55	0.05	0	0
	Rocinela sp. *	S	0	0	4.55	0.05	0	0
Total Richness			6		9		8	
Mean Species Richness			2.37 (2.04 - 2.70)		2.50 (2.14 - 2.86)		2.20 (1.81 - 2.59)	
Total Mean Abundance			55.32 (39.89 - 70.74) ^{a}		85.32 (63.35 - 107.29) ^{a}		21.90 (15.49 - 28.31) ^{b}	
Brillouin Diversity Index			0.38 (0.29 - 0.47) ^{a}		0.20 (0.13 - 0.26) ^{b}		0.34 (0.22 - 0.46) ^{ab}	
Berger-Parker Dominance Index			0.84 (0.79 – 0.89) ^{a}		0.94 (0.91 - 0.96) ^{b}		0.83 (0.76 - 0.90) ^{ab}	

3.4. Trace metals

Biometric data of sharks devoted to trace metals analyses and concentrations of these elements in muscle and liver are shown in **Table 3**. Concentrations of Ni, Cu and Pb were significantly higher in the liver (Wilcoxon test, W = 104, 0 and 9,5, $p < 0.01$). Concentration of Cd was also significantly higher in the liver whereas Zn concentration was higher in the muscle (T-test, *t* = -8,36 and 2.55, $p < 0.015$), and Hg concentrations were similar in both tissues. None of the detected concentrations were above the maximum permitted level in fish muscle by the European Union Commission Regulation (EC) 1881/2006 (EC, 2006) except for Hg, which even doubled the maximum allowed value in half of the individuals. Mercury values were higher in individuals caught off Blanes than in those from off Barcelona (t-test, *t* = - 2.63, p = 0.017). On the contrary, muscle concentrations of zinc and arsenic were significantly higher in fish off Barcelona (*t*-test, *t* = 3.38 and 2.44, p = 0.003 and 0.025, respectively). In the case of liver concentrations, significantly higher values of nickel and zinc were found in fish off Blanes (t-test, *t* = - 2.39 and - 4.22, *p* = 0.028 and 0.001, respectively).

Table 3. Biometric data of *Scyliorhinus canicula* specimens captured off two localities of the Catalan coast and devoted to metallic elements analyses and mean values (followed by standard deviation) of metallic element concentrations in muscle and liver tissues. Metallic elements analysed are nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg). All values are given in $\mu g \cdot g^{-1}$ wet weight. TL = Total length. Significant differences among localities are expressed by different superscript letters.

Locality	Maximum permitted level (EC, 1881/2006)	Blanes	Barcelona	**Catalan Coast**
Depth (m)		355	60	**60 - 355**
n		10	10	**20**
TL (cm)		41.79 (1.57) ^{a}	44.45 (2.14) ^{b}	**43.12 (2.28)**
Ni		0.009 (0.005)	0.007 (0.007)	**0.008 (0.060)**
Cu		0.689 (0.186)	0.633 (0.115)	**0.661 (0.153)**
Zn		12.419 (4.195) ^{a}	18.516 (3.868) ^{b}	**15.468 (5.020)**
As		21.625 (5.576) ^{a}	29.104 (8.217) ^{b}	**25.364 (7.838)**
Cd	0.05	0.002 (0.001)	0.002 (0,001)	**0.002 (0.001)**
Pb	0.3	0.009 (0.005)	0.008 (0.004)	**0.008 (0.005)**
Hg	1	2.533 (0.785) ^{a}	1.700 (0.621) ^{b}	**2.116 (0.811)**
Liver Ni		0.014 (0.004) ^{a}	0.009 (0.006) ^{b}	**0.012 (0.005)**
Liver Cu		4.426 (3.174)	2.788 (2.122)	**3.067 (2.402)**
Liver Zn		14.356 (3.023) ^{a}	9.594 (3.484) ^{b}	**11.975 (3.509)**
Liver As		-	-	**-**
Liver Cd		0.518 (0.252)	0.395 (0.272)	**0.457 (0.243)**
Liver Pb		0.045 (0.020)	0.035 (0.020)	**0.040 (0.020)**
Liver Hg		2.723 (1.512)	1.746 (1.119)	**2.260 (1.397)**

Levels of zinc in muscle samples were positively correlated with fish TL (Rp = 0.74, $p < 0.001$), while zinc liver concentrations were higher in smaller fish (Rs = - 0.67, p = 0.001). Moreover, nickel and zinc concentrations in liver were negatively correlated with liver weight (Rs = - 0.82 and - 0.87, $p < 0.001$). No other metal concentrations were correlated with total length or liver weight.

3.5. Multifactorial analysis

The MFA explained 35.18 % of the variability of the data in the first two axes, with the first axis explaining 21.1 % and the second axis 14.1 % of the total variability **(Fig. 5)**.

The most contributing variables to the first dimension were depth (31.08 % of explained variance), fish length (12.21 %) and fullness index (9.40 %). In the case of parasite abundances, the most contributing was *P. obtusus* (10.68 %). Parasitological indices and AIs related variables were less important in explaining the variability of the data in the first axis. As for the second dimension, the most contributing variables were the abundance of the nematodes *P. baylisi* and, again, *P. obtusus* (12.82 % and 9.79 %, respectively), followed by the AIs total abundance (9.37 %), total length (9.19 %) and hepatosomatic index (8.61 %). Finally, in this second dimension, parasitological indices contributed more than in the first dimension (H = 7.96 % and BPdom = 8.25 %) **(Fig. 5A)**.

A differentiation of individual samples was observed according to the sampling location, being sharks off Barcelona differentiated from those off the other two locations along the first axis, mostly on the basis of a shallower depth of catch, higher fish condition indices (TL, Fullness) and *P. obtusus* higher abundance. Sharks off Blanes were mainly differentiated from those off Ebro Delta along the second axis; mainly due to lower *P. baylisi*, higher *P. obtusus* abundance and lower AIs ingestion rates **(Fig. 5B)**.

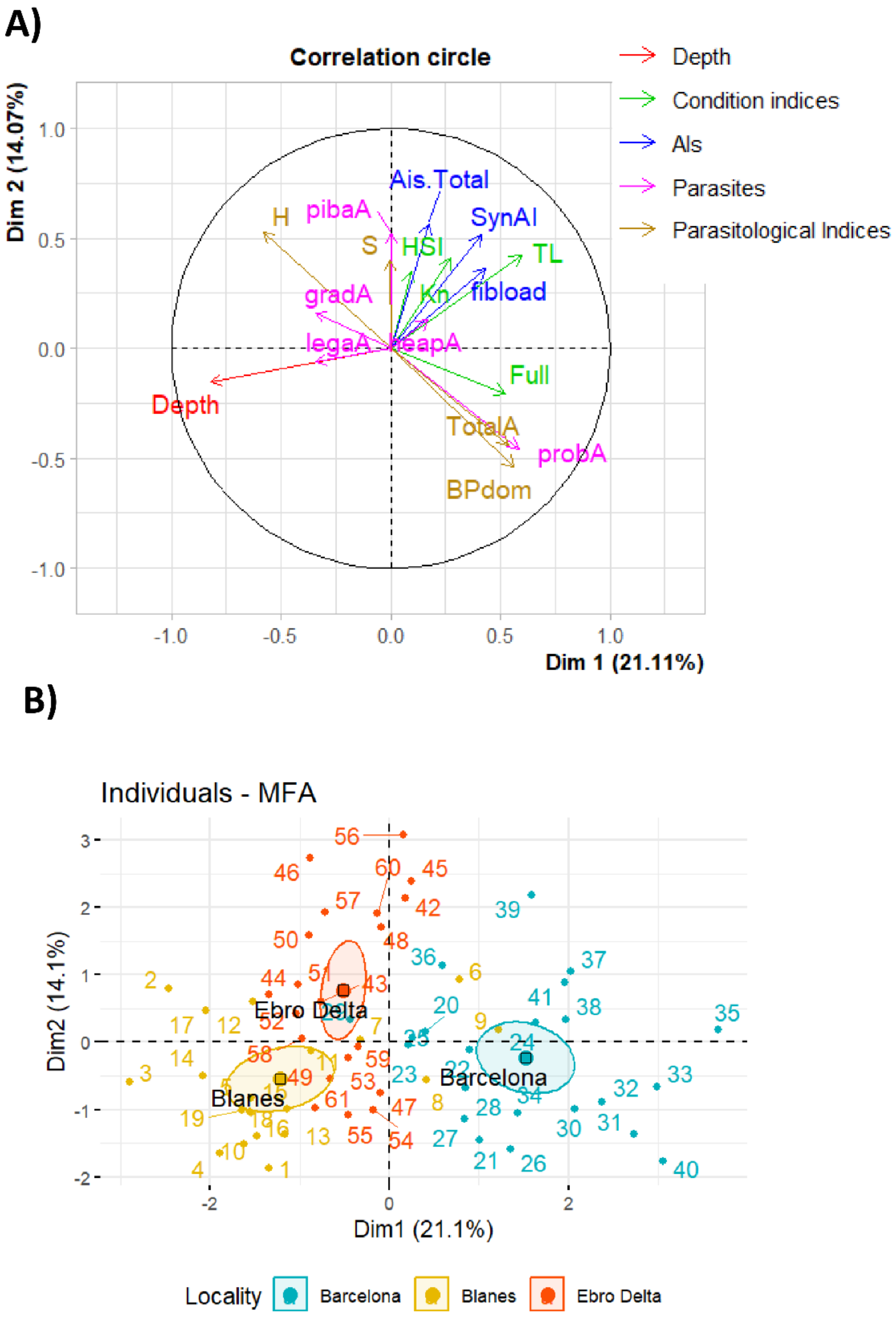


Figure 5. Multiple factor analysis (MFA) among samples of *Scyliorhinus canicula* caught off three different areas of the Catalan coast. A) MFA among body condition indices (total length (TL); Le Cren relative condition index (Kn); stomach fullness (Fullness); hepatosomatic index (HSI)), abundance of anthropogenic items (AIs, (total anthropogenic items (Ais.Total), sum of the length of anthropogenic items (fibload), and abundance of synthetic items (SynAI)), main parasite abundances (*Proleptus obtusus* (probA), *Piscicapillaria baylisi* (pibaA), *Hexabothrium appendiculatum* (heapA), *Lernaeopoda galei* (legaA) and *Grillotia adenoplusia* (gradA)) and parasitological descriptors (total mean abundance (TotalA), species richness (S), Brillouin Diversity Index (H) and Berger-Parker Dominance Index (BPdom)). B) Factor map of the MFA, individuals are represented by dots and locations by colours.

3.6. Liver histology

No major histopathological alterations such as haemorrhagic lesions or degenerative tumours were found. Hepatic alterations linked to parasitic presence were also absent, in accordance with the lack of parasites found in this organ.

Livers presented different degrees of lipid deposition in the cytoplasm of the hepatocytes, being generally homogenous along the parenchyma. The characterization of hepatic structure according to the quantity and size of lipid droplets resulted in 21 % of individuals presenting low, 37 % intermediate and 42 % high lipidic deposition.

Pigmented macrophages (containing intracytoplasmic pigments such as melanin) were found in all livers among the hepatocytes although with variable densities, ranging between 1.67 and 28.33 macrophages per field (mean number of macrophages/field = 7.58). Small inflammatory foci mainly composed by mononuclear cells, macrophages and lymphocytes were found in all livers, usually associated to blood vessels **(Fig. 6A)**. In two mature females (P = 15 %), an abnormal presence of eosinophilic granular cells, compared to other samples, were found associated to inflammatory foci and within blood vessels, **(Fig. 6B)**. A large inflammatory focus of unknown aetiology was detected just in one individual **(Fig. 6C)**.

In the case of females, immature individuals presented lower abundance of pigmented macrophages compared to mature individuals (K–W, $\chi^2 = 6.09$, $p = 0.048$). No significant correlations between pigmented macrophage abundance and lipid deposition with other variables (related to parasites, AI's ingestion or condition indices) were found (All $R_s < 0.65$).

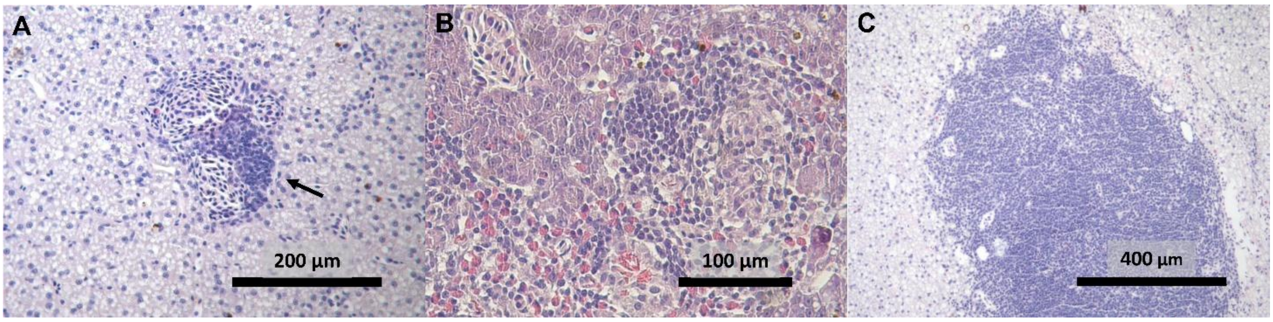


Figure 6. Liver histological results of *Scyliorhinus canicula* captured off the Catalan coast. A) Inflammatory foci associated to a blood vessel (arrow) B) High presence of eosinophilic granular cells within a blood vessel C) Large inflammatory focus.

4. Discussion

The present study provides important insights on threats affecting small-spotted catshark populations from the Balearic Sea, such as anthropogenic items (including microplastics), trace metals and parasites, and evaluates their implications for the species health and human food safety.

Furthermore, it assesses possible local variations, both geographically and bathymetrically. The small-scale geographical differentiation observed in the present study (comprised within a distance range of approximately 215 km), is in accordance with mark–recapture studies that suggest adults do not generally make long migrations (Rodríguez-Cabello et al., 2004) and that in fact display a philopatric behaviour, especially females (Gubili et al., 2014; Sims et al., 2001). Hence, the sedentary pattern observed by this species together with the geographical variability of the results obtained in terms of parasites, fibre ingestion and trace metal accumulation, highlights the importance of monitoring and assessing the health status and the impacts of different pollutants at a small-scale.

4.1. AIs ingestion and characterization

The present study is the first reporting AIs ingestion for the small-spotted catshark in the Balearic Sea. It further reveals the highest AIs prevalence and abundance values in this fish species to date **(Table 4)**.

However, it must be considered that comparisons among studies on this subject can be complex due to the different methodological approaches followed by different researchers. For example, studies using digestion methods for AIs isolation may be neglecting cellulosic fibres that can disintegrate in most cases (Dehaut et al., 2016). Some others only consider stomach contents, thus underestimating the abundance of ingestion (Bellas et al., 2016; Neves et al., 2015). In the present study, not only plastic or synthetic fibres were analysed, but also those non-synthetic like cellulose, which is the most common fibre-type found on sediments (Avio et al., 2020; Sanchez-Vidal et al., 2018; Suaria et al., 2020) and also has an anthropogenic origin. By including cellulosic fibres in present analyses, overall levels of AIs can be higher than those previously reported in *S. canicula* by other authors. When cellulose fibres, which constitute 54 % of all AIs identified, are removed from present results in order to facilitate the comparison with other studies **(see Table 4)**, the resulting ingestion levels (2.43 AIs/ind; 72.13 % of occurrence) are in accordance with other Mediterranean studies that used a digestion protocol like Valente et al. (2019) in the Tyrrhenian Sea (2.5 AIs/ind; 66.7 %) or Mancuso et al. (2022) in SW Sicily (2.4

AIs/ind; 80.3 %) **(Table 4)**. This supports the notion that studies applying digestion protocols are likely underestimating the real ingestion rates of AIs by marine biota.

Table 4 Comparative table on micro-litter ingestion by small-spotted catshark (*Scyliorhinus canicula*) in the Atlantic Ocean and Mediterranean Sea. In brackets, standard deviation. Syn = Results only considering synthetic fibres to easily compare with studies based on digestion analyses. N = number of analysed fish. In bold, mean values for the global area (Catalan Coast).

Sampling area	N	Abundance	Prevalence	Sampling procedure	Reference
Atlantic Ocean					
Portuguese coast	20	0.3	20%	Visual inspection	Neves et al., 2015
Galician coast	24	1	4.2%	NaOH digestion	Bellas et al., 2016
Cantabrian coast	24	1.2 (0.45)	20.8%		
Gulf of Cádiz	24	1.2 (0.45)	20.8%		
North Sea	20	0.3	15%	Visual inspection	Smith, 2018
North-East Atlantic and Celtic Sea	12	1.42	66.6%	KOH digestion	Parton et al., 2020
South-West coast of the UK	200	0.14	6.5%	Visual inspection	Morgan et al., 2021
Mediterranean Sea					
Tyrrhenian Sea	30	2.50 (0.52)	66.7%	KOH digestion	Valente et al., 2019
Tyrrhenian Sea (Gulf of Patti)	12	1.1	33.3%	Visual inspection	Capillo et al., 2020
Mazara del Vallo (SW Sicily)	25	1.56	86.3%	KOH digestion	Mancia et al., 2020
Lampedusa	25	1.24	75.7%		
Tyrrhenian Sea (Gulf of Patti)	27	0.33	22.2%	Visual inspection	Pedà et al., 2020
SW Sicily	61	2.40	80.3%	KOH digestion	Mancuso et al., 2022
Blanes	19	2 (1.9) Syn = 0.68 (0.89)	68.42% Syn = 47.37%	Visual inspection	Present study
Barcelona	20	5.7 (4.1) Syn = 3.68 (3.06)	90.91% Syn = 86.36%		
Ebro Delta	22	7.2 (5.8) Syn = 2.74 (2.62)	100% Syn = 80%		
Balearic Sea	**61**	**5 (4.7)** Syn = 2.43 (2.70)	**86.89%** **Syn = 72.13%**		

Still, *S. canicula* from the present study area, together with those of other Mediterranean regions, are generally characterised by higher microplastic abundances compared to conspecifics from other nearby areas such as the NW Atlantic coast (*e.g.*, off Spain, Portugal or United Kingdom; **Table 4**). The Mediterranean Sea, and especially its western basin, seems to be an area of plastic accumulation (Pham et al., 2014; Sharma et al., 2021). Moreover, Spain is the second leading country on dumping plastics into the Mediterranean (126 tons/day) (UNEP/MAP, 2015), and Barcelona is the third major plastic debris contributing city (1787 tons/year) (Liubartseva et al., 2018; Sharma et al., 2021). Therefore, the high values of AIs observed in the present study are in accordance with the high pollution levels that characterise the Mediterranean area due to its anthropogenic pressure, as already seen in other benthic and pelagic species off the Catalan coast (Carreras-Colom et al., 2018, 2020; Muns-Pujadas et al., 2023; Rodríguez-Romeu et al., 2020, 2022).

All items recovered in the present study were fibre-shaped, likewise the dominance of fibre-shaped items of the marine environmental micro-litter composition (Browne et al., 2011). Polymer composition found in *S. canicula* in the present study (Cellulose > PET > Acrylic > PA > PP) is also in agreement with the proportion of fibre polymeric composition described by Sanchez-Vidal et al. (2018) in southern European seafloor sediments (Cellulose > PET > Acrylic > PA > PE > PP).

Accordingly, benthic fish species are likely to ingest more fibres whereas pelagic species are more prone to ingest particles (fragments or films), that float due to their lower density and the fact that they might remain longer in the water column (*e.g.,* fragments and films; Neves et al., 2015; Rodríguez-Romeu et al., 2022). Moreover, pelagic species feeding by filtering seem not to discriminate food particles and ingest more films and particles floating in the water column (Rodríguez-Romeu et al., 2022).

Regarding small-scale geographical variations, cellulosic fibres were dominant in fish caught off the deepest sampling areas; Blanes and Ebro Delta. This may be due to the higher density of cellulose fibres, which are more likely to sink into deeper environments than other synthetic microfibres. Indeed, they are found in large quantities in deep-sea environments (Sanchez-Vidal et al., 2018). Contrary, a higher percentage of synthetic fibres, more common in densely populated areas (Alomar et al., 2016; Muns-Pujadas et al., 2023), were found off Barcelona. The proximity of the sampling sites off Barcelona to its densely populated coast and the smooth bathymetry of these areas (Durán et al., 2014), together with the inputs of Llobregat and Besós rivers, that flow throughout industrialized and densely urbanized areas, could explain this fact (Derraik, 2002; Jambeck et al., 2015; Rodríguez-Romeu et al., 2020).

Moreover, fibres found in fish from off Ebro Delta were significantly smaller than those found in fish from off Barcelona. The former had ingested more cellulose fibres, which are more brittle and damageable (Liu et al., 2023). Therefore, the possibility of shattering of cellulosic fibres in fish digestive tracts causing overestimations of fibres abundance, might not be discarded. In this sense, the gastrointestinal tract fibre load (TLAI), based on fibres length, would be a more reliable indicator of ingestion rates than fibres abundance. The spiral valve in elasmobranchs increases the area and time for enzymatic digestion and nutrient absorption (Holmgren and Nilsson, 1999) which can also result in a higher retention time of AIs in this organ rather than in the stomach (Valente et al., 2019). This is in accordance with the significantly higher proportion of AIs (72 %) found in the spiral valve.

4.2. Parasitological assessment

In accordance with previous studies, parasite communities of *S. canicula* are overall characterized by low average richness and diversity and by high dominance values (Reinero et al., 2022).

The dominant parasites in sharks off the Catalan coast are *P. obtusus* and *G. adenoplusia*. The nematode *P. obtusus* is reported to be found in *S. canicula* across the Atlantic Ocean and Mediterranean Sea (Bakopoulos et al., 2018; Gangemi et al., 2019; Henderson and Dunne, 1998; Moore, 2001; Reinero et al., 2022; Sanmartin Duran et al., 1989; Silva et al., 2017). However, sharks from the Balearic Sea display higher abundances of this nematode (Casadevall et al., 2010; Dallarés et al., 2017a) with fish from off Barcelona reporting the highest values reported to date. Crustaceans are the intermediate hosts of this parasite (Moravec, 2007) and the main prey of *S. canicula* (Martinho et al., 2012; Olaso et al., 2005; Šantić et al., 2012; Valls et al., 2011) although adult sharks eat less crustaceans than juvenile sharks do (Martinho et al., 2012; Olaso et al., 2005; Šantić et al., 2012; Valls et al., 2011). The effect of depth segregation (with an adult preference for deeper waters), with ontogeny on this shark's feeding strategy and the availability of crustaceans as prey is vital in determining the prevalence and abundance of this parasite species (Silva et al., 2017).

The life cycle of the Trypanorhynch cestode *G. adenoplusia* also includes crustaceans, mainly copepods, as first intermediate hosts, followed by a second intermediate host (schooling teleosts, cephalopods), a paratenic host in some cases (larger fish, mesopredator elasmobranchs) and large elasmobranchs as definitive hosts (Dallarés et al., 2016; Palm, 2004). Santoro et al., (2021) was the first study to report the genus *Grillotia* in the muscle of *S. canicula* in the Gulf of Naples (Tyrrhenian Sea) with a mean abundance of 32.99 ± 30.77 parasites/ind. These higher abundances, compared to present results, may be due to the larger size of fish examined in the Tyrrhenian Sea, as larval forms (plerocercus) accumulate in the host musculature (especially in the tail) through their lifetime until they are predated by their final host (Dallarés et al., 2017a). However, as seen in the present study, geographical patterns, such as environmental conditions and depth-related faunal assemblages, may also be important in explaining parasite infection values (Isbert et al., 2023). In the case of *S. canicula*, spatial comparisons are difficult, since most parasitological studies to date did not include the analysis of muscle tissues, so further studies must consider the musculature as an organ of interest for fully describing parasite communities.

In accordance, the small-scale variability seen in the present study in the composition and structure of parasite assemblages may be attributed to different habitat features and feeding behaviour that determine prey availability, as the most contributing parasites are trophically transmitted. It is noteworthy to mention the difference between sexes in the abundance of the copepod L. *galei*. This parasite is found in the cloaca area, so it can be hypothesized that males are more susceptible to infection since its claspers provide the parasite a higher bonding surface.

4.3. Trace metals accumulation

Concentration levels of Ni, Cd, As, Pb and Hg in muscle tissue obtained in the present study are similar to other reported values in the North-Western Mediterranean for this species (Bouchoucha et al., 2019; Chouvelon et al., 2018; Cresson et al., 2014; Mille et al., 2018). Cu concentrations are also in the range of those found in the Gulf of Lions and Antalya Bay, Turkey (Mille et al., 2018; Türkmen et al., 2009). Zn concentrations are higher than those reported in other Mediterranean and Atlantic areas (Domi et al., 2005; Marques et al., 2021; Mille et al., 2018; Türkmen et al., 2009), but similar to those reported in the Catalan coast in the past (Flos et al., 1979). The mean liver concentrations of Ni and Zn in the Catalan coast resemble the levels reported in nearby areas for Ni (Bouchoucha et al., 2019) and historical levels for Zn within the same region (Flos et al., 1979). Therefore, the observed local variations could be attributed to factors specific to individual fish, such as length or depth of catch.

Over 90 % of the Hg found in muscle tissue of *S. canicula* occurs as methylmercury (MeHg), the most toxic organomercury compound (Storelli et al., 2022). Muscle Hg concentrations in *S. canicula* are much higher in the Mediterranean Sea than in the Atlantic Ocean (Chouvelon et al., 2018; Coelho et al., 2010; Domi et al., 2005; Marques et al., 2021). Moreover, within the Mediterranean Sea, MeHg concentrations in waters of the western basin double those reported in the eastern basin (Cossa et al., 2022). Mediterranean waters have a high methylation potential associated with low oxygen water masses, oligotrophy and high bacterial activity (Chouvelon et al., 2018; Cossa et al., 2009; Cossa and Coquery, 2005). In addition, higher MeHg concentrations are found in long-living, benthic, deep-water organisms and it biomagnifies along the food web (Cossa et al., 2012, 2022; Cossa and Coquery, 2005; Cresson et al., 2014). All these factors make *S. canicula* from the Balearic Sea prone to MeHg accumulation.

4.4. Health assessment

Condition indices and fish biometrics do not reveal any alterations in *S. canicula* populations in the Catalan coast, in relation to the pollutants and parasites analysed. Similarly, in a study from South of Sicily, no correlation between the Kn and AI's ingestion was found in *S. canicula* (Mancuso et al., 2022). Likewise, other studies carried in different species from the same area did not find potential relationships between the Kn and other pollutants, stressors or health descriptors (Carreras-Colom et al., 2022; Muns- Pujadas et al., 2023; Rodríguez-Romeu et al., 2022).

Although high values of AI's ingestion are found in the study area, the lack of meso- and macro-plastic ingestion, which is considered infrequent in demersal and benthic species (Anastasopoulou et al., 2013; Deudero and Alomar, 2015), reduces the possibility of impacting their health, for example by obstructing the gastrointestinal tract. In fact, some studies pointed out that the anatomy of the spiral valve may provide a barrier to macro-litter items, which can be regurgitated, as it happens with undigested residues, such as bones and scales (Morgan et al., 2021; Valente et al., 2019).

Despite the high parasite abundances found, there is no apparent effect on the condition of the host. Generally, abundant and rich parasite communities do not imply a bad health condition for their fish host (Dallarés et al., 2014; Rodríguez-Romeu et al., 2020, 2022). Nonetheless, heavy infections of encysted plerocerci might result in damage of the caudal musculature and loss of its functionality (Dallarés et al., 2017b; Isbert et al., 2023).

Concerning trace metals, although adverse health effects and stress responses on *S. canicula* have been well documented in experimental studies upon exposure to high concentrations of different trace metals (Crespo and Balasch, 1980; Hernández-Pascual and Tort, 1989; Torres et al., 1987; Tort et al., 1982, 1984; Tort and Torres, 1988), research in the wild, where concentrations are low, is limited and potential effects of current environmental concentrations are not well understood (Merly et al., 2019). For instance, results obtained in blood levels of heavy metals in the white shark (*Caracharodon carcharias*) suggested that sharks can stand levels of metals which would be toxic to teleosts, and there might be certain physiological features which make them more resilient to their potential negative effects (Merly et al., 2019). In accordance with condition indices and liver histology, no major alteration in the health status of *S. canicula* from the Catalan coast has been detected in the present study, and they thus seem to effectively cope with the current environmental levels of trace metals. However, chronic exposure, which has not been so well documented in laboratory conditions may still raise concern and there is still an important gap of knowledge in this area.

Studies on the histopathological features of elasmobranchs are scarce, compared to those of teleosts (Yancheva et al., 2016). Liver is a target organ for histopathology due to its importance in xenobiotic detoxification (Costa, 2018), and in the case of sharks, is an important organ for buoyancy through lipid storage (specifically as squalene, Ballantyne, 2014). These lipids, accumulated in fat vacuoles within the hepatocytes, may constitute up to 80 % of the liver (Gajíc et al., 2020). However, 21 % of our samples presented a low lipid content. Some authors have related the depletion or absence of lipid stores in the liver to a poor nutritional condition and emaciation (Garner, 2013; Stedman and Garner, 2018), but in the present study there is no apparent relation to emaciation reflected by condition indices. Thus, this low lipidic content may be related to other factors like the mobilization of lipid reserves for reproduction, especially in females (*e.g.,* for the formation of egg yolk), as other authors in the Catalan coast suggested (Valls et al., 2016). In the present study, most individuals with low lipidic content were maturing or mature females, but studies with more individuals assessed are needed to reveal the relation of lipid mobilization with reproduction.

Melanomacrophage centres (MMC) present in various fish species, principally in hematopoietic tissues (Agius, 1980; Agius and Roberts, 2003; Ferguson, 2006), are widely used as biomarkers of fish health (Carreras-Colom et al., 2022; Fournie et al., 2001) due to their potential association with several stressors like nutritional deficiencies, starvation (Agius and Roberts, 1981; Rios et al., 2007; Wolke, 1992), variations in temperature (Blazer et al., 1987), parasitic infections (Pérez-i-García et al., 2017) and exposure to pollutants among others (Carrassón et al., 2008; Lindesjöö et al., 1996; Qualhato et al., 2018; Sayed and Younes, 2017), but also natural process like senescence (Brown and George, 1985). However, just few studies have reported pigmented macrophage levels on sharks despite being long living organisms capable of bioaccumulating different toxins, and therefore good sentinel species for monitoring (Agius and Agbede, 1984; Borucinska et al., 2009; Gajíc et al., 2020; Pulsford et al., 1992).

Although some authors used the term MMC to describe the pigmented macrophages present in chondrichthyan and agnatha species (Borucinska et al., 2009; Gajíc et al., 2020), they refer to scattered, solitary and pigmented macrophages rather than the well-organized aggregations in Osteichtyes (Agius, 1980). This has been also confirmed in the present study, with single macrophages, or aggregations of two or three cells found randomly distributed in the liver of *S. canicula*. The density of these macrophages in our samples is lower than those reported by Gajíc et al., 2020 in *S. canicula* from the Adriatic Sea (mean = 8.41; range = 4.4–13.7; 400× magnification), and also to those reported in three large pelagic sharks from the Atlantic Ocean (Borucinska et al., 2009). Both studies considered the examined sharks to be healthy and the difference in the density of macrophages could be attributed to the larger size, and therefore probably older sharks, or interspecific differences.

The lack of relevant histological alterations together with the lack of correlations between pigmented macrophage abundance and lipid deposition with other analysed variables (parasites, micro-litter) indicate that *S. canicula* from off Girona are not affected by the present levels of stressors found in fish. Unfortunately, direct correlations between heavy metal concentrations and liver histology of the same individual were not possible in the present study due to different sampling pools. Further studies linking heavy metals to histopathology should be undertaken to shed light into the effects of these pollutants in sharks.

4.5. Human consumption risk assessment

In relation to human consumption, gastrointestinal tracts of sharks, where AIs are found, are discarded before cooking. Considering that AIs found in this study are unlikely to translocate to musculature (the edible part) due to their relatively large size (Burns and Boxall, 2018), eating shark meat does not pose a threat to humans in this sense.

Regarding trace metals, As, whose toxic inorganic portion (iAs) can vary a lot depending on the species (Fattorini et al., 2006), does not have an established concentration threshold for fish meat in the European Union. However, the EU established a limit of 25 ppm with a moisture proportion of 12 % of As on products intended for animal fed (EC, 2002). *Scyliorhinus canicula* is reported to be used in oil and fishmeal production across Europe (Ebert and Stehmann, 2013) and with the values found in this work, that well pass over this limit (103.68 ppm dry wet of As), this species would not be suitable for feed production.

The most important concern derived from present results is that all *S. canicula* but one, exceed the limits of Hg levels in the muscle allowed for consumption (EC, 2006), which may pose a risk for human health upon consuming its meat. In fact, Hg levels in muscle of *S. canicula* observed in the present study are only exceeded by those reported by Chouvelon et al., 2018 (2.86 ± 2.01 $\mu g \cdot g^{-1}$ ww) in the Gulf of Lions, also in the North-Western Mediterranean Sea. The specimens analysed in the latter study were larger (48 ± 5 cm TL) and caught in deeper grounds than those examined herein. These two factors (*e.g.,* fish size and depth range) are known to favour the accumulation of Hg in fish (Koenig et al., 2013). The depth factor can also explain the higher values obtained in fish from off Blanes (355 m) rather than on those from off Barcelona (60 m) in the present study.

Due to human health concerns of exposure to trace metals, more attention needs to be paid to current levels, particularly of Hg, in fish species landed in the North-Western Mediterranean. Specifically, studies determining Hg concentrations at different bathymetrical ranges as well as in specimens of different sizes should be urgently carried out. Given the increasing rates of Hg global concentrations in the last years as a result of anthropogenic activities, future monitoring is also needed (Cossa et al., 2022). Finally, other high-trophic level, long-living, benthic and deep-sea organisms (*e.g., Galeus melastomus*, which is also consumed) are of special interest for future studies.

The only zoonotic parasite found in sharks of the present study was a single *Anisakis* larvae. Higher prevalences of *Anisakis* larvae were found in other areas, *e.g.*, in the eastern Solent (United Kingdom) or in the Aegean Sea (13.3 % and 26.9 %, respectively; Bakopoulos et al., 2018; Moore, 2001). Therefore, the low prevalence of zoonotic parasites in *S. canicula* fom the Catalan Coast indicates a minimal health concern for consumers. However, the cestode larvae affecting the musculature should be taken into consideration for human consumption since, although it is not considered a zoonotic species, they diminish the quality of the flesh. More research on larger specimens would be necessary to assess if expected higher abundances can compromise both the health of the fish and its consumption.

5. Conclusions

The population of *S. canicula* in the Catalan Coast does not seem to be negatively affected by any major pathology nor by the levels of pollutants reported herein. This is supported by the population trend in the North-Western Mediterranean, which seem to have been growing throughout the present century (Ramírez-Amaro et al., 2020). Even though present levels of AIs found in sharks' gastrointestinal tract do not seem to pose a threat to their health, monitoring should be established in the upcoming future due to the expected increase of litter entering the oceans and its potential negative impacts on marine fauna. The high levels of trace metals (especially Hg) and the encysted larvae of *G. adenoplusia* in the muscle tissue are of certain concern for consumers. Further studies should focus on bigger specimens as both Hg and cestode larvae tend to accumulate during the shark's lifetime.

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CRediT authorship contribution statement

Andrea Higueruelo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Maria Constenla:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Francesc Padrós:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Paula Sánchez-Marín:** Writing – review & editing, Methodology, Investigation. **Maite Carrassón:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Anna Soler-Membrives:** Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Sara Dallarés:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Andrea Higueruelo reports financial support was provided by Government of Catalonia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Table S1. Environmental parameters (temperature. salinity. turbidity. and oxygen concentration) of the near bottom layer (0 – 5 m above the seafloor) measured in the three sampled localities.

Sampling point		Zone	Depth range (m)	Temperature [ºC]	Salinity [g/L]	Turbidity [FTU]	Oxygen [mg/l]
G1 i G3	GCTD2	Upper slope	399 - 404	13.76	38.63	0.43	6.52
G2	GCTD5	Continental shelf	68.5 - 73.5	14.12	38.19	0.48	8.42
B1 B2 i B3	BCTD1	Continental shelf	37.5 - 42.5	17.20	38.01	0.26	9.14
D1	DCTD2	Upper slope	344.5 - 349.5	13.69	38.59	1.21	6.69

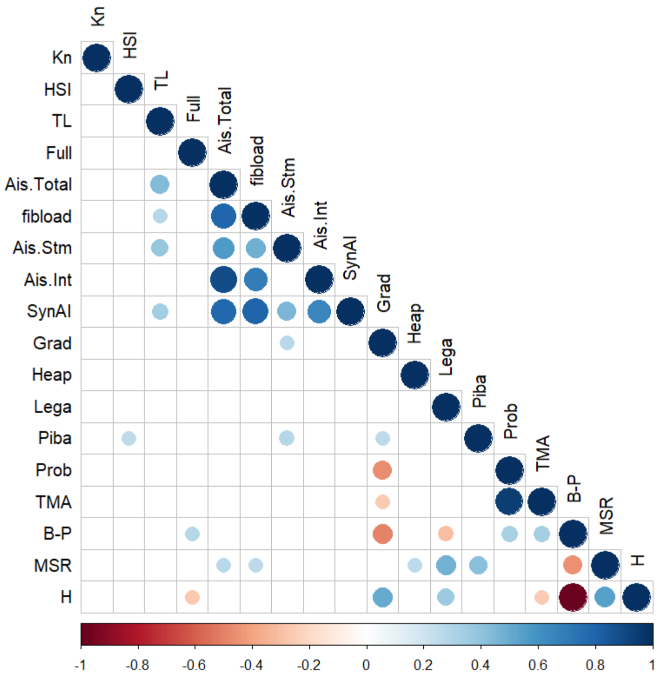

Figure S1. Correlation matrix Correlation matrix indicating positive (blue) and negative (red) significant ($p < 0.05$) correlations among Le Cren relative condition Index (Kn), hepatosomatic index (HIS), fish total length (TL), stomach fullness (Full), total AIs ingested and Ais from the stomach and the spiral valve (Ais.Total, Ais.Stm, Ais.Int), sum of the length of anthropogenic items (fibload), abundance of synthetic items (SynAI), the abundance of the main parasites [*Grillotia adenoplusia* (Grad), *Hexabothrium appendiculatum* (Heap), *Lernaeopoda galei* (Lega), *Piscicapillaria baylisi* (Piba) and *Proleptus obusus* (Prob)], total mean abundance of parasites (TMA), Berger-Parker dominance index (B-P), mean species richness (MSR) and Brillouin's diversity index (H). Circle size and colour intensity are proportional to the value of the correlation coefficient.

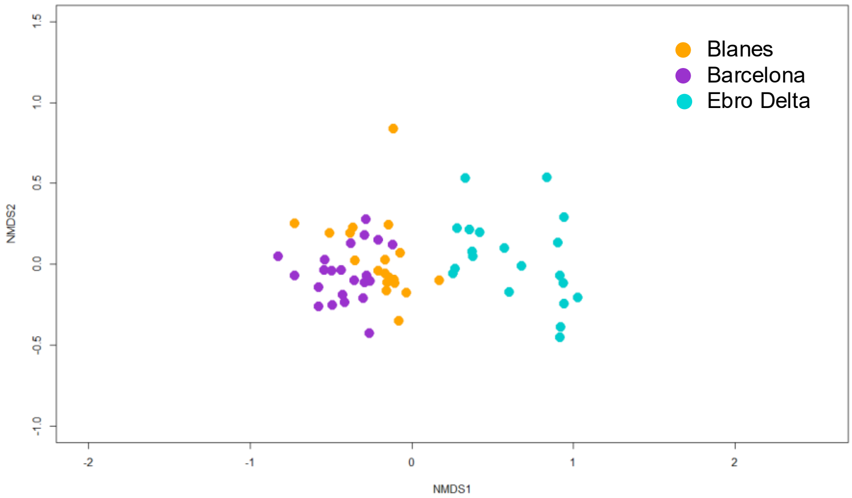

Figure S2. Non-metric multidimensional scaling (nMDS) ordination plot. The parasite species assemblage of each locality is represented with variables log transformed and analysis performed according to Bray-Curtis dissimilarity matrix. Every dot corresponding to a shark individual (n = 62). A stress estimate of 0.103 was obtained.

CHAPTER 3

A “toxic trio” (mercury, lead and cadmium) metal assessment in marine commercial species from Northwestern Mediterranean Sea: risk and recommendations

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Abstract

Mercury (Hg), lead (Pb), and cadmium (Cd), commonly termed “the toxic trio", are highly toxic metals regulated in food by the European Union for consumer safety. This study examined the biological and environmental factors influencing their accumulation in marine organisms by analysing trace metal concentrations in the muscle tissue of 10 species with varied habitat preferences (seven teleosts, two elasmobranchs, and one crustacean) caught in the Northwestern Mediterranean. Shark samples across different size ranges were analysed to identify accumulation patterns. Geographical variability was evaluated using the small-spotted catshark (*Scyliorhinus canicula*) as a biomonitor, comparing Mediterranean results with data from Atlantic Spanish regions and published values. Compliance with European regulations and associated consumer risks were also assessed. Hg accumulation showed interspecific variation linked to habitat use, with the highest levels in benthic species, and intraspecific accumulation positively correlated with size. Geographically, Hg levels were higher in the Northwestern Mediterranean, reflecting the region’s high methylation potential. While Pb and Cd remained within European consumption limits, over one-third of benthic samples exceeded Hg thresholds, and 92.45 % of adult sharks surpassed safe levels, compromising their commercialization. Mediterranean benthic fish should be consumed sparingly, particularly by pregnant women and children, as recommended for top predator species. This study highlights how habitat use and body size drive Hg accumulation, establishes *S. canicula* as a biomonitor for contamination, and underscores the role of regional environmental factors in shaping metal distribution and bioavailability, contributing to a better understanding of Hg fate in marine ecosystems and its potential impact.

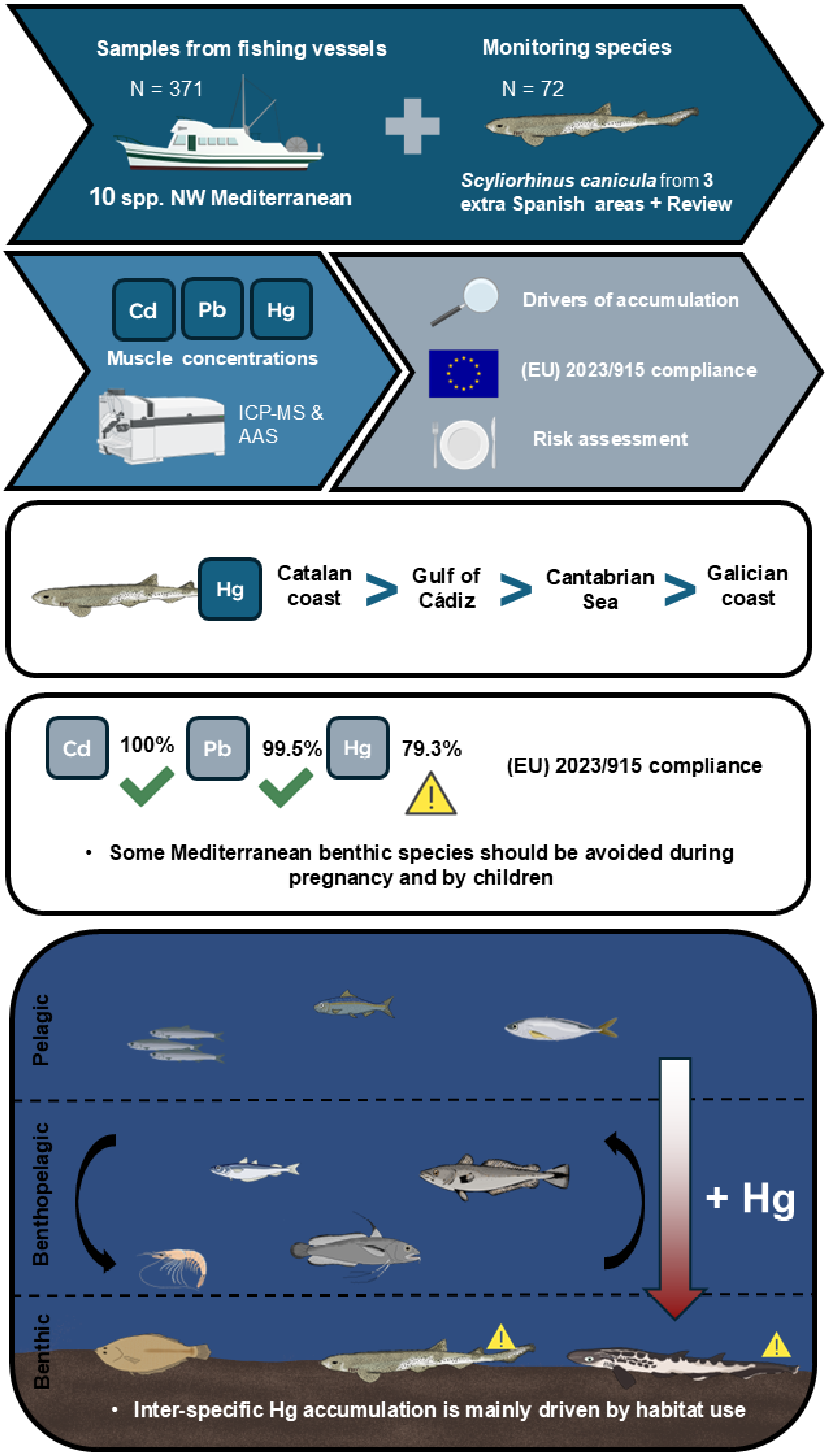
Keywords

Trace metals, Habitat use, Risk assessment, Food safety, Marine pollution

Highlights

- Inter-specific Hg accumulation is mainly driven by habitat use
- *Scyliorhinus canicula* is a suitable biomonitor for Hg
- 92.45 % of benthic adult sharks analysed were above European consumption limits for Hg
- Muscle Pb and Cd levels in selected species are below European consumption limits
- Mediterranean methylation potential results in high Hg levels in marine species

Graphical abstract



Samples from fishing vessels

N = 371

10 spp. NW Mediterranean

Monitoring species

N = 72

Scyliorhinus canicula from **3 extra Spanish areas + Review**

Cd Pb Hg

Muscle concentrations

ICP-MS & AAS

Drivers of accumulation

(EU) 2023/915 compliance

Risk assessment

Hg Catalan coast > Gulf of Cádiz > Cantabrian Sea > Galician coast

Cd 100% ✓ Pb 99.5% ✓ Hg 79.3% ⚠ (EU) 2023/915 compliance

- **Some Mediterranean benthic species should be avoided during pregnancy and by children**

- **Inter-specific Hg accumulation is mainly driven by habitat use**

Hg, Pb and Cd = Mercury, Lead and Cadmium
ICP-MS = Inductively coupled plasma mass spectrometry
AAS = atomic absorption spectrometry

1. Introduction

To safeguard ocean health, one of the Sustainable Development Goals set by the United Nations' 2030 Agenda calls for preventing and significantly reducing marine pollution by 2025 (United Nations, 2015). Among pollutants threatening marine aquatic ecosystems, trace metal contamination stands out as a major global concern (Piwowarska et al., 2024). Trace metals occur in aquatic environments through natural processes, but anthropogenic activities, such as mining, industrial and agricultural activities and untreated sewage discharge, have significantly contributed to increasing their concentrations (Zhou et al., 2008).

The Mediterranean Sea is characterized by being a semi-enclosed mass of water, which naturally communicates with the Atlantic through the Strait of Gibraltar. It is also a concentration basin with a negative hydrographic balance due to high evaporation rates. Currently, it faces significant anthropogenic pressures which, in combination with the particular characteristics commented above, it is especially susceptible to the impacts of most pollutants (Danovaro, 2003; Durrieu de Madron et al., 2011). Specifically, the Mediterranean Sea experiences extremely high trace metal inputs through atmospheric deposition compared to the open ocean. This is primarily attributed to the input of anthropogenic aerosols from industrial and domestic activities due to its densely populated shores, in addition to the deposition of Sahara mineral dust (Cerro et al., 2020). Moreover, available data on trace metal concentrations in the environment suggest that the NW Mediterranean is the Mediterranean area under the greatest pollution stress (Danovaro, 2003; Middag et al., 2022).

The accumulation of trace metals in biota is determined by numerous physiological, biological and ecological factors (Madgett et al., 2021; Signa et al., 2017). Moreover, geographic location and the associated environmental conditions are key determinants of metal uptake, as they influence metal bioavailability and speciation (Luoma and Rainbow, 2008). Particularly, there is a concern regarding the Mediterranean Sea's high Mercury (Hg) methylation potential, attributed to a combination of ecological and biochemical factors, which has been formally named as the "Mediterranean mercury anomaly". This phenomenon renders methylmercury (MeHg), a highly toxic mercury species, available within the food chain (Cossa and Coquery, 2005; Sandheinrich and Wiener, 2011). In line with this, MeHg water concentrations are twice as high in the western basin compared to the eastern basin (Cossa et al., 2022). Since MeHg concentrations in animals are influenced by the levels of their foraging zones, this difference is also mirrored by organisms of both basins, resulting in higher concentrations in animals from the western basin (Cossa et al., 2022). Once in the marine environment, MeHg and other trace metals can bioaccumulate in marine organisms and biomagnify towards the upper levels of marine trophic webs, potentially reaching humans through seafood consumption (Environmental Protection Agency, 2024).

Among trace metals, Hg, lead (Pb), and cadmium (Cd) are commonly referred to as “the toxic trio” due to their high degree of toxicity. Not only can they adversely affect marine organisms (Garai et al., 2021), but exposure to these metals can also pose serious implications for human health, particularly for vulnerable groups such as pregnant women and children (WHO, 2019, 2023, 2024). Exposure to these metals has been associated with various health issues including cancer, neurological damage, gastrointestinal, cardiovascular and renal damage, immunosuppression, reproductive defects and endocrine disruption among others (WHO, 2019, 2023, 2024).

Consequently, the European Commission (EC) establishes Hg, Pb and Cd thresholds in commercialized marine species (Regulation 2023/915 of the EC) (EC, 2023). Different studies have reported Hg concentrations exceeding these limits in several marine species, some of which of commercial interest, caught in the Mediterranean Sea, particularly in the western basin (Barone et al., 2018; Cresson et al., 2014; Higueruelo et al., 2024; Koenig et al., 2013a; Llull et al., 2017; Storelli and Barone, 2013). For instance, certain species doubled or even tripled the Hg levels set by the EC in the Balearic Sea (Higueruelo et al., 2024; Llull et al., 2017). However, no restrictive policies have been adopted concerning the sale of these organisms.

Despite this, several benefits for human health derived from marine species consumption are recognised, as these are a dietary source of energy, protein, and other nutrients important for health, also during pregnancy and childhood (FAO/WHO, 2023). The recent Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Consultation on Risks and Benefits of Fish Consumption concluded that risk-benefit assessments for fish consumption at regional, national, or subnational levels are needed to refine recommendations considering different fish species contamination levels and nutrient contents, and local consumption habits (FAO/WHO, 2023). Currently, the Spanish Agency for Food Safety and Nutrition (AESAN) is the national authority in Spain monitoring trace metals and other contaminants in food. However, although its reports include the percentage of legal compliance for fish and other seafood products sold in Spain, the data is reported in a generic manner without specific data on species or geographical origin. Hence, and considering the particularities of the Mediterranean Sea commented above, it is of major importance to evaluate and report trace metal contents on different consumed species in this area.

In the present study, 10 representative commercial marine species, caught off the Catalan coast (Northwestern Mediterranean, Balearic Sea), displaying different habitat use traits were chosen. These species represent over a third of the total fishery catches in terms of biomass in Catalonia (Idescat, 2023a). The main objectives of the present study are: i) to reveal the concentration of Hg, Pb and Cd in the muscle tissue of specimens belonging to the aforementioned selected species caught off the Catalan coast, ii) to investigate the potential influence of biological factors (*i.e.*, fish size, sex) and habitat (including depth) on their accumulation patterns in muscle, iii) to assess geographical variations on the assessed metal contents within the Catalan coast and between the latter and locations off the Spanish Atlantic coast through the use of the small-spotted catshark (*Scyliorhinus canicula* Linnaeus, 1758) as a pollution bioindicator species, and iv) to evaluate the risk of consuming these marine species for humans through the Estimated Weekly Intake (EWI) compared to the Tolerable Weekly Intake (TWI) set by the European Food Safety Authority (EFSA) considering different scenarios. Based on these assessments, recommendations are finally provided for safe consumption.

2.Materials and methods

2.1. Study area and sample collection

For the present study, 10 representative commercial species were chosen: spotted flounder (*Citharus linguatula* Linnaeus, 1758), European anchovy (*Engraulis encrasicolus* Linnaeus, 1758), blackmouth catshark (*Galeus melastomus* Rafinesque, 1810), European hake (*Merluccius merluccius* Linnaeus, 1758), blue whiting (*Micromesistius poutassou* Risso, 1827), deep-water rose shrimp (*Parapenaeus longirostris* Lucas, 1846), greater forkbeard (*Phycis blennoides* Brünnich, 1768), round sardinella (*Sardinella aurita* Valenciennes, 1847), small-spotted catshark (*S. canicula*) and Mediterranean horse mackerel (*Trachurus mediterraneus* Steindachner, 1868). Specimens were captured by commercial fishing trawlers and purse seiner vessels between summer 2019 and summer 2023 off the Catalan Coast (NW Mediterranean). A total of 371 individuals from three areas were collected (off Barcelona, Ebro Delta and Blanes) at depths comprised between 38 and 727 m **(Fig. 1a)**. Between 26 and 30 individuals were collected per species, with the exception of *S. aurita*, for which only 10 specimens were obtained. In the case of sharks, efforts were made to sample individuals across a wide range of sizes to enable subsequent analyses of size-related metal accumulation patterns, an in the case of *S. canicula*, small-scale geographical comparisons (*G. melastomus*, *n* =69; *S. canicula*, *n* =89) **(Table 1)**. Collected individuals were preserved frozen at -20 ◦C upon capture. The Barcelona sampling site receives the inputs of the Besós and Llobregat rivers (58 and 170 km long; 104.1 and 312.1 hm^3/y, respectively) (Idescat, 2023b). The city of Barcelona accommodates a population of 1.6 million in the nearby urban coastal area (Idescat, 2024), being one of the most important commercial and touristic ports on the Mediterranean coast and characterized by a substantial industrial history (Tatjer, 2006). The Ebro River is the second largest river in Spain (910 km long, 14,000 hm3/y), receiving the inputs of industrial and agricultural activities that might also impact the Ebro Delta sampling site (Galimany et al., 2019). In contrast, the sampling point near the city of Blanes, influenced by the relatively small Tordera River (54 km long; 12.78 hm3/y Idescat, 2023b), is considered the least affected by anthropogenic activities.

In addition to Mediterranean samples, 71 female specimens of *S. canicula* collected in 2013 from three Spanish Atlantic areas were obtained for geographical comparison. Specifically, 23 individuals were collected in the Gulf of Cádiz (southern Spain), 24 nearby the Galician coast (between the city of Baiona and the cape of Estaca de Bares, NW Spain) and 24 from the Cantabrian Sea (between the cape Cabo Peñas and the river Bidasoa, in northern Spain) during the Oceanographic campaigns DEMERSALES and ARSA held by the Spanish Institute of Oceanography (IEO) in 2013 **(Fig. 1b)**.

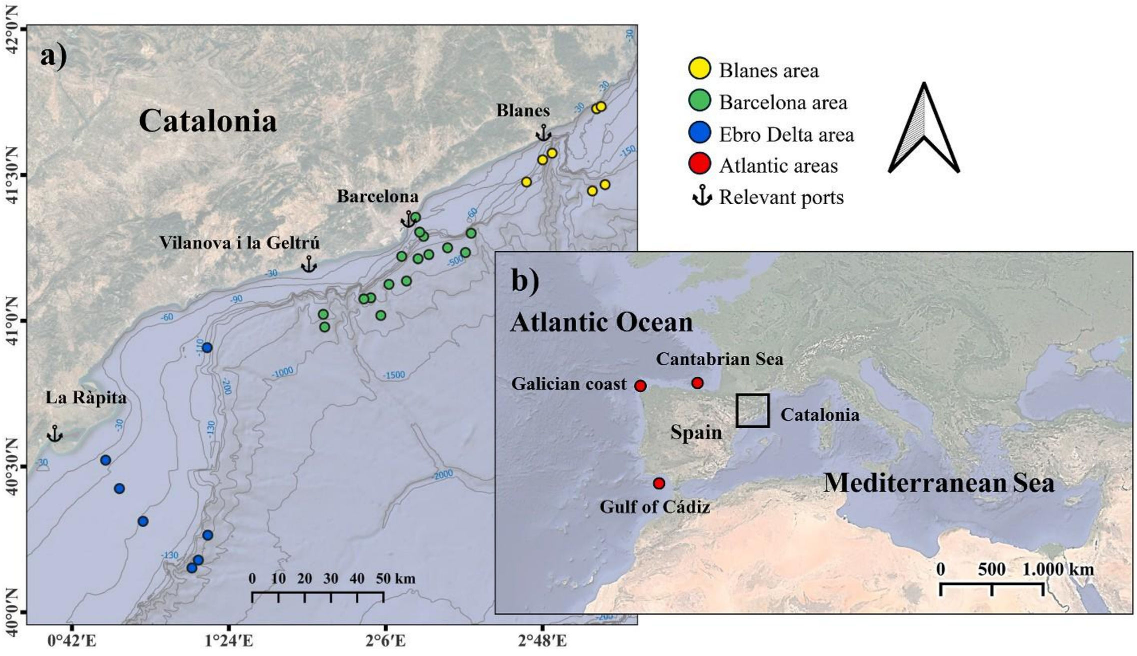


Figure 1. Map of the study area. Sampling points from three different areas off the Catalan coast and additional sampling points from the Atlantic Ocean.

Table 1. Mean length and muscle concentrations of mercury (Hg), lead (Pb) and cadmium (Cd) (standard deviation) of selected species sampled off the Catalan coast arranged according to their habitat use. EC limit refers to the maximum levels for certain contaminants in food established by the European Commission, Regulation 2023/915. In the case of sharks, values are provided separately for juveniles and adults according to the size at maturity reported by Ramírez-Amaro et al. (2020) and unpublished data by present authors. All values are given in µg/g wet weight (ww). Length refers to standard length in the case of teleosts, total length in the case of sharks and cephalothorax length in the case of crustaceans. N = Sample size. In bold, values above the EC limit.

	N	Length (cm)	Hg (µg/g ww)	EC Hg limit	Pb (µg/g ww)	EC Pb limit	Cd (µg/g ww)	EC Cd limit
Pelagic								
Engraulis encrasicolus	30	11.52 (0.62)	0.10 (0.02)	0.30	0.006 (0.003)	0.30	0.0041 (0.0029)	0.25
Trachurus mediterraneus	30	19.13 (1.76)	0.15 (0.10)	0.50	0.006 (0.004)	0.30	0.0009 (0.0006)	0.050
Sardinella aurita	10	14.97 (0.84)	0.09 (0.02)	0.30	0.042 (0.027)	0.30	0.0006 (0.0001)	0.050
Benthopelagic								
Merluccius merluccius	29	18.87 (4.38)	0.07 (0.03)	0.50	0.037 (0.061)	0.30	0.0009 (0.0003)	0.050
Micromesistius poutassou	30	22.01 (1.29)	0.16 (0.04)	0.50	0.062 (0.094)	0.30	0.0013 (0.0004)	0.050
Phycis blennoides	26	18.96 (2.41)	0.26 (0.06)	0.50	0.003 (0.002)	0.30	0.0001 (0.0001)	0.050
Parapenaeus longirostris	30	3.03 (0.36)	0.25 (0.15)	0.50	0.014 (0.011)	0.50	0.0046 (0.0022)	0.50
Benthic								
Citharus linguatula	28	16.34 (2.00)	0.45 (0.36)	0.50	0.004 (0.002)	0.30	0.0001 (0.0001)	0.050
Galeus melastomus $_{\text{juvenile}}$	50	36.28 (8.80)	0.70 (0.81)	1.0	0.007 (0.008)	0.30	0.0017 (0.0024)	0.050
Scyliorhinus canicula $_{\text{juvenile}}$	46	29.76 (5.58)	0.77 (0.33)	1.0	0.023 (0.029)	0.30	0.0031 (0.0041)	0.050
Galeus melastomus $_{\text{adult}}$	19	52.81 (1.89)	**1.70** (0.51)	1.0	0.006 (0.004)	0.30	0.0010 (0.0003)	0.050
Scyliorhinus canicula $_{\text{adult}}$	43	43.37 (2.70)	**1.98** (0.81)	1.0	0.009 (0.005)	0.30	0.0018 (0.0008)	0.050

2.2. Trace metal analyses

A portion of approximately two to 20 g of edible muscle was obtained for each individual. Sharks were carefully skinned in order to get clean muscle samples. Previously, length (total length for sharks, standard length for teleosts or cephalothorax length for crustaceans), total weight and, when possible, sex was recorded. Samples were analysed in the facilities of the IEO VIGO (Instituto Español de Oceanografia – Centro Oceanográfico de Vigo) as follows. Muscle portions were freeze-dried, ground to a fine powder and homogenized. They were weighed before and after lyophilization to determine the percentage of water on each sample. Digestion was carried out using 300 mg of homogenized sample with concentrated HNO3 (PlasmaPURE, SCP Sciences) on Teflon reactors in a microwave digestion system (MARS6, CEM) using the procedure described in Besada et al. (2014). In cases when metal concentrations were near the limit of quantification (LOQ), analyses were repeated using a hotplate digestion method as described in Sánchez-Marín et al. (2023). Metal concentrations of Pb, Cd and additionally, nickel (Ni), copper (Cu), zinc (Zn) and arsenic (As) were obtained using inductively coupled plasma mass spectrometry (Agilent 8900 ICP-MS) as described in Sánchez-Marín et al. (2023). Exception- ally, the analyses of these metals in *M. poutassou* and *M. merluccius* muscle samples were carried out in the Chemical Analysis Service from the Autonomous University of Barcelona (SAQ), following the protocol described in Carreras-Colom et al. (2022). Trace metal analyses for *S. canicula* captured in Atlantic areas in 2013 were performed as described in Besada et al. (2011a). Total Hg was determined in all samples by pyrolysis atomic absorption spectrometry with gold amalgamation (employing an AMA254 Advanced Mercury Analyzer (LECO Instruments), as described in Belmonte et al. (2021).

Procedural blanks and certified reference material of fish muscle (DORM-2, DORM-5 or ERM-BB422) were also included in each batch. Obtained recoveries were between 86 % and 116 % in all cases except for Ni in DORM-5, that was 77 %. The limits of detection (LOD) associated to the different analyses are reported in **Table S1**.

2.3. Consumption risk assessment

Obtained concentrations of selected metallic elements were compared to the maximum consumption levels set by the EC (EC, 2023). For the risk assessment, total Hg was converted to MeHg with a conversion factor of 1 for fish and of 0.8 for the crustacean *P. longirostris*, following the conservative approach established by the EFSA (EFSA, 2012). Consumers exposure to MeHg, Pb and Cd for each species was estimated by calculating the EWI (µg/kg · body weight) according to the following equation: $EWI = C \times IR/BW$; where C is the mean metal concentration in each given species (µg/g wet weight), BW is the consumers body weight (kg·bw) and IR is the weekly ingestion rate (g/week). EWI was further compared to the TWI for each metal as the percentage of ingestion (%TWI) calculated as $100 \cdot EWI/TWI$. The EFSA set a TWI for MeHg and Cd of 1.3 and 2.5 µg/kg·bw, respectively (EFSA, 2009, 2012). In the case of Pb, the FAO/WHO Joint Expert Committee on Food Additives set a provisional tolerable weekly intake (PTWI) of 25 µg/kg·bw (FAO/WHO, 1999). Different possible scenarios have been considered for these calculations. On the one hand, three types of consumers are considered (a child of 35 kg, and adults of 60 and 75 kg) and on the other hand, the portions of fish consumed per week (between one and three; a portion corresponding to 150 g for adults and 75 g for children) (AESAN, 2008, 2022a).

2.4. Data analysis

Habitat use and trophic level for each species were determined according to information available on Fish Base (Froese and Pauly, 2024), and size at sexual maturity of sharks was obtained from unpublished data by present authors for *S. canicula* and from Ramírez-Amaro et al. (2020) for *G. melastomus*. Data distribution of quantitative variables was plotted for visual assessment and tested for normality and homoscedasticity using the Shapiro-Wilk test and Levene's test, respectively. When necessary, variables were log or square-root transformed to comply with normality and homoscedasticity requirements and outliers were excluded for some statistical analysis when required.

The Spearman rank correlation coefficient (rho) was employed for detecting associations between concentrations of each metal and length of each individual and trophic level. The potential correlation among the different metals were also tested within each species. In the species where sex differentiation was possible, differences in metal concentrations between sexes were tested using a Wilcoxon test; except for Hg, which was tested using a linear model considering length as a covariate. In the case of *S. canicula* differences in Pb and Cd concentrations between very small (<25 cm) and large (≥25 cm) individuals were compared using a Wilcoxon test. Differences in metal concentrations among habitats and, in the case of *S. canicula,* among locations, were tested using Generalized linear mixed models (GLMM) fit by maximum likelihood and considering the species as a random effect and trophic level as a covariate. In the case of exponential correlations, fitted curves were obtained following the equation $[Hg] = a \exp(b \cdot TL)$; where a and b were adjusted parameters. A logistic regression was built to infer the length at which Hg concentration exceeded the limit for consumption. The variables were fitted to a logit function with the formula: $P = 1/ (1+\exp(-\beta0+\beta1*X))$, where P is the probability of an individual exceeding the Hg limit at a specific length, β0 is the intercept and β1 is the slope, both estimated parameters. The lengths at which 50 % and 95 % of the individuals exceeded this limit were inferred based on this formula. In the case of *S. canicula*, subsamples of individuals of similar size (groups of at least 10 individuals within a size range of approximately 5 cm) were used to test differences among sampled areas off the Catalan coast and between depths of capture in the case of Barcelona sampling site, as well as for comparison with samples from the Atlantic, with Student's t-test and, when normality was not satisfied, with Wilcoxon test.

All data analyses were performed with RStudio Statistical Software (v. 4.2.3). Correlations were considered strong when the coefficient (rho) was higher than 0.60. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Metal concentrations in marine species off the catalan coast

Mean muscle concentrations of Hg, Pb and Cd in 10 different species caught off the Catalan coast are given in **Table 1**, along with the maximum levels permitted in marketed food as established by EC Regulation 2023/915 (EC, 2023). Highest concentrations of Hg, Pb and Cd were found in S. *canicula, M. poutassou* and P. *longirostris*, respectively, while lowest concentrations were found in *M. merluccius* for Hg and in *P. blennoides* for Pb and Cd.

Additionally, mean concentrations of Ni, Cu, Zn and As are reported in Supplementary material **(Table S2)**. In short, these values ranged from 0.006 to 0.05, from 0.14 to 4.21, from 2.54 to 11.97 and from 3.63 to 24.82 µg/g ww, respectively. Overall, the highest mean concentrations were observed in the crustacean *P. longirostris*, while the lowest were found in *P. blennoides*, except for As, which was found at lowest concentrations in *M. merluccius*.

For sexed species (*P. longirostris, S. canicula, and G. melastomus*), the effect of sex on metal concentrations was analysed, finding no significant differences for any of the trace metals. Similarly, no significant correlations were found among the concentrations of Hg, Pb and Cd within any individual species.

3.2. Influence of biological and environmental factors on trace metal concentrations

No correlations were found between trophic level and metal concentrations for any species (rho <0.60 or p >0.05). Hg was the only trace metal showing significant positive correlations with length for the teleosts *T. mediterraneus* (rho = 0.69), *M. merluccius* (rho = 0.60) and *C. linguatula* (rho = 0.71) and the crustacean *P. longirostris* (rho = 0.73) (p <0.001 in all cases).

Hg was also strongly correlated with TL of both sharks, *S. canicula* and *G. melastomus* (rho =0.79 and 0.91, respectively; p <0.001 in both cases), with an exponential increase in Hg concentration with size (R2 = 0.62 and 0.77, respectively) **(Fig. 2a and b)**. For Pb and Cd concentrations, no strong significant correlations with shark length were observed (rho <0.60 or p >0.05). Nonetheless, significantly higher levels of Cd and Pb were found in very small individuals (<25 cm) of *S. canicula* (Wilcoxon-test; W = 77 and 78, respectively; p <0.001 in both cases) than in larger ones (≥25 cm) **(Fig. 2c and d)**.

Hg mean concentration was significantly higher in benthic than in benthopelagic species (GLMM; z = 4.343, p <0.001) and pelagic species (GLMM; z = 5.164, p <0.001), while there were no significant differences in the concentration of Pb and Cd among groups of species with differing habitat use (p >0.05 in all cases) **(Fig. 3)**. Trophic level, which ranged from 3.1 to 4.4, had no significant effect on the models (p >0.05) and was therefore excluded.

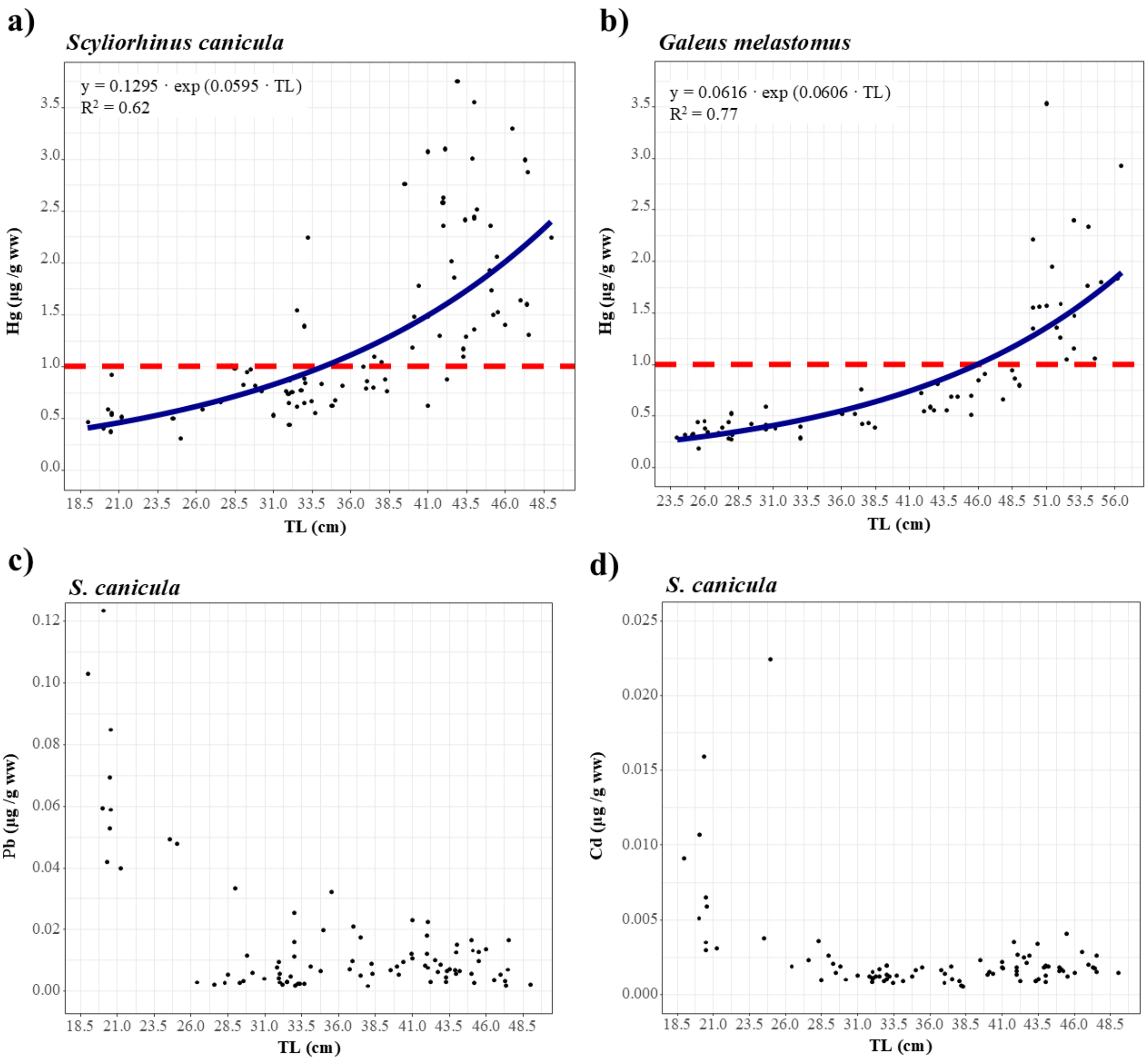


Figure 2. Correlation plots between trace metals muscle concentrations and total length of sharks. Correlation of mercury (Hg) concentration in muscle tissue of a) *Scyliorhinus canicula* and b) *Galeus melastomus* with total length (TL; cm). Correlation of c) lead (Pb) and d) cadmium (Cd) concentrations in *S. canicula* with TL. Trace metal concentration values are given in µg/g · wet weight (ww). Red dashed line corresponds to the limit of 1 µg/g ww of Hg set for consumption stablished by the European Comission.

3.3. Deeper focus on *S. canicula* trace metal concentrations

3.3.1. Small-scale variability in the Catalan coast

Metal concentrations among areas off the Catalan coast were compared using individuals within the same size range to minimize the effect of total length on metal accumulation levels. No

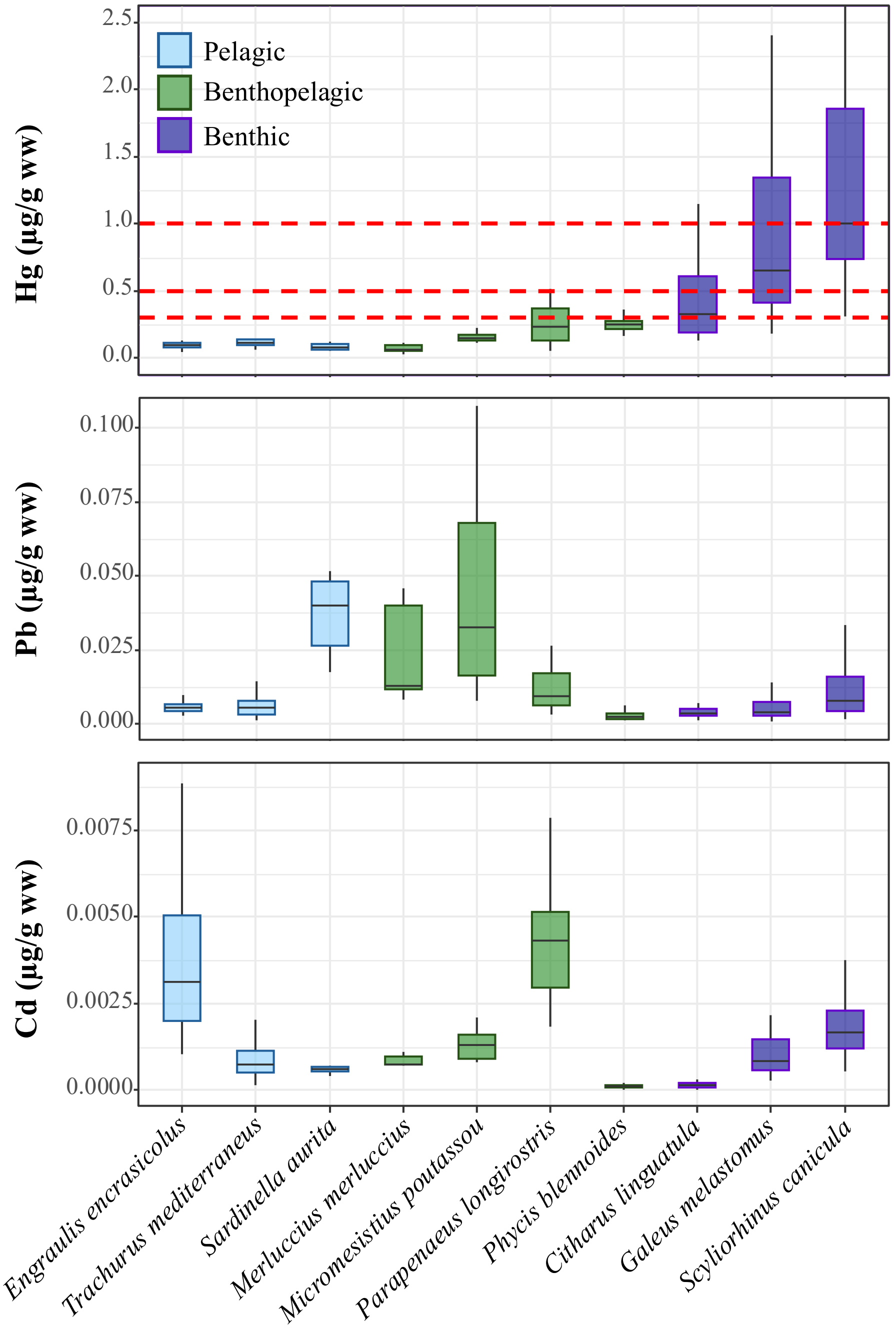


Figure 3. Boxplots displaying concentrations of mercury (Hg), lead (Pb) and cadmium (Cd) in the different target species, grouped according to their habitat distribution. All values are given in µg / g · wet weight (ww). Red dashed lines indicate the maximum permitted Hg limits for consumption established by the European Commission, being 0.3 µg / g ww for *Engraulis encrasicolus* and *Sardinella aurita;* 0.5 µg / g ww for *Trachurus mediterraneus, Merluccius merluccius, Micromesistius poutassou, Phycis blennoides, Parapenaeus longirostris* and *Citharus linguatula,* and 1 µg / g ww for *Scyliorhinus canicula* and *Galeus melastomus*. The limits for Pb and Cd are not illustrated, as the measured concentrations are several orders of magnitude lower.

significant differences in Hg, Pb, and Cd concentrations were observed between Barcelona and Ebro Delta samples ($p > 0.05$ in all cases). For Blanes and Barcelona, Pb levels showed no significant differences ($p > 0.05$), while Cd and Hg concentrations were significantly higher in individuals caught off Blanes (Cd: t = -2.14, p = 0.04; Hg t = -2.31, p = 0.03) **(Table 2)**. Within the same size range (40–50 cm in total length) and for individuals from Barcelona, no differences in metal concentration were found between those caught at different depths (60 versus 266–311 m) (p >0.05 in all cases).

3.3.2. Variability across Mediterranean and Atlantic areas

Within the same size range, Hg concentrations in *S. canicula* were different in all analysed areas (K-W; χ2 = 70.64, p <0.001) with fish from off the Catalan coast showing the highest concentrations, followed by those from off Cádiz and the Cantabrian Sea, and fish off Galicia displaying the lowest concentrations **(Table 2)**. Contrary, Pb concentrations did not differ among Spanish study areas (K-W; χ2 = 4.74, p = 0.19). In the case of Cd, the only differences observed occurred between fish from the Catalan coast and those from the rest of localities, the former displaying lower concentrations (K-W; χ2 = 29.36, p < 0.001) **(Table 2)**. Additionally, concentrations of As, Cu and Zn from individuals caught off Cádiz, Cantabrian Sea and Galicia are reported on **Table S2**. In this case, similar mean values of Cu (0.35–0.37 µg/g ww) and Zn (6.96–7.24 µg/g ww) were obtained in samples from all Atlantic areas, while higher mean values of As were obtained in samples from Gulf of Cadiz (16.19 µg/g ww), followed by those of the Cantabrian Sea (12.29 µg/g ww) and the Galician coast (9.96 µg/g ww).

Table 2. Values of mercury (Hg), lead (Pb) and cadmium (Cd) in muscle tissue of *Scyliorhinus canicula* caught off different areas off Spain. Mean values (standard deviation) (minimum – maximum) are expressed in µg / g wet weight (ww). Samples within the same size range where selected. N = sample size. Significant differences among localities are expressed by different superscript letters. *Analytical data below the limit of detection (LOD) were replaced by LOD/2 to avoid missing data.

	N	Length (cm)	Hg (µg/g ww)	Pb (µg/g ww)	Cd (µg/g ww)
Small-scale variability					
Barcelona	13	31.50 (1.54) (29.0 – 33.4)	0.89 (0.30)^{a} (0.44 – 1.54)	0.008 (0.010)^{a} (0.002 – 0.033)	0.0015 (0.0005)^{a} (0.0008 – 0.0026)
Ebro Delta	10	31.99 (1.62) (31.25 – 33.7)	0.87 (0.50)^{a} (0.53 – 2.24)	0.007 (0.005)^{a} (0.002 – 0.016)	0.0012 (0.0003)^{a} (0.0009 – 0.0019)
Blanes	10	41.79 (1.57) (39.5 – 44.2)	2.53 (0.79)^{a} (1.30 – 3.75)	0.009 (0.005)^{a} (0.006 – 0.023)	0.0016 (0.0006)^{a} (0.0014 – 0.0035)
Barcelona	16	42.68 (1.24) (40 – 44)	1.80 (0.79)^{b} (0.62 – 3.55)	0.010 (0.006)^{a} (0.003 – 0.023)	0.0022 (0.0007)^{a} (0.0009 – 0.0034)
Large-scale variability					
Gulf of Cádiz	23	33.12 (1.91)^{ab} (30 – 35.8)	0.35 (0.08)^{a} (0.21 – 0.56)	0.006 (0.002)^{a*} (0.005 – 0.017)	0.0106 (0.0068)^{a} (0.0020 – 0.0270)
Cantabrian Sea	24	33.70 (1.50)^{a} (30.8 – 35.9)	0.207 (0.05)^{b} (0.15 – 0.35)	0.008 (0.009)^{a*} (0.005 – 0.048)	0.0085 (0.0043)^{a} (0.0020 – 0.0220)
Galician coast	24	32.08 (1.58)^{b} (29 – 35.4)	0.12 (0.03)^{c} (0.07 – 0.19)	0.007 (0.004)^{a*} (0.005 – 0.018)	0.0076 (0.0024)^{a} (0.0040 – 0.0130)
Catalan coast	11	32.80 (0.98)^{ab} (31.8 – 34.8)	0.86 (0.33)^{d} (0.44 – 1.54)	0.006 (0.007)^{a} (0.002 – 0.025)	0.0012 (0.0004)^{b} (0.0008 – 0.0020)

3.4. Risk assessment

Muscle metal concentrations of all pelagic and benthopelagic bony fish species were below the Hg limit for consumption established by the EU **(Table 1)**. In the case of the crustacean *P. longirostris,* five individuals (16.66 % of the total sample) showed concentrations close to the EC limit (>0.4 µg/g ww) and only one individual (3.33 % of the total sample) was above it (>0.5 µg/g ww). As for benthic species, 32.14 % of the specimens of *C. linguatula* displayed muscle concentrations above the maximum permitted levels for consumption. In the case of sharks, 31.88 % of *G. melastomus* and 50.56 % of *S. canicula* showed Hg concentrations above the established EC limit of 1 µg/g ww. A logistic regression on the proportion of sharks above the Hg EC limits for consumption at each given body length was fitted for each shark species to infer the lengths at which 50 % and 95 % of individuals surpassed the limit. For *S. canicula,* individuals larger than 37.03 and 43.85 cm had a 50 % and 95 % probability, respectively, of exceeding the consumption limit (R2 = 0.73). Similarly, for *G. melastomus,* individuals larger than 49.48 and 49.57 cm had a 50 % and 95 % probability of exceeding the limit (R2 = 1).

Regarding Pb, two out of 30 *M. poutassou* specimens had concentrations above the 0.3 µg/g ww limit set by the EC. Finally, none of the analysed individuals exceeded or approached the Cd limit for consumption.

In all the different scenarios approached for assessing consumer's exposure to trace metals (adult of 75 kg, adult of 60 kg or child of 35 kg eating once to three times a week a portion of a given species), the EWI for Pb and Cd were far below the PTWI of 25 and TWI of 2.5 µg/kg bw for both children and adults. The estimated %PTWI of Pb per portion of selected species ranged from 0.02 to 0.62 % **(Table S3)** and the %TWI of Cd ranged from 0.01 to 0.46 % **(Table S4)**. However, for MeHg, the estimated intake per portion varied considerably across species, with sharks exceeding the TWI of 1.3 µg/kg bw set by EFSA (EFSA, 2012) across all consumer groups **(Fig. 4; Table 3)**. Several scenarios assessing %TWI based on consumer group and intake frequency also surpassed the safety threshold. Estimated values ranged from 2.72 % TWI corresponding to a 75 kg adult consuming *M. merluccius* once a month to as high as 1144 % TWI for a 60 kg adult consuming mature *S. canicula* three times per week **(Table 3)**.

4. Discussion

4.1. Mercury (Hg) concentration drivers among marine species

The trophic level of a certain species within the food web is a well-established determinant of its Hg concentration, primarily due to biomagnification processes (Fitzgerald et al., 2007;

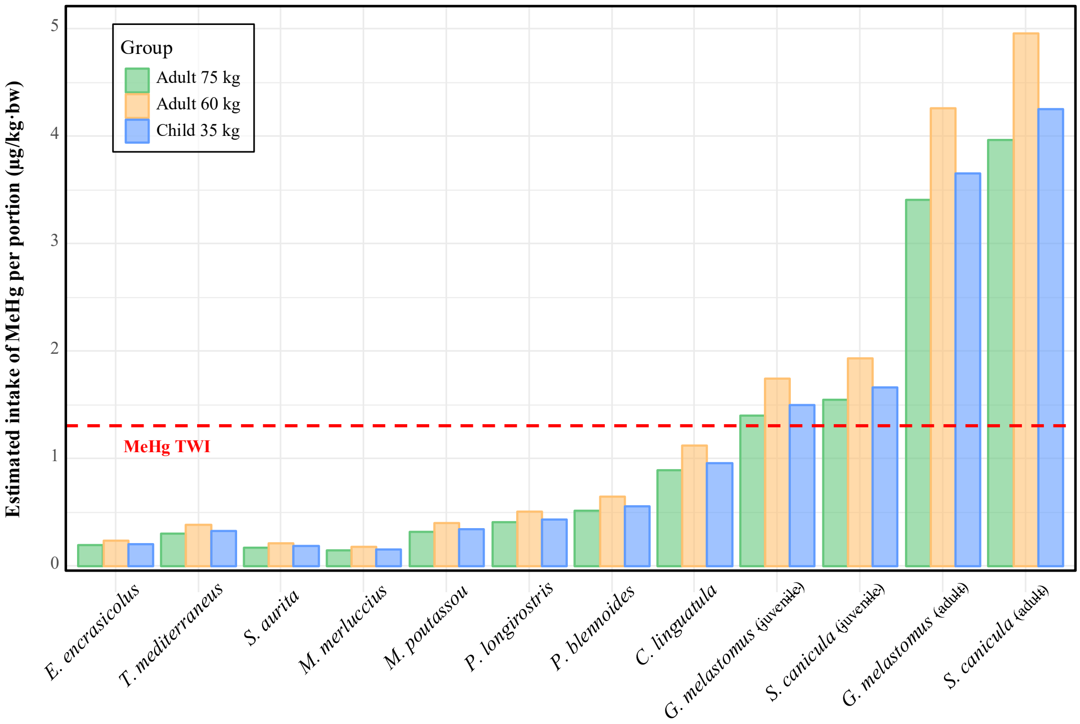


Figure 4. Estimated methyl mercury (MeHg) intake per portion (corresponding to 150 g for adults and 75 g for children) of the different analysed species from off the Catalan coast, expressed in µg/kg · body weight (bw) depending on the consumer weight (an adult of 75 or 60 kg, or a child of 35 kg). In the case of sharks, values are provided separately for juveniles and adults according to their size at maturity reported by Ramírez-Amaro et al. (2020) and unpublished data by present authors. The red dashed line corresponds to the MeHg Tolerable Weekly Intake (TWI) of 1.3 µg/kg bw set by the European Food Safety Authority (EFSA).

Madgett et al., 2021). However, Hg accumulation is also significantly influenced by the species habitat and depth range, along with the habitat of its preferred prey. This relationship is highlighted in the present study and is consistent with findings from other studies from the Mediterranean Sea (Cresson et al., 2014; Koenig et al., 2013a) and the Atlantic and Pacific oceans (Chouvelon et al., 2012; Choy et al., 2009). MeHg is a toxic form of Hg that easily accumulates in marine organisms (Cossa et al., 2022). The methylation processes that convert inorganic mercury into MeHg occur predominantly in low-oxygen zones with elevated bacterial activity. Moreover, bacterial demethylation processes are low in deep marine ecosystems (Blum et al., 2013; Cossa et al., 2009). In addition, sediments serve as a significant source of contamination as methylated Hg is absorbed by sinking particles and, once in the sediments, enters benthic trophic webs through burrowing species (Cresson et al., 2014). Therefore, benthic species are particularly susceptible to MeHg accumulation, as evidenced by the high concentrations found in the present study. Conversely, pelagic species that inhabit and feed primarily above the thermohalocline, where MeHg is less available, are less exposed to its

Table 3. Estimated methyl mercury (MeHg) intake per portion (corresponding to 150 g for adults and 75 g for children) of the different species from off the Catalan coast in µg/kg body weight and corresponding percentage of the Tolerable Weekly Intake (%TWI) depending on the consumer (an adult weighing 75, 60 kg or a child 35 kg) and frequency of consumption (once a month, once a week and three times per week). In the case of sharks, values are provided separately for juveniles and adults according to the size at maturity reported by Ramírez-Amaro et al. (2020) and unpublished data by present authors. In bold, values above the TWI set by the EFSA (EFSA, 2012).

	Estimated intake of MeHg per portion (µg/kg·bw)			**% TWI**								
				1 portion a month			**1 portion a week**			**3 portions a week**		
Species	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg
Engraulis encrasicolus	0.19	0.24	0.20	3.68	4.60	3.9	14.7	18.4	15.8	44.1	55.2	47.3
Trachurus mediterraneus	0.30	0.38	0.33	5.85	7.31	6.3	23.4	29.2	25.1	70.2	87.7	75.2
Sardinella aurita	0.17	0.21	0.18	3.29	4.11	3.5	13.2	16.4	14.1	39.5	49.3	42.3
Merluccius merluccius	0.14	0.18	0.15	2.72	3.40	2.9	10.9	13.6	11.7	32.6	40.8	35.0
Micromesistius poutassou	0.32	0.40	0.34	6.12	7.65	6.6	24.5	30.6	26.2	73.4	91.8	78.7
Parapenaeus longirostris	0.40	0.50	0.43	7.77	9.71	8.3	31.1	38.8	33.3	93.2	**117**	**100**
Phycis blennoides	0.52	0.64	0.55	9.90	12.4	10.6	39.6	49.5	42.5	**119**	**149**	**127**
Citharus linguatula	0.89	1.12	0.96	17.2	21.5	18.4	68.8	86.0	73.7	**206**	**258**	**221**
Galeus melastomus juvenile	1.40	1.74	1.49	26.8	33.5	28.8	**107**	**134**	**115**	**322**	**402**	**345**
Scyliorhinus canicula juvenile	1.55	1.93	1.66	29.8	37.9	31.9	**119**	**149**	**127**	**357**	**446**	**382**
Galeus melastomus adult	3.41	4.26	3.65	65.5	81.9	70.2	**262**	**328**	**281**	**786**	**983**	**843**
Scyliorhinus canicula adult	3.97	4.96	4.25	76.3	95.4	81.7	**305**	**381**	**327**	**915**	**1144**	**981**

accumulation. For instance, pelagic apex predators from the Balearic Sea such as swordfish (*Xiphias gladius* Linnaeus, 1758), Atlantic bluefin Tuna (*Thunnus thynnus* Linnaeus, 1758), albacore (*Thunnus alalunga* Bonnaterre, 1788) or skipjack tuna (*Katsuwonus pelamis* Linnaeus, 1758) show lower Hg concentrations (0.8 ± 0.5; 0.7 ± 0.0; 0.2 ± 0.1 and 0.1 ± 0.0 µg/g ww, respectively) (Belmonte et al., 2021; Chanto-García et al., 2021; Girolametti et al., 2023) compared to the demersal species from this study, despite occupying higher trophic levels. Similarly, benthopelagic species like *M. poutassou,* which feed mainly on myctophids (which in turn feed on planktonic organisms) and other pelagic organisms (Mir-Arguimbau et al., 2020), are also less exposed to Hg uptake through its diet compared to other benthopelagic species that mostly feed on suprabenthic and epibenthic species.

Intra-specific variability has been mainly associated with body size, as a proxy for age and hence, exposure time, (Storelli et al., 2007), since MeHg readily bioaccumulates in muscle tissue (Watanabe et al., 2012). Current results suggest a positive correlation between body length and MeHg in most teleost species and even in the decapod *P. longirostris*. However, limited size variability and low sample numbers available for these species limits the statistical significance of these results. In the case of sharks, an exponential increase of MeHg accumulation with age is clearly observed; a tendency already reported in several fish species (Cresson et al., 2014; Magalhães et al., 2007) and crustaceans (Barghigiani et al., 2000). This exponential accumulation has been attributed to faster growth rates in immature individuals, which allows Hg to be more effectively diluted through the synthesis of new tissue (Cresson et al., 2014). Upon reaching maturity, energy shifts towards reproduction, slowing growth and increasing bioaccumulation rates (Barghigiani et al., 2000). Furthermore, ontogenic dietary shifts towards higher trophic level prey could contribute to increase Hg accumulation (Cossa et al., 2012). For instance, juvenile individuals of *G. melastomus* or *S. canicula* primarily feed on crustaceans, while adults prey on fish and cephalopods (Carrassón et al., 1992; Šantić et al., 2012). This dietary shift also helps explaining the lower Hg concentrations observed in juveniles of *M. merluccius*, typically caught in the continental shelf of the Catalan coast (Muns-Pujadas et al., 2024), compared to those reported in by Llull et al. (2017)(0.071 vs 0.3 µg/g ww), as their study sampled more offshore waters, capturing older individuals (Mean TL =18.9 vs 33.8 cm).

4.2. Lead (Pb) and cadmium (Cd) in marine species

Neither habitat use nor individual body length explained Cd or Pb accumulation patterns, according to present results. Mean concentrations for these elements across species were low, ranging from 0.0001 to 0.0046 µg/g ww for Cd and from 0.003 to 0.062 µg/g ww for Pb. This is primarily due to the fact that these metals accumulate in organs such as the kidney and liver (or hepatopancreas) due to their roles in detoxification processes, and gills, which are in direct contact with the environment, with concentrations in muscle tissue typically being trace (Castro-González and Méndez-Armenta, 2008; Dang and Wang, 2009; Moiseenko and Gashkina, 2020; Storelli and Marcotrigiano, 2004). For example, a study on P. *longirostris* from the Ionian Sea found Cd levels to be 10 times higher in the cephalothorax, which contains the hepatopancreas, compared to muscle tissue (Soultani et al., 2019).

In the present study, the highest Cd concentrations were observed in *P. longirostris*, while *M. poutassou* exhibited the highest Pb levels (0.062 µg/g ww), consistent with values reported in southern Italy (0.06 µg/g ww) (Storelli et al., 2020). As seen in other studies, fish species generally show lower Cd and Pb levels in muscle compared to shellfish, particularly bivalve molluscs and gastropods, which often accumulate these metals at higher magnitudes (Falcó et al., 2006; Jureša and Blanuša, 2003; Olmedo et al., 2013). Nonetheless, some chondrichthyans such as *Etmopterus spinax* Linnaeus, 1758 and *Chimaera monstrosa* Linnaeus, 1758 have been reported to exceed the EU Cd and Pb thresholds, highlighting species-specific accumulation patterns (Carrasco-Puig et al., 2024). No correlation between Pb or Cd levels in muscle tissue and size was detected in any of the species studied here. In contrast, Sánchez-Marín & Beiras (2008) reported size-dependent Pb accumulation in bivalve molluscs, which may be primarily attributed to the fact that Pb was measured in the entire individual, thereby including those organs that preferentially accumulate this metal.

Although habitat use did not significantly relate to Cd and Pb concentrations in this study, future research should consider the proximity to coastal zones and estuaries, as water metal concentrations in these areas can be several orders of magnitude higher due to weathering and anthropogenic inputs (Suárez-Serrano et al., 2010; Vicente-Martorell et al., 2009). This might explain the higher concentrations of Cd and Pb in very small individuals of *S. canicula* (<25 cm), which are typically found in shallower coastal areas, where egg-laying occurs (Ebert et al., 2021). Changes in diet and variations in the mechanisms of metabolization and detoxification during growth may also contribute to this surprising pattern (Gallo et al., 2023). In addition, environmental factors such as salinity, pH and temperature may affect the bioavailability and bioaccumulation of these metals (Belivermis¸ et al., 2020; Moiseenko and Gashkina, 2020; Zhang and Reynolds, 2019).

4.3. *Scyliorhinus canicula* as a biomonitoring species

No small-scale differences among the three areas from off the Catalan coast regarding Pb and Cd were found, while, surprisingly, individuals from off Blanes displayed higher Hg concentrations compared to those from Barcelona. Submarine canyons, due to their shape and proximity to the coast, act as preferential pathways for the transport of sediments, particulate matter, and pollutants, including heavy metals (Company et al., 2012; Dumas et al., 2014; Palanques et al., 2022). In the Catalan margin, the Palamós and Blanes canyons are considered major hotspots for suspended and downgradient sediment fluxes (Company et al., 2012; Zúñiga et al., 2009). This enhanced transport may explain the higher Hg exposure observed in individuals caught off Blanes (see Blanes canyon on **Fig. 1**). Similarly, as contaminants tend to accumulate at the head of the canyon (Jesus et al., 2010; Koenig et al., 2013b; Richter et al., 2009), the reduced Hg concentration observed in *G. melastomus,* compared to *S. canicula*, which inhabits shallower waters (Massutí and Moranta, 2003), could be related to these differential depth ranges among sharks. Although other studies have reported positive correlations between muscle Hg concentrations and depth (Cresson et al., 2014; Magalhães et al., 2007), no such relationship was observed in *S. canicula*. In the water column of the Northwestern Mediterranean, dimethylmercury (DMHg, one of the methylated forms of MeHg) was found to be maximum at 500 m depth, where apparent oxygen utilization (AOU), an indicator of bacterial activity, is highest (Cossa and Coquery, 2005). Further research is needed to explore in deeper detail the role of depth in Hg bioaccumulation and determine if there is a specific depth at which maximum Hg accumulation occurs, followed by a subsequent decline.

The comparison of trace metal concentrations in *S. canicula* from the Mediterranean Sea with those from Atlantic areas revealed no significant differences in Pb concentrations. Obtained Pb concentrations were consistent with those reported in other studies of the same species (Mille et al., 2018), although some inconsistencies exist across the literature (Bouchoucha et al., 2019; Filice et al., 2023; Türkmen et al., 2009). In contrast, Cd levels were significantly higher in all Atlantic areas compared to the Catalan coast. This may be due to elevated dissolved Cd in waters as observed in the Gulf of Cádiz (Laiz et al., 2020) or natural upwelling processes occurring in the Galician coast (Besada et al., 2011b). However, since Cd primarily accumulates in organs such as the liver and kidneys rather than in muscle tissue, the muscle Cd levels may not fully reflect the total Cd burden in the organisms and, therefore, caution is needed when interpreting these patterns.

Regarding Hg, significant differences were found among all sites, with the highest concentrations observed in samples from off the Catalan Coast, followed by those from individuals captured in the Gulf of Cádiz, the Cantabrian Sea, and the Galician coast. As previously mentioned, this may be attributed to the so-called “Mediterranean mercury anomaly” whereby the Mediterranean Sea exhibits high methylation potentials that increase Hg availability within trophic webs (Cossa et al., 2022). In addition, the oligotrophic nature of the Mediterranean Sea results in lower primary productivity, reducing the "biodilution" of contaminants within trophic webs (Cresson et al., 2014). This oligotrophy also leads to slower growth rates, meaning that individuals in the Mediterranean have longer exposure times to Hg compared to their Atlantic counterparts of the same size (Cossa et al., 2012). This may explain the higher Hg concentration found in *S. canicula* from the Catalan coast and other nearby areas such as the Gulf of Lion and Bay of Marseille, where maximum Hg levels of 7.13 and 8.96 µg/g ww, respectively, have been documented (Bouchoucha et al., 2019; Chouvelon et al., 2018; Cresson et al., 2014) **(Table 4)**. Studies from various Italian regions generally report lower Hg concentrations in *S. canicula* compared to the Northwestern Mediterranean, although these still are much higher than those found in the Atlantic Ocean **(Table 4)**. The elevated Hg levels observed in samples from the Gulf of Cádiz compared to the other two Atlantic areas can likely be attributed to the discharge of two major rivers, the Guadiana and Guadalquivir, as well as the Tinto-Odiel river system, which have been historically impacted by mining activities (Besada et al., 2022; González-Ortegón et al., 2018). In addition, the region receives Hg influx from the Mediterranean Sea through the Strait of Gibraltar (Cossa et al., 2022). Further studies with larger sample sizes and standardized body sizes are needed to better support the small and large-scale variability in Hg concentrations observed in the present study. In addition, studies on *S. canicula* Hg accumulation in the Levantine basin and southern part of the Mediterranean are needed to fully uncover geographic patterns across its distribution range.

Biomonitors are biological processes, organisms or communities of organisms that can provide measurable data on the quality of an environment, including heavy metals, and that can be used to establish geographical and temporal comparisons (Markert et al., 2003; Luoma and Rainbow, 2008). Elasmobranchs, due to their long lifespan and high-trophic level within marine food webs, can be used as reliable indicators of certain pollutants due to their bioaccumulation potential (Alves et al., 2022).

Scyliorhinus canicula is a benthic elasmobranch with a broad bathymetric and geographic distribution throughout the Eastern Atlantic and Mediterranean Sea (Ebert et al., 2021; Massutí and Moranta, 2003). Its high abundance and resilience to fishing pressure, being a common by-catch species normally discarded (Carbonell et al., 2003; ICATMAR, 2024; Rodríguez-Cabello et al., 2005), offers the opportunity to collect samples from fisheries without the need for dedicated scientific sampling efforts. These characteristics, together with its sedentary and philopatric behaviour, especially in females (which helps detection of small-scale geographical differences) (Rodríguez-Cabello et al., 2004; Sims et al., 2001), and its significant Hg bioaccumulation capacity, make *S. canicula* an ideal species for monitoring Hg levels and

Table 4. Comparison of mercury (Hg) concentrations in muscle tissue of *Scyliorhinus canicula* caught off different areas reported in the present and other studies. Mean values are followed by standard deviation and range of values (minimum – maximum). Hg concentrations expressed in µg / g wet weight (ww). For concentrations given as a function of dry weight transformations to wet weight were calculated either using the percentage of water content reported in each given study or assuming a 75% of water content if this information was absent. ND = no data available.

Sampling area	TL (cm)	N	Hg concentration (µg / g ww)	Sampling year	Reference
Mediterranean Sea					
Alicante	26.7 ± 2.5	11	0.05 ± 0.02 (0.03 - 0.08)	2018 - 2021	Capodiferro et al., 2022
Ebro Delta	32.0 ± 1.6 (28.5 - 33.7)	10	0.87 ± 0.50 (0.53 - 2.24)	2019	Present study
Barcelona coast	36.2 ± 8.8 (19 - 49)	69	1.18 ± 0.77 (0.31 - 3.55)	2019 - 2023	Present study
Blanes coast	41.8 ± 1.6 (39.5 - 44.2)	10	2.53 ± 0.80 (1.30 - 3.75)	2019	Present study
Catalan coast (adults)	43.4 ± 2.7	43	1.98 ± 0.81 (0.62 - 3.75)	2019 - 2023	Present study
Catalan coast (juveniles)	29.8 ± 5.6	46	0.77 ± 0.51 (0.31 - 2.24)	2019 - 2023	Present study
Balearic Islands	ND	8	0.78 (0.39 - 1.5)	2014 - 2016	Llull et al., 2017
Balearic Islands	39.4 ± 6.3	12	1.32 ± 1.19 (0.39 - 3.77)	2018 - 2021	Capodiferro et al., 2022
Balearic Islands (Ciutadella)	ND	ND	3.8	2014	Junqué et al., 2017
Balearic Islands (Maó)	ND	ND	1.1	2014	Junqué et al., 2017
Gulf of Lions	38.2 ± 12.7 (16.4 - 56.9)	17	1.88 ± 1.87 (- 7.13)	2012	Cresson et al., 2014
Gulf of Lions	48 ± 5 (41 - 57)	9	2.86 ± 2.01 (1.22 - 7.13)	2012	Chouvelon et al., 2018
Cassidaigne canyon (Bay of Marseille)	41.7 ± 5.9 (24.4 - 50.9)	63	1.61 ± 1.34 (0.23 - 8.96)	2015	Bouchoucha et al., 2019
Stoechades Canyon (Gulf of Hyères)	37 ± 6.2 (24.4 - 50)	82	0.85 ± 0.31 (0.00 - 1.86)	2015	Bouchoucha et al., 2019
Different Italian areas	ND	14	1.17 (0.17 - 2.32)	2009 - 2011	Brambilla et al., 2013
Markets from Apulian region (South Italy)	ND	49	0.61 ± 0.04	2020	Storelli et al., 2022
Adriatic Sea	43.7 ± 4.6 (36.5 - 49)	70	1.49 ± 0.61 (0.79 - 2.56)	1999	Storelli et al., 2002
Adriatic Sea	ND	12	1.1 ± 0.62 (0.26 - 2.06)	2003	Storelli et al., 2005
Atlantic Ocean					
Atlantic Ocean	ND	48	(0.13 - 0.8)	2007	Coelho et al., 2010
Scotland	ND	44	0.49 (0.26 - 0.99)	2015 - 2017	Madgett et al., 2021
Celtic Sea	61.1 ± 3 (55 - 64)	8	0.48 ± 0.20 (0.20 - 0.78)	2000	Domi et al., 2005
Bay of Biscay North (CIEM VIIIa)	49 ± 7.5 (36 - 62)	25	0.51 ± 0.15 (0.29 - 0.95)	1986	Cossa et al., 1990
Mancha occidental (CIEM VIIe)	59.6 ± 4.7 (48.5 - 66)	25	0.64 ± 0.30 (0.25 - 1.28)	1987	Cossa et al.,1990
Mancha oriental (CIEM VIId)	58.9 ± 3.2 (53.5 - 65)	24	1.05 ± 0.35 (0.51 - 1.91)	1988	Cossa et al., 1990
Bay of Biscay	57.9 ± 3.1 (53 - 63)	10	0.56 ± 0.31 (0.25 - 1.22)	2008	Chouvelon et al., 2018
Cantabrian Sea	33.7 ± 1.5 (30.8 - 35.9)	24	0.21 ± 0.05 (0.15 - 0.35)	2013	Present study
Galician coast	32.1 ± 1.6 (29 - 35.4)	24	0.12 ± 0.03 (0.07 - 0.19)	2013	Present study
Portuguese coast (adults)	53.3 ± 3 (50 - 64)	41	0.28 ± 0.17	2018	Marques et al., 2021
Portuguese coast (juveniles)	44.9 ± 3.9 (31.5 - 49.5)	33	0.10 ± 0.11	2018	Marques et al., 2021

identifying areas with high methylation potential as evidenced in the present study. As discussed earlier, Pb and Cd accumulate in the liver rather than muscle tissue (Castro-González and M´endez-Armenta, 2008; Dang and Wang, 2009; Moiseenko and Gashkina, 2020; Storelli and Marcotrigiano, 2004). For instance, Higueruelo et al. (2024) reported Cd and Pb concentrations in the liver of *S. canicula* to be 228 and five times higher, respectively, than in the muscle tissue. Consequently, the liver would be a more suitable target organ for environmental monitoring studies. In contrast, for food safety assessments, the liver might not be such a good representative as it is not typically consumed.

4.4. Risk assessment and recommendations for consumption

Results from the present study, in agreement with previous research, raise concerns regarding the safety associated with the consumption of benthic species from the Northwestern Mediterranean Sea (Cresson et al., 2014; Koenig et al., 2013a; Storelli and Barone, 2013). Various studies on commercially important species in the Mediterranean have reported individuals with Hg values exceeding the established limits. Notable examples include the red shrimp (*Aristeus antennatus* Risso, 1816), Norway lobster (*Nephrops norvegicus* Linnaeus, 1758), blackbellied angler (*Lophius budegassa* Spinola, 1807), angler (*Lophius piscatorius* Linnaeus, 1758)*,* dusky grouper *(Epinephelus marginatus* Lowe, 1834)*,* red mullet (*Mullus barbatus* Linnaeus, 1758), and common sole *(Solea solea* Linnaeus, 1758) (Capodiferro et al., 2022; Cresson et al., 2014; Junqué et al., 2017; Koenig et al., 2013a; Llull et al., 2017; Storelli and Barone, 2013).

According to our data, there is a high likelihood that an adult *S. canicula* or *G. melastomus* caught off the Catalan coast may exceed the Hg concentration limits set by the European Union (Regulation 2023/ 915). In accordance with the current legislation, individuals larger than 37 and 49.5 cm, respectively, caught off the Catalan coast, would not be suitable for commercialisation. Similarly, individuals of *C. linguatula* larger than 19 cm would not be suitable for consumption either. In contrast, the pelagic and benthopelagic fish species analysed herein comply with the current legislation. Only two individuals of *M. poutassou* showed Pb levels above the European legislation, indicating a 99,46 % compliance across all the species analysed for this metal. In the case of Cd, present results indicate a 100 % of compliance. These findings align with previous studies reporting safe Pb and Cd concentrations in fish and shellfish species from the Catalan coast (Nadal et al., 2008) and sold in Catalan markets (Falcó et al., 2006; González et al., 2019).

The latest report from AESAN, reporting values of several contaminants in different foodstuffs as part of the "*Program 11: Control of Contaminants in Food*" (AESAN, 2022b), highlights that mercury is the contaminant with the highest rate of non-compliance. Specifically, 4.15 % of the analysed samples exceeded the permissible regulatory limits for Hg, detected exclusively in fish and fish-derived products, though the report did not specify the species or the region of origin. In the present study, 20.75 % of the samples from NW Mediterranean surpassed the permissible limits for Hg. In the same AESAN report, a high percentage of compliance with legislation regarding Pb and Cd in fish and fish-derived products is reported, similarly to this study. To improve risk assessments and optimize monitoring efforts, future reports should include more detailed data on the fishing area and specific species exceeding EU limits, which would allow more accurate consumption recommendations.

The risk assessment presented in the present study has revealed that the consumption of fish from the Catalan coast, at least for the selected species, contributes minimally to the maximum intake of Cd and Pb recommended by the authorities (EFSA, 2009; FAO/WHO, 1999). In the worst-case scenarios, an adult of 60 kg eating three portions of *M. poutassou* a week would ingest only 1.9 % of Pb PTWI (0.47 µg/kg bw), and consuming three portions of *P. longirostris* would correspond to 1.38 % of Cd TWI (0.04 µg/kg bw) **(Tables S3 and S4)**.

In contrast, the weekly intake of MeHg raises more significant concerns **(Table 4)**. In the case of sharks, even one portion of a juvenile shark per week exceeds the TWI of 1.3 µg/kg bw, for both children and adults, while a single portion of adult shark meat per week would be close to the limit for an adult weighing 60 kg (EFSA, 2012). In particular, consuming a single portion of adult *S. canicula* once a week would represent more than three times the TWI for both adults and children. Hypothesizing a high consumption of three portions a week, an adult weighing 60 kg would be ingesting more than 10 times the recommended tolerable intake. The consumption of other benthic species like *C. linguatula,* should be limited to once a week. Present results also suggest that consumption of *P. longirostris* and *P. blennoides* should be limited to twice a week, especially during pregnancy and childhood.

The consumption of shark meat in the Catalan coast is relatively low, and the most captured elasmobranchs in the NW Mediterranean, *S. canicula* and *G. melastomus*, are usually discarded (Carbonell et al., 2003; ICATMAR, 2024). Nonetheless, in the Mediterranean and many other regions worldwide, elasmobranch species are frequently sold under mislabelled or generic names, which can lead to consumers unknowingly purchasing shark and batoid meat (Bornatowski et al., 2013; Giovos et al., 2020, 2021; Pardo and Jiménez, 2020). A recent study revealed that misidentification of elasmobranchs caught off the Catalan coast is very common, with species often being landed skinned, without head and viscera, making proper identification challenging. Additionally, 55 % of captured rays are classified under “*Raja* sp.”, a designation that includes species not only from the genus *Raja,* but also from several different families (Barría and Colmenero, 2019). Future studies on trace metal concentrations in the Mediterranean Sea should consider including ray species, as they are also benthic consumed species, that may potentially contain elevated Hg levels. Proper identification of sharks and ray meat is paramount to ensure a safe and informed consumption of these species.

In general, benthic species from the Mediterranean Sea, especially elasmobranchs, should be avoided by pregnant women and children. As the current European legislation does not account for the specific characteristics of the Mediterranean in terms of methylation potential, Hg levels observed in the NW Mediterranean indicate that large specimens of benthic sharks, such as *S. canicula* or *G. melastomus,* are not in compliance with the existing regulation and shall not be sold for consumption.

Having said that, fish and seafood remain an important dietary source of energy, protein, and a range of other nutrients, including the long-chain n-3 polyunsaturated fatty acids (omega-3), which are beneficial to health (FAO/WHO, 2023). Even during pregnancy, eating fish reduces the risk of suboptimal neurodevelopment in offspring, and consumption of low-mercury fish is recommended (FDA, 2024). For instance, small pelagic fish from the Catalan coast have been found to be both safe and healthy, and its regular consumption is recommended (Muns-Pujadas et al., 2025; Rodríguez-Romeu et al., 2022). In terms of Pb and Cd, muscle concentrations of selected species from the Catalan coast are within safe levels for consumption, although the ingestion of brown meat in crustaceans (*i.e.*, meat from the cephalothorax), which contains elevated Cd levels, should be limited (AESAN, 2011).

5.Conclusions

Interspecific variability in muscular Hg accumulation among marine organisms appears to be largely influenced by their habitat use, while intra-specific differences strongly correlate with body size. In contrast, Cd and Pb do not significantly accumulate in muscle tissue, with reported concentrations remaining low. *Scyliorhinus canicula* emerges as a suitable biomonitoring species for detecting Hg hotspots. For monitoring Cd and Pb, other organs such as liver, or alternative species like shellfish should be prioritised. A literature review on Hg muscle concentrations in *S. canicula* highlighted notably high concentrations in the Northwestern Mediterranean, raising concerns about the consumption of this species, regardless of its size and site of capture, as well as of other benthic organisms in the area. Notably, present findings show a substantial proportion of benthic samples exceeding European safety limits, thereby questioning their suitability for commercialization. This underscores the need for further research focusing on other benthic commercially important species that could also represent a potential risk to consumers. Nonetheless, as with top predators such as tunas, the consumption of benthic species from the Mediterranean should be limited, especially during pregnancy and by children, as a precautionary measure. Overall, the present study highlights the importance of integrating regional environmental conditions and biological factors to accurately assess trace metal accumulation patterns in marine species.

CRediT authorship contribution statement

Andrea Higueruelo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Victoria Besada:** Writing – review & editing, Methodology, Investigation. **Paula S´anchez-Marín:** Writing – review & editing, Methodology, Investigation. **Laura Muns-Pujadas:** Writing – review & editing, Methodology. **Maria Constenla:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Sara Dallar´es:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Ester Carreras- Colom:** Writing – review & editing, Methodology, Investigation. **Oriol Rodríguez-Romeu:** Writing – review & editing, Methodology. **Anna Soler-Membrives:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Table S1. Limits of detection (LOD) obtained in the different analyses carried out by the Chemical Analysis Service from the Autonomous University of Barcelona (SAQ) and the Spanish Institute of Oceanography (IEO). Obtained results are expressed in micrograms per gram of dry weight (µg/g dw).

	Ni	Cu	Zn	As	Cd	Pb	Hg
IEO MARS 6	< 0.024	< 0.012	< 3.77	< 0.014	< 0.0007	< 0.008	< 0.003
IEO Hot plate	0.003	0.0005	0.005	0.0003	0.0003	0.0008	0.0008
SAQ - UAB	< 0.019	< 0.093	< 0.93	< 0.019	< 0.0009	< 0.009	-
IEO 2013	-	0.5	0.3	0.3	0.005	0.05	0.003

Table S2. Mean total length and muscle concentrations of nickel (Ni), copper (Cu), zinc (Zn) and arsenic (As) (standard deviation) of different species caught off the Catalan coast. In the case of *Scyliorhinus canicula*. values from samples caught off Atlantic areas are also reported. All values are given in µg / g wet weight (ww). In the case of crustaceans. total length refers to cephalothorax length.

	Total length (cm)	Ni (µg/g ww)	Cu (µg/g ww)	Zn (µg/g ww)	As (µg/g ww)
Engraulis encrasicolus	11.52 (0.62)	0.019 (0.023)	1.45 (0.27)	11.31 (2.77)	5.22 (1.10)
Trachurus mediterraneus	19.13 (1.76)	0.007 (0.006)	0.54 (0.15)	3.37 (1.44)	1.77 (0.78)
Sardinella aurita	14.97 (0.84)	0.009 (0.004)	1.17 (0.22)	5.26 (0.56)	6.14 (2.22)
Merluccius merluccius	18.87 (4.38)	0.103 (0.243)	0.22 (0.07)	3.08 (0.25)	3.62 (0.8)
Micromesistius poutassou	22.01 (1.29)	0.051 (0.062)	0.17 (0.03)	2.92 (0.36)	7.76 (2.05)
Parapenaeus longirostris	3.03 (0.36)	0.052 (0.069)	4.21 (0.71)	11.97 (0.99)	24.82 (9.53)
Phycis blennoides	18.96 (2.41)	0.006 (0.003)	0.14 (0.02)	2.54 (0.23)	10.30 (2.59)
Citharus linguatula	16.34 (2.00)	0.023 (0.060)	0.22 (0.36)	3.25 (0.28)	6.34 (1.80)
Galeus melastomus	40.83 (10.59)	0.008 (0.009)	0.25 (0.08)	3.09 (0.64)	17.39 (8.22)
Scyliorhinus canicula **Catalan coast**	36.24 (8.13)	0.023 (0.031)	0.49 (0.19)	10.22 (4.33)	24.15 (8.06)
S. canicula **Gulf of Cádiz**	33.12 (1.91)	-	0.35 (0.15)	7.01 (0.89)	16.19 (3.59)
S. canicula **Galicia coast**	33.70 (1.50)	-	0.37 (0.07)	6.96 (0.73)	9.96 (2.06)
S. canicula **Cantabrian Sea**	32.08 (1.58)	-	0.36 (0.07)	7.24 (0.81)	12.29 (2.23)

Table S3. Estimated lead (Pb) intake per portion (corresponding to 150 g for adults and 75 g for children) of the different species from off the Catalan coast in µg/kg body weight and corresponding percentage of the Potential Tolerable Weekly Intake (% PTWI) depending on the consumer (an adult weighing 75, 60 kg or a child 35 kg) and frequency of consumption (once a month, once a week and three times per week). In the case of sharks, values are provided separately for juveniles and adults according to the size at maturity reported by Ramírez-Amaro et al. (2020) and unpublished data by present authors.

	Estimated intake of Pb per portion (µg/kg·bw)			**% PTWI**								
				1 portion a month			**1 portion a week**			**3 portions a week**		
Species	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg
Engraulis encrasicolus	0.012	0.015	0.012	0.01	0.01	0.01	0.05	0.06	0.05	0.14	0.17	0.15
Trachurus mediterraneus	0.012	0.015	0.013	0.01	0.02	0.01	0.05	0.06	0.05	0.15	0.18	0.16
Sardinella aurita	0.085	0.106	0.091	0.08	0.11	0.09	0.34	0.42	0.36	1.02	1.27	1.09
Merluccius merluccius	0.075	0.093	0.080	0.07	0.09	0.08	0.30	0.37	0.32	0.90	1.12	0.96
Micromesistius poutassou	0.125	0.156	0.134	0.12	0.16	0.13	0.50	0.62	0.53	1.50	1.87	1.60
Parapenaeus longirostris	0.027	0.034	0.029	0.03	0.03	0.03	0.11	0.14	0.12	0.33	0.41	0.35
Phycis blennoides	0.006	0.008	0.007	0.01	0.01	0.01	0.02	0.03	0.03	0.07	0.09	0.08
Citharus linguatula	0.008	0.010	0.009	0.01	0.01	0.01	0.03	0.04	0.04	0.10	0.12	0.11
Galeus melastomus juvenile	0.013	0.017	0.014	0.01	0.02	0.01	0.05	0.07	0.06	0.16	0.20	0.17
Scyliorhinus canicula juvenile	0.045	0.056	0.048	0.05	0.06	0.05	0.18	0.23	0.19	0.54	0.68	0.58
Galeus melastomus adult	0.011	0.014	0.012	0.01	0.01	0.01	0.04	0.06	0.05	0.13	0.17	0.14
Scyliorhinus canicula adult	0.018	0.022	0.019	0.02	0.02	0.02	0.07	0.09	0.08	0.21	0.27	0.23

Table S4. Estimated cadmium (Cd) intake per portion (corresponding to 150 g for adults and 75 g for children) of the different species from off the Catalan coast in µg/kg body weight and corresponding percentage of the Potential Tolerable Weekly Intake (% TWI) depending on the consumer (an adult weighing 75, 60 kg or a child 35 kg) and frequency of consumption (once a month, once a week and three times per week). In the case of sharks, values are provided separately for juveniles and adults according to the size at maturity reported by Ramírez-Amaro et al. (2020) and unpublished data by present authors.

	Estimated intake of Cd per portion (µg/kg·bw)			**% TWI**								
				1 portion a month			**1 portion a week**			**3 portions a week**		
Species	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg	Adult 75 kg	Adult 60 kg	Child 35 kg
Engraulis encrasicolus	0.008	0.010	0.009	0.08	0.10	0.09	0.33	0.41	0.35	0.98	1.23	1.05
Trachurus mediterraneus	0.002	0.002	0.002	0.02	0.02	0.02	0.07	0.09	0.08	0.22	0.27	0.23
Sardinella aurita	0.001	0.002	0.001	0.01	0.02	0.01	0.05	0.06	0.05	0.14	0.18	0.15
Merluccius merluccius	0.002	0.002	0.002	0.02	0.02	0.02	0.07	0.09	0.08	0.22	0.27	0.23
Micromesistius poutassou	0.003	0.003	0.003	0.03	0.03	0.03	0.10	0.13	0.11	0.31	0.39	0.33
Parapenaeus longirostris	0.009	0.012	0.010	0.09	0.12	0.10	0.37	0.46	0.39	1.10	1.38	1.18
Phycis blennoides	0.000	0.000	0.000	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.03	0.03
Citharus linguatula	0.000	0.000	0.000	0.00	0.00	0.00	0.01	0.01	0.01	0.02	0.03	0.03
Galeus melastomus juvenile	0.003	0.004	0.004	0.03	0.04	0.04	0.14	0.17	0.15	0.41	0.51	0.44
Scyliorhinus canicula juvenile	0.006	0.008	0.007	0.06	0.08	0.07	0.25	0.31	0.27	0.74	0.93	0.80
Galeus melastomus adult	0.002	0.003	0.002	0.02	0.03	0.02	0.08	0.10	0.09	0.24	0.30	0.26
Scyliorhinus canicula adult	0.004	0.005	0.004	0.04	0.05	0.04	0.14	0.18	0.15	0.43	0.54	0.46

CHAPTER 4

Testimonial of ecological and biogeographic patterns: parasite assemblages of deepwater catsharks (Pentanchidae) in Icelandic waters

Andrea Higueruelo, Bjoern C. Schaeffner, Anna Soler-Membrives and Sara Dallarés

Abstract

Pentanchids (Elasmobranchii) are among the most species-rich groups of chondrichthyans. In the North Atlantic Ocean, the Icelandic catshark [*Apristurus laurussonii* (Saemundsson)], white ghost catshark (*Apristurus aphyodes* Nakaya & Stehmann), and mouse catshark [*Galeus murinus* (Collett)] are commonly found in deepwater habitats. However, information on their parasite communities remains scarce. This study provides the first comprehensive characterisation of the metazoan parasite communities of the three pentanchid species in Iceland. In total, 17 specimens of *A. aphyodes*, 14 *A. laurussonii*, and 25 *G. murinus* were collected at depths of 466–1,322 m during autumns of 2023 and 2024 in southern and western Icelandic waters. Sharks were examined using standardised parasitological protocols, including morphological and molecular identification methods. Parasites infection patterns were assessed in relation to host size, maturity, Le Cren body condition index (Kn, as a proxy of hosts health condition) and capture area. Parasite loads in all sharks ranged from 2 to 227 individuals, comprising 15 different taxa and resulting in 27 new parasite-host records, some of which likely representing new species. Eight out of nine commonly found parasites (prevalence > 25% in at least one host species) did not display a high degree of host-specificity, indicating similar feeding habits, niche, and trophic position of these sympatric species. Nonetheless, multivariate analyses revealed significant differences in the structure and composition of their parasite assemblages, with some parasites representing indicator species and occurring more abundantly and frequently in a certain deepwater catshark species. Significant small-scale geographic differences were found in parasite assemblages of *A. aphyodes*. At a broader geographical scale, North Atlantic pentanchids showed higher parasite richness and diversity, and lower dominance compared to standardised data from Mediterranean counterparts. Ecological factors underlying these patterns on host-parasite dynamics in (deepwater) catsharks are discussed.

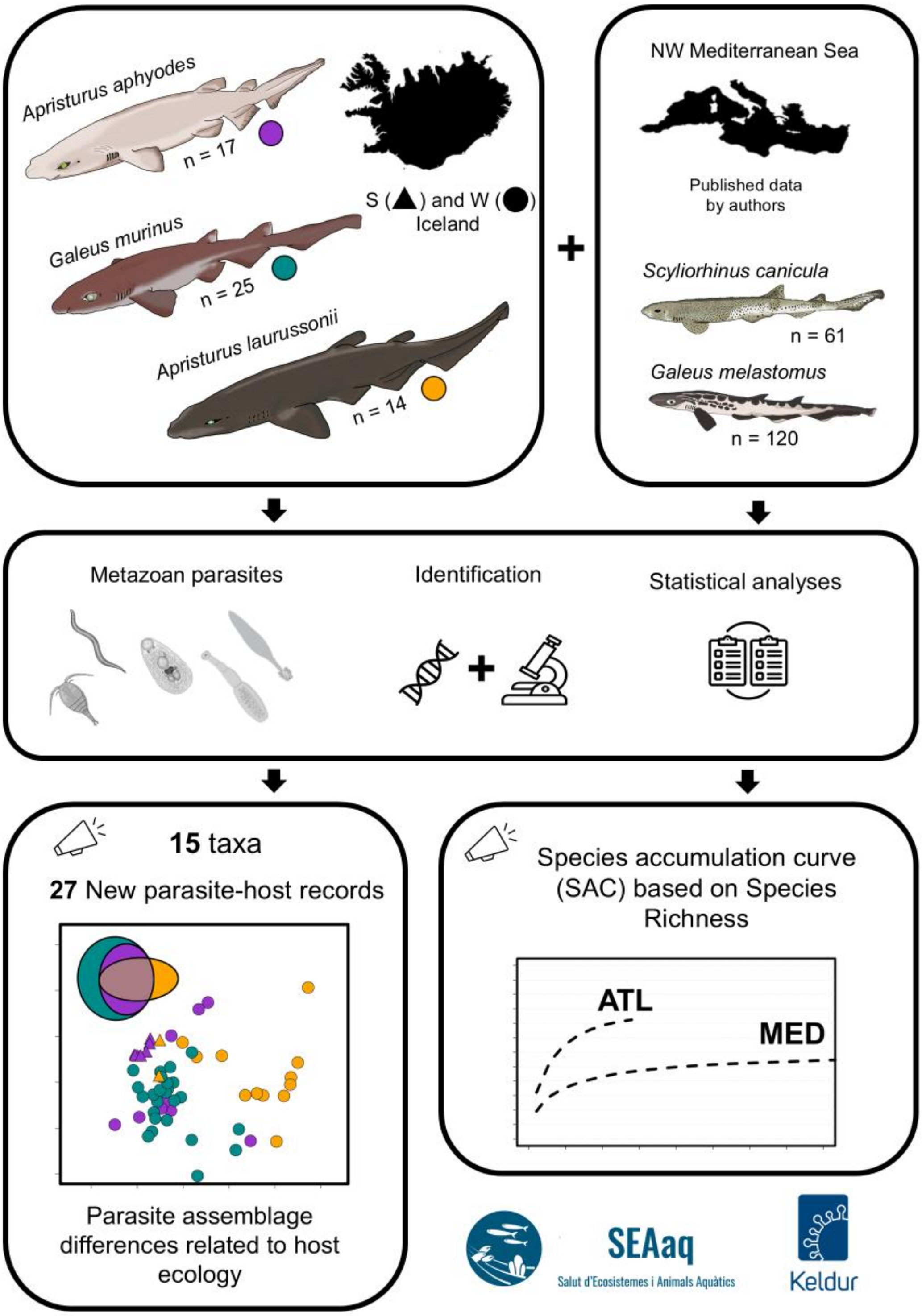
Keywords

Pentanchidae, Elasmobranchii, Parasites, Parasite ecology, North Atlantic Ocean, Epidemiology

Highlights

- Hidden parasite diversity in three Icelandic pentanchid sharks was uncovered
- 15 parasite taxa were detected with 27 new parasite-host records
- Parasite community differed geographically between West and South Iceland
- Icelandic sharks showed higher parasite richness than Mediterranean counterparts
- Parasites of deep-sea sharks are indicators of the host biology and ecology

Graphical abstract



1. Introduction

Catsharks are a diverse group of small, bottom-dwelling, generally non-migratory sharks characterised by their elongated, cat-like eyes, adapted for seeing in low light conditions (Compagno, 1984). They were originally classified within the family Scyliorhinidae. However, following a taxonomic revision that distinguished between catsharks and deepwater catsharks, the latter were transferred into a separate family, the Pentanchidae (Iglésias et al., 2005), now considered the largest family of living sharks. Despite this speciosity, many pentanchid sharks are poorly known, most likely due to their life history in deep waters, where research is scarce and still expanding (Ebert et al., 2021). Although the deep sea comprises over 90% of world oceans and represents the largest biome on this planet, vast areas remain unknown and discovery rates of new species are high (Ramirez-Llodra et al., 2010; Selbach & Paterson, 2025).

Despite the ecological significance and unique marine environment of Icelandic waters (NE Atlantic Ocean), their marine biodiversity remains relatively understudied (Omarsdottir et al., 2012). In this region, three pentanchids, namely the Iceland catshark [*Apristurus laurussonii* (Saemundsson)], the white ghost catshark (*Apristurus aphyodes* Nakaya & Stehmann) and the mouse catshark [*Galeus murinus* (Collett)] are among the most frequent chondrichthyans (Jakobsdóttir et al., 2023). They are small (*i.e.,* less than 80 cm in length), bottom dwelling species distributed in the North-East Atlantic Ocean (*A. laurussonii* shows the broadest distribution encompassing North and Central Atlantic waters) and found across a wide depth range in the continental slopes (between 380 and 2,060 m, see Ebert et al., 2021). Currently classified as "Least Concern" in the International Union for Conservation of Nature's Red List of Threatened Species, they are generally attributed stable population trends (*A. laurussoni* and *G. murinus*) (Iglésias, 2015; Kulka et al., 2020; Walls, 2015). Despite their abundance and ecological importance in North Atlantic waters, knowledge on their basic biology (*i.e.*, diet, behaviour) or parasite infections, among others, is still extremely limited.

Parasites represent a predominant portion of living organisms (Price, 1980) and play a significant role in determining the structure of communities and ecosystems through interactions with their hosts, influencing their behaviour and fitness and ultimately regulating their populations (Price et al., 1986; Thomas et al., 1998; Wood et al., 2007). They are also useful bioindicators, being able to provide valuable information on their host species, such as trophic interactions and migration patterns (and thus habitat preferences based on prey availability) (Alarcos & Timi, 2013; Dallarés et al., 2017; Williams et al., 1992), or to reveal responses of free-living populations and communities to environmental impacts (MacKenzie, 1999; Vidal-Martínez et al., 2010). They have been used for many decades as indicators of fish population stocks, to address host phylogenetic relationships (Locke et al., 2013; MacKenzie & Abaunza, 1998) and, more recently, to even help assessing the effectiveness of protected conservation areas (Braicovich et al., 2021). Despite playing a vital role in marine ecosystems and constituting an important component of Ocean's biodiversity, fish parasites have often been neglected in biodiversity and ecosystemic studies (Klimpel et al., 2006).

As for many other North Atlantic elasmobranch species, studies on parasite communities of Icelandic deepwater catsharks are almost entirely absent. For instance, only a single parasite species has been cited from *A. laurussonii* and *A. aphyodes*, the cestodes *Ditrachybothridium macrocephalum* Rees, 1959 and *Yamaguticestus kuchtai* Caira, Bueno & Jensen, 2021 (Bray & Olson, 2004; Caira et al., 2021), respectively, while parasite records from *G. murinus* are entirely lacking.

In contrast, there are a considerable number of studies on different ecological aspects of the two most common catsharks distributed not only in the Atlantic Ocean but also in the Mediterranean Sea, namely the blackmouth catshark (*Galeus melastomus* Rafinesque) and the small-spotted catshark [*Scyliorhinus canicula* (L.)] (Follesa et al., 2019; Massutí & Moranta, 2003). Their parasite community is particularly well-known and characterised, with 20 and 29 parasite species, respectively, reported across their respective distribution ranges, including monogeneans, cestodes, trematodes, nematodes, copepods and isopods (see Pollerspöck & Straube, 2025 for a complete list of references). In the Balearic Sea alone, 15 and 12 parasite species have been reported infecting *G. melastomus* and *S. canicula*, respectively (Dallarés et al., 2017; Higueruelo et al., 2024). Given the high diversity of parasites found in Mediterranean catsharks, and considering the higher biomass, species richness and abundance of deep-sea fish assemblages in the Atlantic (Massutí et al., 2004), it is likely that North Atlantic catsharks will reveal broader parasite communities with potentially new species yet to be discovered.

Although molecular ecology and the use of genetic tools are still poorly applied in parasitology compared to free-living organisms (Selbach et al., 2019), molecular tools are of great interest for addressing parasite species identification and host specificity (Criscione et al., 2005). These tools are highly advisable for the characterisation of parasite assemblages, where larval forms (impossible to identify solely based on morphological features), cryptic species and phenotypic plasticity frequently occur. Therefore, combining traditional parasitological techniques based on morphology with molecular analyses is the most effective approach for studying parasite communities across different ecological and geographical contexts.

Parasitological investigations play a critical role in deepening our understanding of biodiversity and the complex interactions within the marine environment. In order to broaden the available knowledge on parasite infection patterns in catsharks from an ecological perspective, the parasite communities infecting *A. laurussonii*, *A. aphyodes*, and *G. murinus* from Icelandic waters were characterised and described for the first time in the present study. In addition, differences among these parasite assemblages as a function of different factors (*e.g.,* host species, host maturity, area of capture) were assessed and parasitological descriptors and infection patterns were analysed jointly with data from Mediterranean catsharks and discussed from an ecosystemic approach.

2. Materials and methods

2.1. Study area and sample collection

A total of 17 specimens of *A. aphyodes*, 14 *A. laurussonii* and 25 G. murinus were collected in autumn of 2023 and 2024 at depths ranging between 466 and 1,322 m **(Table 1)** in southern and western Icelandic waters (North Atlantic Ocean). Samples were collected in the frame of the annual Icelandic Autumn Groundfish Surveys from the Marine and Freshwater Research Institute Iceland (MFRI) on board of the research vessels Árni Friðriksson and Breki. For comparative analysis, the sampling stations were divided into southern and western areas **(Figure 1)**.

Immediately upon capture, a photograph of each individual was taken and records of total length (TL, in cm), total weight (TW, in g) and sex were obtained for each shark individual. Five spiral valves from *A. aphyodes* were immediately preserved in 95% EtOH and five and four spiral valves, from *A. laurussonii* and *A. aphyodes*, respectively, were preserved in 4% buffered formalin for molecular and morphological parasite identification purposes. Specimens were frozen at -20 ºC for further examination.

2.2. Dissection procedure and parasitological study

Prior to dissection, the external surfaces of each individual were examined macroscopically for ectoparasites. After removal of the abdominal organs (*i.e.,* liver, gonads, stomach, spiral valve, spleen, and pancreas), which were preserved separately for further examination, the eviscerated weight (EW) was recorded. Subsequently, the remaining organs (*i.e.*, nostrils, gills, heart, kidneys, and brain) were also removed. Maturity was inferred from the overall appearance of

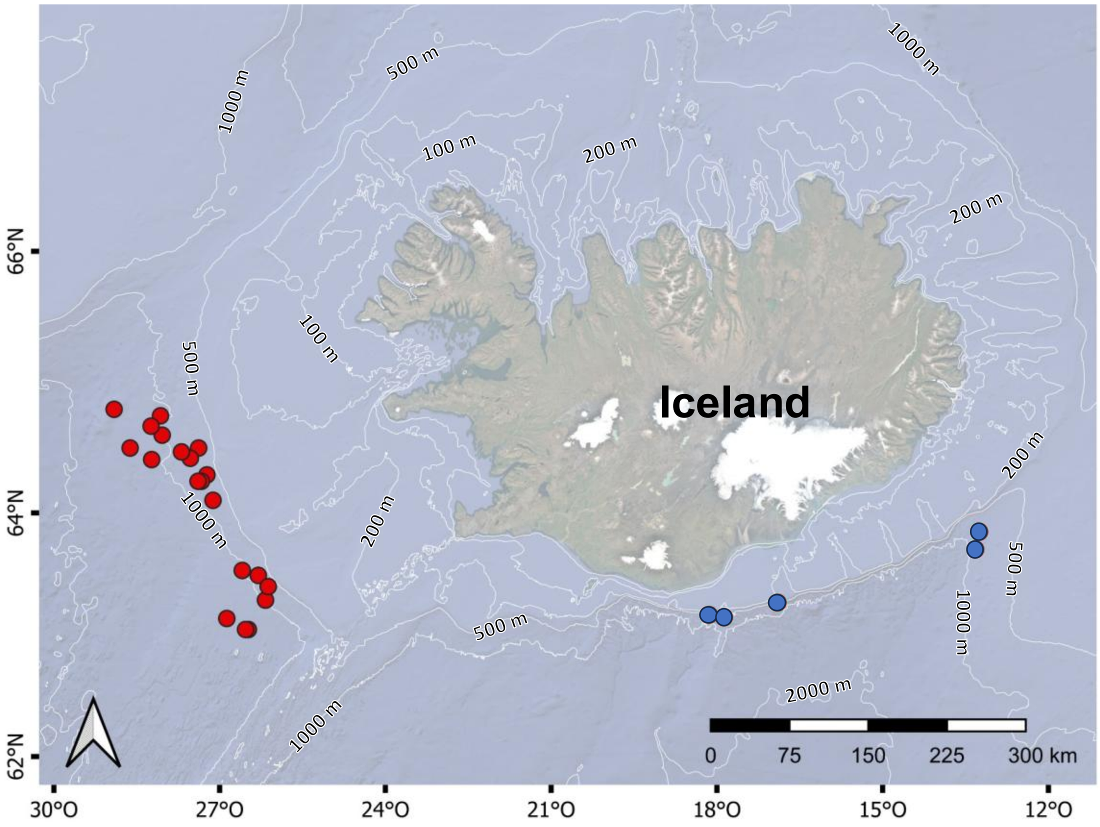


Figure 1. Map of the study area. Dots indicate the sampling stations where the pentanchid sharks were collected. Red dots: western area; blue dots: southern area.

Table 1. Biometric data of *Apristurus aphyodes*, *Apristurus laurussonii* and *Galeus murinus* sampled in Icelandic waters. N: sample size; (*): number of females; Maturity: Percentage of sexually mature individuals. Mean values followed by standard deviation and range values (minimum - maximum) of depth of collection, total length (TL), total weight (TW) and Le Cren relative condition index (Kn). Different superscript letters indicate statistically significant differences among host species.

	A. aphyodes	*A. laurussonii*	*G. murinus*
N (*)	17 (9)	14 (2)	25 (21)
Maturity (%)	94.1	92.9	68.0
Depth (m)	745 (233) 546–1,278	1,112 (159) 796–1,281	852 (197) 466–1322
TL (cm)	60.9 (7.8)^{a} 51.5–71.0	67.3 (3.7)^{a} 61.5–75.3	49.5 (6.5)^{b} 30.5–54.9
TW (g)	798.4 (206.9) 472.0–1,086.0	941.3 (165.0) 672.0–1,180.0	453.5 (141.4) 91.5–622.2
Kn	1.01 (0.11) 0.84–1.31	1.01 (0.12) 0.76–1.17	0.97 (0.07) 0.83–1.11

reproductive organs, the degree of clasper calcification in males, and the presence of egg capsules in females (Higueruelo et al., *in press*).

All organs were examined for metazoan parasites under a stereomicroscope. In nine individuals, the liver or gonads were discarded on board for reasons beyond the authors' control and were therefore unavailable for examination or inclusion in subsequent analyses. Mouth- and abdominal cavity were washed with 0.9% saline solution to recover detached parasites potentially present in these cavities. The musculature between pectoral and caudal fins was cut into thin slices and thoroughly inspected for potential encysted endoparasites. All recovered parasites were counted and stored in 70% ethanol.

For morphological identification, platyhelminths were stained either with Delafield’s haematoxylin or iron acetocarmine, dehydrated through a graded series of ethanol, cleared in clove oil and permanently mounted in Canada balsam on microscope slides. Nematodes were examined as semi-permanent mounts in pure glycerine. All parasites were identified to the lowest possible taxonomic level.

Parasites selected for molecular identification were preserved in 95% EtOH in the freezer. When possible, hologenophores (sensu Pleijel et al., 2008) were prepared. When specimens were too small, a morphologically identical voucher was selected. Representative voucher specimens were deposited in the parasitological collection of the Zoology unit of the Universitat Autònoma de Barcelona (Barcelona, Spain) (Accession numbers: M3 to M8, C47 to C51 and D9 to D10).

Genomic DNA was extracted using a QIAgen DNA extraction kit or a QIAcube HT system, following the manufacturer’s protocol. Mitochondrial cytochrome oxidase 1 (mtCOI) and Internal transcribed spacer (ITS) for nematodes and partial nuclear large subunit ribosomal DNA (28S rDNA) for the rest of parasites were amplified by polymerase chain reaction (PCR) amplifications. These were performed as described in Constenla et al. (2014) or Brabec et al. (2012), respectively, adjusted for Taqman Expression mastermix. The PCR-products were analysed by capillary electrophoresis using a High-Resolution DNA kit in a Qiaxcel Advanced Instrument and viewed in the Qiaxcel ScreenGel (Qiagen) or analysed on RedGel-stained 1 % TAE agarose gels. Sequencing of PCR products was performed by Genewiz-Azenta or Macrogen Inc. using either the Sanger method or capillary electrophoresis, respectively. Obtained sequences were aligned using BioEdit 7.7.1 (Hall, 1999) checked visually for accuracy, and compared to available sequences in Genbank with Mega v.11 (Tamura et al., 2021).

2.3. Data analysis

Parasite prevalence (P), mean abundance (MA), mean species richness (MSR) and species richness (S) were calculated for each host species following Bush et al. (1997). A 95 % confidence interval for the mean abundance was calculated with the software Quantitative Parasitology

(QPweb) (Reiczigel et al., 2019). Parasite diversity was estimated by Brillouin's index (H) and calculated with PRIMER 6 software (Anderson et al., 2008). The Berger-Parker dominance index (B-P dom) was calculated as the proportion of individuals belonging to the most abundant parasite species relative to the total number of parasites in each individual host. Le Cren's relative body condition index (Kn) was calculated separately for each shark species with the formula Kn = EW / (α X TLβ), where α and β are the slope and the intercept of the weight-length relationship, of the entire dataset of sampled fish (Le Cren, 1951). Parasite taxa with a prevalence < 5% in all hosts were considered accidental, while parasite taxa with >25 % prevalence in at least one host species were considered common.

Fish biometric data (TL and Kn) and parasite infection parameters were tested for normality and homoscedasticity using the Shapiro-Wilk test and Levene's test, respectively. Data distribution was also plotted for visual assessment. When necessary, variables were log or square root transformed to comply with normality and homoscedasticity requirements for parametric tests.

To detect potential associations in each host species between individual fish biological data and parasitological descriptors (*i.e.,* richness, total abundance, abundance of common parasite taxa and diversity), Pearson's or Spearman's correlation tests (the latter when normality was not satisfied) were used. Differences in parasitological descriptors among the three host species were tested using ANOVA for parametric data and Wilcoxon or Kruskal–Wallis tests for non-parametric data, with post hoc pairwise comparisons performed using TukeyHSD and Dunn's tests (Bonferroni-adjusted), respectively. Fisher Exact Test and subsequent pairwise comparisons with the function *pairwiseNominalIndependence* were performed to test differences among the three host species for common parasites' prevalence. Whenever sample size was high enough (with at least eight individuals in each group), these potential differences were also tested between immature and mature individuals (for *G. murinus*) and between western and southern sampling areas (for *A. aphyodes*). Differences among areas were tested after ruling out potential interactions with host TL, which were evaluated using Generalized Linear Models (GLMs). Distributions were selected according to data type: Poisson for count data (*e.g.*, S), binomial with a logit link function for prevalence data, and Gaussian or Gamma for parametric and non-parametric variables, respectively.

Ordination of parasite infracommunities (*i.e.,* all parasite taxa infecting a given individual host) according to the different hosts and sampling areas was visualised with a non-metric multidimensional scaling (nMDS) based on a Bray-Curtis dissimilarity matrix calculated from log + 1 transformed species abundance data. An Euler diagram was also constructed to illustrate the amount of specific or shared parasite taxa among host species. A PERMANOVA (Permutational Analysis of Variance) was conducted using parasite abundance and prevalence data (using a Bray-Curtis and Jaccard dissimilarity matrix, respectively) to reveal potential differences in the composition and structure of parasite assemblages across hosts and areas (the latter only for *A. aphyodes*). PERMANOVA analyses were performed using the *Adonis2* function, followed by pairwise tests, with 999 unrestricted permutations of raw data. The Indicator Value Index (IndVal) (Dufrêne & Legendre, 1997) was then applied to identify the most representative parasite species for each host species and of each area in the case of *A. aphyodes*.

Previously published data by present authors on parasite infection parameters of the two most common Mediterranean catsharks (*i.e., S. canicula* and *G. melastomus*) (Dallarés et al., 2017; Higueruelo et al., 2024) were used to explore large-scale geographic patterns, something possible because parasitological protocols matched those followed in the present study. Differences on parasitological indices (*i.e.*, MA, S, H, B-P dom and MA of each parasite phylum) between Mediterranean and Atlantic catsharks were tested with Wilcoxon and Pearson's Chi-squared Test. Species accumulation curves (SACs) were used to predict and compare total species numbers for each host and study area using the *specaccum* function with random method and 999 permutations. Statistical analyses were conducted with R version 4.4.1. Correlations were considered strong when the correlation coefficient (R) was higher than 0.65. Statistical significance was set at 0.05.

3. Results

A total of 56 pentanchid shark individuals were examined for parasites, 82.1% of them being sexually mature. Overall, sharks TL ranged between 30.5 and 75.3 cm, with *G. murinus* being significantly smaller than *Apristurus* species (K-W, $\chi^2 = 33.92$, $p < 0.001$) **(Table 1)**.

3.1. Parasite composition and parasitological descriptors of Icelandic pentanchids

A total of 2,780 metazoan parasites belonging to 15 different taxa were recovered from the three analysed shark species, including one nematode, one cirriped, two copepods, four monogeneans, five cestodes and two digeneans **(Table 2)**. These findings represent 27 new parasite-host records. All sharks were parasitised by at least one parasite, with parasite abundance ranging from 2 to 227.

Table 2. Descriptors of parasite component populations (i.e., all parasites of a given species infecting a given host population) on the three pentanchid species captured off Iceland. Developmental stage, location within host, prevalence (P %) and mean abundance (MA, followed, in parentheses, by a 95% confidence interval) are provided for the parasites found in *Apristurus aphyodes, Apristurus laurussonii* and *Galeus murinus*. For *A. aphyodes*, values are also presented separately for two areas of capture: west and south of Iceland. Abbreviations for infection sites within host: BC, body cavity; G, gills; GO, gonad; L, liver; M, muscle; N, nostrils, P, pancreas; S, stomach; SK, skin; SP, spleen and SV, spiral valve. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Mt, metacercaria; Pd, plerocercoid; Ps, plerocercus. Different superscript lowercase and capital letters show significant differences in the mean abundance and prevalence, respectively, of parasite populations among host species.

				A. aphyodes (West)		***A. aphyodes*** (South)		***A. aphyodes***		***A. laurussonii***		***G. murinus***	
Parasite taxa		**Stage**	**Infection site**	**P%**	**MA (95% CI)**	**P%**	**MA (95% CI)**	**P%**	**MA (95% CI)**	**P%**	**MA (95% CI)**	**P%**	**MA (95% CI)**
Nematoda													
	Anisakis Type I *	L3	BC, GO, K, L, M, P, S, SP, SV	100	28.3 (14.3–47 .2)	100	68.9 (41.6–94.8)	100 ^{A}	47.4 ^{a} (31.7–68.2)	57.1^{B}	14.3 ^{b} (4.1–45.6)	100 ^{A}	45.7 ^{a} (33.2–64.8)
Crustacea													
Thecostraca	*Anelasma squalicola*	A	SK, L									12.0	0.1 (0–0.2)
Copepoda	*Lernaeopodina* sp.	A	G									4.0	0.04 (0–0.1)
	Taeniacanthidae gen. sp.	A	N									4.0	0.04 (0–0.1)
Platyhelminthes													
Monogenea	*Calicotyle* sp. *	J, A	BC, SP	77.8	3.9 (1.3–7.8)			41.2	2.1 (0.6–5.1)	14.3	0.1 (0–0.4)	32.0	1.0 (0.4–1.9)
	Cathariotrematinae gen. sp. *	J, A	N							71.4	4.5 (2.6–6.8)		
	Hexabothriidae gen. sp. *	J, A	G	55.6	3.6 (1.3–6.1)			29.4 ^{A}	1.9 ^{a} (0.6–3.7)	78.6^{B}	3.0 ^{ab} (1.6–4.9)	96.0 ^{B}	5.7 ^{b} (4.1–8.1)
	Squalotrema sp.	J, A	N									16.0	0.2 (0–0.3)
Cestoda	*Ditrachybothridium macrocephalum* *	A	SV	66.7	3.6 (1.0–7.8)			35.3	1.9 (0.5–4.7)			48.0	4.2 (1.9–8.5)
	Grillotia sp *	Ps	M	44.4	0.6 (0.1–1.0)			23.5	0.3 (0.1–0.6)			36.0	0.8 (0.3–2.2)
	Hepatoxylon trichiuri *	Pd	GO, L, S	22.2	0.3 (0–0.9)			11.8	0.2 (0–0.5)	7.1	0.1 (0–0.2)	32.0	0.6 (0.2–1.0)
	Heterosphyriocephalus tergestinus	Pd	S									4.0	0.04 (0–0.1)
	Yamaguticestus kuchtai *	A	SV	44.4	7.6 (1.7–23.9)			23.5	4.0 ^{ab} (0.8–16.7)	50.0	0.7 ^{a} (0.3–1.1)	12.0	0.1 ^{b} (0–0.2)
Trematoda	*Otodistomum cestoides* *	Mt	S	22.2	0.2 (0–0.4)			11.8	0.1 (0–0.3)			48.0	0.6 (0.1–2.3)
	Digenea indet.	A	BC							14.3	0.1 (0 – 0.3)		

Table 3. Descriptors of parasite component communities on the three pentanchid species captured off Iceland. Total richness (S), mean species richness (MSR), total mean abundance (TMA), Brillouin Diversity Index (H) and Berger-Parker Dominance Index (B-P dom) are displayed for parasite assemblages characterised in sharks captured off Iceland (*Apristurus aphyodes, Apristurus laurussonii and Galeus murinus*) and in the Balearic Sea (*Galeus melastomus* and *Scyliorhinus canicula*) (Dallarés et al., 2017; Higueruelo et al., 2024). For A. aphyodes, values are also presented separately for two areas of capture: west and south of Iceland. N = number of individuals. 95% confidence interval is shown in brackets for MSR, TMA, H and B-P dom. Different superscript letters indicate statistically significant differences among host species.

	Iceland (present study)					**Mediterranean Sea** (Dallarés et al., 2017; Higueruelo et al., 2024)	
	A. aphyodes (West)	***A. aphyodes*** (South)	***A. aphyodes***	***A. laurussonii***	***G. murinus***	***G. melastomus***	***S. canicula***
N	9	8	17	14	25	120	61
S	8	1	8	7	13	15	12
MSR	4.3 (3.4–5.3)	1	2.8 (1.8–3.8)^{ac}	2.9 (2.3–3.6)^{ab}	4.1 (3.5–4.7)^{b}	1.7 (1.5–1.9)^{c}	2.4 (2.2–2.6)^{a}
TMA	48.0 (5.2–69.4)	68.9 (34.9–102.8)	57.8 (39.8–75.8)^{ac}	22.9 (5.6–40.1)^{ab}	59.1 (40.0–78.2)^{ac}	40.8 (30.9–50.8)^{b}	55.2 (43.9–66.4)^{c}
H	0.72 (0.56–0.88)	0	0.38 (0.18–0.58)^{ac}	0.56 (0.38–0.73)^{ac}	0.59 (0.48–0.70)^{a}	0.15 (0.11–0.18)^{b}	0.30 (0.25–0.36)^{c}
B-P dom	0.68 (0.57–0.80)	1	0.83 (0.73–0.93)^{abc}	0.69 (0.58–0.79)^{ac}	0.75 (0.69–0.82)^{a}	0.93 (0.90–0.95)^{b}	0.87 (0.84–0.90)^{c}

Among the recovered parasites, five taxa were found in all three analysed hosts. *Anisakis* Type I (sensu Berland, 1961), found as third stage larvae encysted in several organs and displaying the highest prevalence (89.3% overall prevalence) was identified as *Anisakis simplex* (Rudolphi, 1809) in the three hosts (GenBank accession numbers: PV933132, PX101432). Shared monogeneans consisted of a yet undescribed species of *Calicotyle* Diesing, 1850 infecting the rectum, and a potentially new species of the family Hexabothriidae infecting the gills (Gen bank accession numbers: PV972204, PV972205, PV972206), with overall prevalences of 30.4 and 71.4%, respectively. Plerocercoids of the cestode *Hepatoxylon trichiuri* (Holten, 1802) (Gen bank accession number: PV972208) were found encysted in the gonad, liver and stomach wall with a prevalence of 19.6%, while adult specimens of *Yamaguticestus kuchtai* Caira, Pickering & Jensen, 2021 (Gen bank accession numbers: PV972202, PV972203) were found infecting the spiral valves in 25% of examined sharks.

In *A. aphyodes* and *G. murinus*, trypanorhynch plerocerci and a metacercariae encysted in the tail musculature and stomach wall, respectively, were also commonly found and genetically identified as *Grillotia adenoplusia* (Gen bank accession number: PV972201) and *Otodistomum cestoides* (Van Beneden, 1870) (Gen bank accession number: PV972207).

In total, eight parasite taxa were found in *A. aphyodes*, none of which were exclusive to this species (Table 2). The most prevalent and abundant parasite was *A. simplex*. The yet undescribed species of *Calicotyle* (*Calicotyle* sp.) showed the highest prevalence and abundance in this host, with up to 13 parasites found in a single shark individual. For this host, a strong positive correlation between Berger-Parker dominance index and fish TL was found (rho = 0.91, $p < 0.001$) while parasite richness and Brillouin's index were negatively associated with fish TL (rho = - 0.79 and -0.70, $p < 0.002$).

In *A. laurussonii*, the most prevalent parasites were the monogeneans Hexabothriidae gen. sp. and Cathariotrematinae gen. sp. (Genbank accession number: PV972210); the latter found infecting the nostrils and exclusively in this species. The abundance of *A. simplex* in *A. laurussonii* was positively correlated with fish TL (rho = 0.71, p = 0.005) and parasite richness with fish Kn (rho = 0.67, p = 0.008).

All examined specimens of *G. murinus* were infected with *A. simplex*, and all but one individual with Hexabothriidae gen. sp.. These were the two most abundant parasites in this host, reaching maximum abundances of 182 and 19 parasites, respectively. Five parasite taxa were exclusive to *G. murinus*: the monogenean *Squalotrema* sp., the copepods *Lernaeopodina* sp. and Taeniacanthidae gen. sp., the cestode *Heterosphyriocephalus tergestinus* (Pintner, 1913) and the cirriped *Anelasma squalicola* Darwin, 1852 (Genbank accession number: PV972209). The latter species was typically found externally attached near the mouth and, interestingly, in one case the parasite had perforated the skin and was found in the liver. Total length of *G. murinus* was positively correlated with parasite total abundance and with abundance of *A. simplex* (rho = 0.65 and 0.70, $p < 0.001$). Concordantly, significant differences in the same two parasitological descriptors were observed between juvenile and adult host specimens, with higher values found in mature individuals (t-test, $t = -3.73$ and $t = -4.06$, respectively, $p < 0.003$ in both cases). Parasite assemblages of adult sharks also displayed a higher dominance index (Wilcoxon test, $W = 30.50$, $p = 0.031$).

3.2. Host-related and geographical patterns of parasite communities of Icelandic pentanchid sharks

The nMDS ordination plot based on parasite abundance data (stress = 0.167) showed a grouping pattern based on host identity **(Figure 2)**. The most similar intraspecific parasite assemblages were those of *G. murinus* and *A. aphyodes*, which showed highest mean intraspecific Bray-Curtis similarity indices. Regarding interspecific comparisons, *A. aphyodes* and *G. murinus* displayed the most similar assemblages while *A. laurussonii* assemblages were the most differentiated. PERMANOVA analyses revealed significant differences in both the structure (Bray-Curtis similarity index, $F = 10.70$, $p < 0.001$) and composition (Jaccard similarity index, $F = 12.67$, $p < 0.001$) of parasite communities among the three hosts. Subsequent pairwise comparisons confirmed that these differences were present across the three host species ($F = 4.69$–14.93, $p < 0.003$ in all cases). The Euler diagram **(Figure 2)** illustrated that out of the 15 parasite taxa identified, seven were exclusively found in one host, six of these classified as uncommon or accidental ($P < 25\%$). The indicator value analysis identified Cathariotrematinae gen. sp. as strongly associated with its single host *A. laurussonii* (IndVal = 0.71, $p = 0.001$) while Hexabothriidae gen. sp and *D. macrocephalum* were moderately associated with *G. murinus,* indicating that they occur more frequently and abundantly in this species (IndVal = 0.52, $p = 0.003$ and IndVal = 0.33, $p = 0.045$, respectively). No significant indicator species were detected for *A. aphyodes.*

Parasite mean species richness was significantly higher in *G. murinus* compared to *A. aphyodes* (K-W, $\chi^2 = 8.39$, $p = 0.015$) and total parasite abundance was lower in *A. laurussonii* compared to the other two hosts (ANOVA, $F = 6.44$, $p = 0.003$). Similarly, the abundance and prevalence of *A. simplex* was significantly lower in *A. laurussonii* (ANOVA, $F = 8.55$, $p<0.001$; Fisher Test, $p < .001$). The prevalence of Hexabothriidae gen. sp. was lower in *A. aphyodes* (Fisher Test, p <

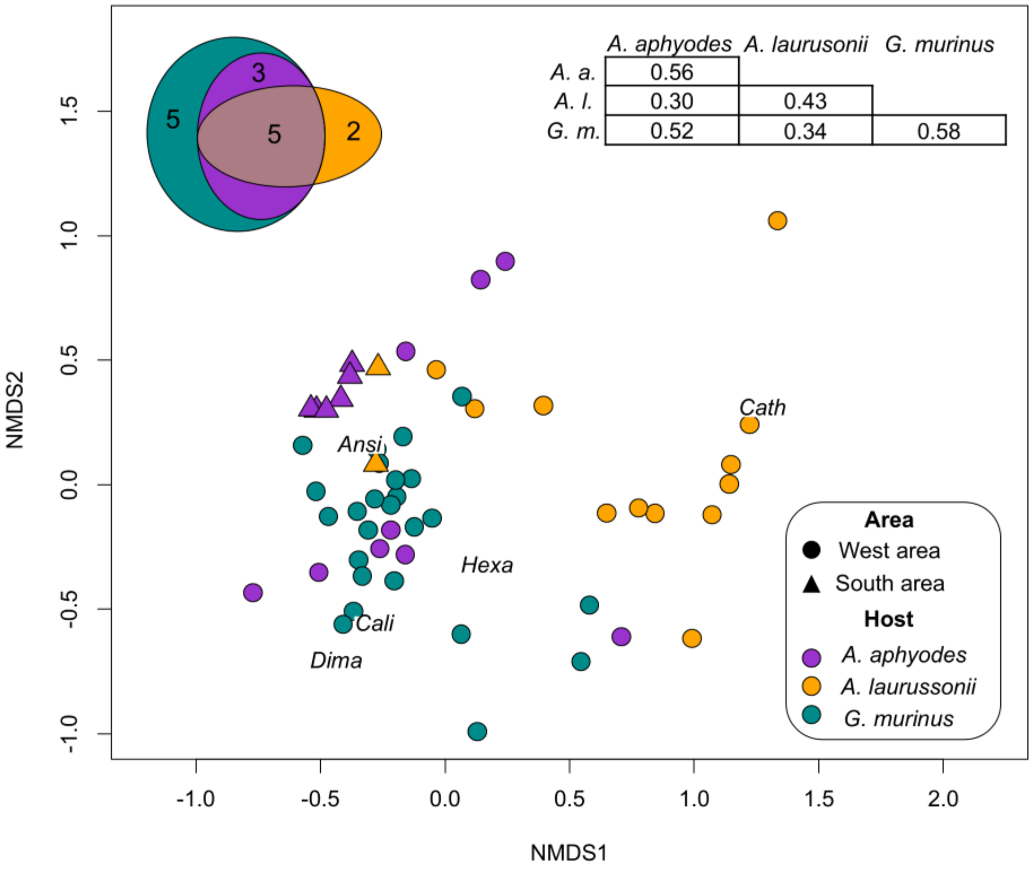


Figure 2. Non-metric multidimensional scaling (nMDS) illustrating the ordination of parasite assemblages according to host species and area of capture. The analysis is based on a Bray-Curtis dissimilarity matrix calculated from log-transformed (log (x + 1)) parasites abundance data. A Bray-Curtis similarity matrix displaying mean similarities within/among hosts (top right) and a Euler diagram depicting distribution of parasite taxa across hosts (top left) are also shown. Abbreviations for representative species according to Indicator value analyses are shown in the plot: Ansi, *Anisakis simplex*; Cali, *Calicotyle* sp.; Cath, Cathariotrematinae gen. sp.; Dima, *Ditrachybothridium macrocephalum*; Hexa, Hexabothriidae gen. sp.

0.001) and its abundance was lower compared to *G. murinus* (K-W, χ^2 = 11.96, p = 0.003). Additionally, the abundance of *Y. kuchtai* was lower in *G. murinus* than in *A. laurussonii* (K-W, χ^2 = 6.40, p = 0.041). No significant differences were found between pentanchid parasite communities with respect to B-P dom or H.

In the case of *A. aphyodes*, geographic differences in parasite assemblages were identified. The nMDS showed a separation between samples caught from the southern and western areas of Iceland **(Figure 2)**. Parasite communities of catshark individuals from the southern region appeared more tightly clustered together (Bray-Curtis similarity index = 61%) compared to the more dispersed communities in the western region (Bray-Curtis similarity index = 36%), although the overall similarity between both areas was only slightly higher (Bray-Curtis similarity index = 39%). There were significant geographical differences on the abundance and presence of parasites according to PERMANOVA analyses (PERMANOVA, F = 9.25 and F = 22.20, respectively, $p < 0.001$ in both cases). The indicator value analyses associated *Calicotyle* sp., *D. macrocephalum* and Hexabothriidae gen. sp with sharks from the western area (IndVal = 0.78, 0.67 and 0.56; $p < 0.026$ in all cases) and *A. simplex* with those of the southern area (IndVal = 0.71, p = 0.030). Although sharks from the South were significantly larger than those from the West (Wilcoxon test, W = 0, $p < 0.001$), no interactions were found between host length and parasitological descriptors when assessing regional differences. Consequently, fish TL was not included as covariate in subsequent analyses. Despite the lack of differences in total parasite abundance ($p > 0.05$), southern pentanchids exhibited lower MSR and H (Wilcoxon test, W = 72, $p < 0.001$ for both tests) and a higher B-P dom as well as *A. simplex* abundance (t-test, t = -5.71 and -2.42, $p < 0.033$).

3.3. Large-scale geographic comparison of catshark parasite assemblages

When comparing the parasite assemblages of the most common Mediterranean and Atlantic catsharks, no significant difference in total parasite abundance was found among hosts ($p > 0.05$). However, when considering parasites grouped by phylum, nematodes were more abundant and prevalent in hosts from the Atlantic Ocean (Wilcoxon test, W = 7863; Chi-squared, $\chi^2 = 43.7$, respectively, $p < 0.001$ in both cases) while crustaceans showed a higher abundance and prevalence in those from the Mediterranean Sea (Wilcoxon test , W = 3852, $p < 0.001$; Chi-squared, $\chi^2 = 10.13$, p = 0.001, respectively). In the case of platyhelminths, no significant differences were observed among hosts of both regions in terms of prevalence and abundance. Differences in parasitological indices were also found to be significant, with Atlantic catsharks displaying a lower B-P dom and a higher H and MSR (Wilcoxon test, W= 2460.5, 8071 and 7711.5, respectively; $p < 0.001$ in all cases) **(Table 3)**.

The greater parasite richness occurring in the Atlantic Ocean compared to the Mediterranean Sea was reflected in the species accumulation curves (SACs) shown in **Figure 3**. In general, the curves followed a typical accumulation pattern, with a steep initial increase that gradually flattened, although the curve associated to *A. laurussonii* did not stabilise. The three catshark species sampled in the Atlantic Ocean showed steeper slopes than those from the Mediterranean Sea.

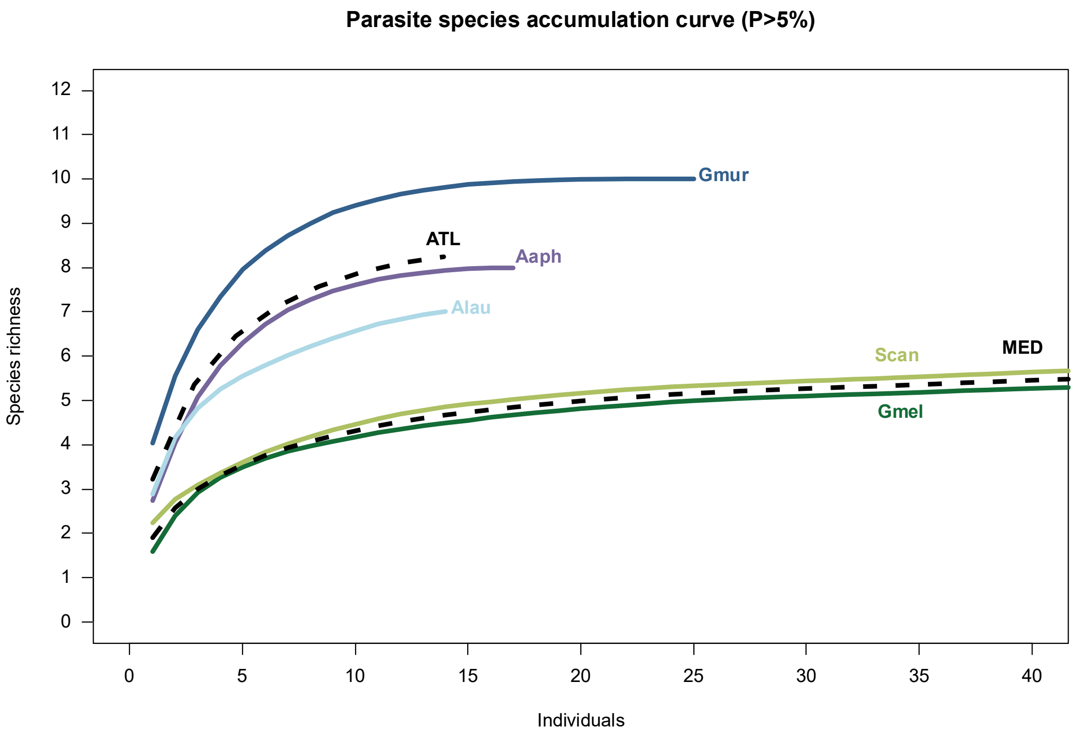


Figure 3. Species accumulation curves showing accumulation of parasite species by host and region. Hosts (solid lines): Aaph, *Apristurus aphyodes*; Alau, *Apristurus laurussonii*; Gmel, *Galeus melastomus*; Gmur, *Galeus murinus*; Scan, *Scyliorhinus canicula*. Regions (mean values, dashed lines): ATL, Atlantic Ocean; MED, Mediterranean Sea.

4. Discussion

This is the first study characterising the parasite communities of deepwater catsharks from North Atlantic waters. Present findings provide valuable data on the parasite assemblages of three of the most common Icelandic pentanchids, uncovering 27 new host-parasite records and providing baseline data for future research on parasite ecology and environmental parasitology, two especially growing fields in the context of global change (Palm & Mehlhorn, 2011; Poulin, 2021; Sures et al., 2023).

The three pentanchid species assessed herein hosted relatively diverse and abundant parasite communities, dominated by generalist taxa. This is consistent with previous observations, in which parasite diversity decreases with depth but increases again near the sea floor (Marcogliese, 2002). The wide depth range, combined with a diverse diet, enables benthodemersal species to harbour a species-rich parasite fauna, especially compared to meso- and bathypelagic fish (Klimpel, 2006, 2009, 2010).

New findings concerning the only two previously recorded parasites in these three catshark species are reported. *Ditrachybothridium macrocephalum* had only been recorded in *A. laurussonii* in its plerocercoid form (Bray & Olson, 2004). However, in the present study, mature specimens were identified infecting *A. aphyodes* and *G. murinus*, supporting the hypothesis proposed by Faliex et al. (2000) that deepwater catsharks serve as definitive hosts for species *Ditrachybothridium* Rees, 1959. The second previously reported parasite, *Y. kuchtai*, recently described in *A. aphyodes* as its type host (Caira et al., 2021), was also found in *A. laurussonii* and *Galeus murinus*. Therefore, the known host range is expanded for both cestode species.

Plerocercoids found in the tail musculature of *A. aphyodes* and *G. murinus* were tentatively identified as *G. adenoplusia* (Pinter, 1903) based on molecular results, which indicated conspecificity with *Grillotia* larvae from the Balearic Sea that had been identified as *G. adenoplusia* based on the study of oncotaxis (Dallarés et al., 2017; Isbert et al., 2023). Molecular characterisation of adult specimens of this parasite, which will allow confirming unequivocally its identity, is still to be done. The definitive host of this parasite is known to be the bluntnose sixgill shark, *Hexanchus griseus* (Bonnaterre), a widely distributed species and capable of long-distance migrations (Ebert & Stehmann, 2013). This finding, together with the genetic structure population results of Vella & Vella (2017), who found shared haplotypes in *H. griseus* from the North-East Atlantic and central Mediterranean Sea, suggests potential connectivity between the Atlantic and Mediterranean populations. Similarly, *Hepatoxylon trichiuri* has been reported in both the Atlantic and Pacific Oceans (Palm, 2004), while *O. cestoides* is known from the Atlantic Ocean and the Mediterranean Sea (Pollerspöck & Straube, 2025). These parasites use various large elasmobranchs as definitive hosts (Pollerspöck & Straube, 2025 and references therein). The frequent occurrence of these larval forms in Icelandic pentanchids suggests predation of these catsharks by larger sharks, a frequent phenomenon (Ebert, 1994, Dedman et al., 2025) that suggests intricate trophic interactions in the region that remain to be fully understood. *Anelasma squalicola* is a cirriped barnacle that directly extracts nutrients from its shark host, a trait that has drawn scientific interest (Ommundsen et al., 2016; Rees et al., 2014, 2019; Sabadel et al., 2022). Rees et al. (2019) concluded that this unique feeding strategy, described as a 'de novo innovation', triggered its global expansion, occurring so quickly that it didn’t have time to evolve into separate species. Molecular data from the present study, showing conspecificity with previously published sequences, further supports this hypothesis by extending the known host range of *A. squalicola* to include *G. murinus*, and its geographic distribution northward into Icelandic waters. In addition, a barnacle was found inside the shark’s body cavity for the first time, where it was attached to the liver after penetrating the skin. Yano & Musick (2000) reported that *A. squalicola* is able retard the development of the reproductive organs of male sharks.

Further studies monitoring this intriguing parasite and its potential effects on shark health would be welcome, especially considering its apparent rapid expansion.

The higher parasite loads, particularly of *A. simplex*, found in mature individuals, together with the correlation observed between TL and parasite abundance, is consistent with the life cycle of this species. Indeed, *Anisakis* species, like many other parasites larval forms, accumulate throughout the lifespan of their paratenic or intermediate host (Mattiucci et al., 2017), potentially becoming more dominant over time. The life cycle of anisakid nematodes involves aquatic invertebrates as first intermediate hosts and cephalopods and fishes as second or paratenic hosts (Klimpel et al., 2004). Fishes, due to their longer lifespans and trophic positions, are more likely to carry *Anisakis* larvae compared to smaller, shorter-lived organisms such as crustaceans (Münster et al., 2015). Thus, the present findings may reflect an ontogenic shift of adult sharks towards higher-trophic-level prey items and a more diversified diet; patterns also reported in other catsharks (Carrassón et al., 1992; Van der Heever et al., 2020). Nonetheless, such a diet shift is usually associated with a higher parasite richness (Poulin, 2004; Timi & Lanfranchi, 2013) which was not observed herein. To reliably detect dietary patterns, studies with a broader size range and a larger sample size would be necessary.

Regarding monogeneans, Cathariotrematinae gen. sp. and *Squalotrema* sp. were exclusively found infecting the nostrils (also referred to as the olfactory bulbs) of *A. laurussonii* and *G. murinus*, respectively. Both species belong to Cathariotrematinae, a monophyletic group of monocotylids known to parasitise shark nostrils (Bullard et al., 2021) and reported herein for the first time from pentanchid sharks. Contrary to the general believe that monogeneans were highly specific taxa,(Poulin, 1992) there is growing evidence that various monocotylid species exhibit low host specificity (Bullard et al., 2021; Chisholm & Whittington, 1996; Kritsky et al., 2017). This contradicts present findings, according to which closely related parasite species sampled from the same area show increased host specificity in the nostrils.

Research on specific parasite groups often leads to selective necropsy practices (*e.g.*, cestode-focused studies specifically targeting the spiral intestine) (Caira & Healy, 2004). While this kind of studies are clearly justified from a taxonomically-based approach, they can also leave the full parasite diversity in elasmobranchs heavily underappreciated because of the dismission of other body regions than the selected ones, such as the nostrils in the case sharks. Including these often neglected organs and tissues in routine necropsies could reveal a broader range of metazoan parasites than currently recognised, highly benefiting the knowledge on general parasite biodiversity.

Among the commonly found parasite species (P > 25%) across the three hosts, it is noteworthy that eight out of nine species were not host-specific and were present in at least two hosts. Among these, six are trophically transmitted parasites, while the remaining two (Hexabothriidae gen. sp. and *Calicotyle* sp.) are ectoparasites found in all three shark species. These findings support the notion that the studied sharks are sympatric species sharing similar feeding habits, having a similar trophic position and habitat preferences (Klimpel et al., 2003; Williams et al., 1992). Parasites have also been recognised as effective indicators of hosts' evolutionary history, with phylogenetically related host species typically sharing more parasite species (Lima et al., 2016; Poulin, 2010). Yet, the parasite community of *A. aphyodes* was more similar to that of *G. murinus* than to its congener *A. laurussonii*. This difference can be mainly attributed to the high prevalence and abundance of Cathariotrematinae gen. sp. along with the overall lower parasite burden, especially *A. simplex*, observed in *A. laurussonii*. While evolutionary history is a contributing factor in shaping parasite communities, it is the ongoing ecological interactions during the species' lifespan that most directly account for the observed patterns (Poulin, 1995).

Concerning the potential impact of parasite infections on the health condition of the studied hosts, the only significant correlation observed with the Kn was with MSR in *A. laurussonii*, suggesting that parasite burden have no major negative impact on the host's condition. Although condition indices can fluctuate due to a variety of factors, complicating the identification of clear relationships, the long-term coevolution between parasites and sharks (Hoberg & Klassen, 2002) may have resulted in an increased host tolerance to parasitism, limiting the fitness costs of infection without necessarily preventing it (Råberg, 2014). Consistent with the present data, some studies pointed out that healthier fish often harbour more abundant and diverse parasite communities (Dallarés et al., 2014; Falkenberg et al., 2024).

The comparative data on *A. aphyodes* sampled off the west and south of Iceland revealed interesting differences in terms of parasite assemblages' composition and structure despite the limited sample size. These differences are mainly attributed to a lower MSR and H, as well as higher dominance and abundance of *A. simplex* in the southern sampling area. This area lies closer to the coast, with steeper topography and greater substrate heterogeneity, whereas the western sampling area is characterised by a gentler slope and more homogeneous substrate (ICES, 2022; EMODnet, 2025). The higher dominance of *Anisakis* in the southern area could potentially be linked to a preference of some cetaceans to productive coastal shelf areas (Pike et al., 2005). However, various environmental factors, along with the distribution of intermediate and definitive host species, influence small-scale spatial differences in parasite communities, making it difficult to clearly determine the causes of the observed patterns with the limited data available. Iceland is influenced by a complex system of converging oceanic water masses (Logemann et al., 2013). In this context, analysing more samples from a broader range of localities would be of great interest, as it could reveal a greater diversity of parasite species associated with these pentanchid hosts and contribute to a better understanding of the biological and ecological complexity of the region. Samples from northern Iceland would be of particular interest, since it is considered a different subarea within the Icelandic Waters ecoregion with influence of cold, low salinity Arctic waters compared to the relative warm and saline Atlantic waters influence in the southern subareas (ICES, 2022). In this sense, a preliminary study identified differences in the parasite composition of Atlantic wolffish (*Anarhichas lupus* L.) when comparing fish from the southern and northern areas (Elfarsson, 2023).

Accurately assessing parasite diversity requires consistent and thorough sampling practices. The standardised methodologies applied in this study enable a comprehensive approach and facilitate reliable comparisons. A large-scale geographic comparison is something often difficult to achieve, as many surveys either overlook specific host organs (*e.g.*, nostrils or musculature) or concentrate solely on particular parasite groups, as explained above. Based on the joint analysis of present results with data obtained during the last years in the Mediterranean Sea by authors of the present study (Dallarés et al., 2017; Higueruelo et al., 2024), some differences in the parasite composition of catsharks with similar ecological characteristics have been detected when comparing both study areas.

The higher prevalence and abundance of nematodes in Atlantic catsharks is mainly attributed to *Anisakis* infections. In the Mediterranean Sea, *A. pegreffii* is the dominant species whereas *A. simplex*, an Arctic boreal species with a circumpolar distribution, prevails in colder waters (Mattiucci et al., 2018). Despite their different distributions, both nematode species use cetaceans as definitive hosts (Mattiucci et al., 2018). Although multiple biotic and abiotic factors influence the biogeography and infection dynamics of *Anisakis* species, the distribution and demography of their definitive hosts is a major relevant factor in explaining infection levels (Kuhn et al., 2016). The high productivity of waters around Iceland due to the confluence of warm and cold waters, among others, makes the region an important feeding ground for cetaceans (Charles et al., 2025), with 23 species recorded, of which 12 are considered regular inhabitants (Víkingsson et al., 2015). This likely contributes to the elevated *Anisakis* larval infections observed in Icelandic catsharks compared to those from the Mediterranean Sea, a pattern well documented in several teleost species (Debenedetti et al., 2019; Levsen et al., 2018; Valero et al., 2006). In contrast, the higher prevalence and abundance of crustaceans in Mediterranean catsharks is mainly attributed to the high occurrence of the copepod *Eudactylina vilelai* in *G. melastomus* (Dallarés et al., 2017), and therefore broader generalisations cannot be drawn from present results.

Regarding SACs generated in this study, the curve for *A. laurussonii*, the species with the smallest sample size, does not reach a plateau, suggesting that additional parasite species may remain undetected and that the observed diversity is likely underestimated. In addition, and consistently with previous observations on different fish species, present results indicate greater parasite species richness in Atlantic species than in their Mediterranean counterparts (Constenla et al., 2015; Mattiucci et al., 2014). Smaller fish sizes, reduced food consumptions, and lower biomass and abundance of animal communities in the Mediterranean have been proposed as potential factors contributing to this pattern (Constenla et al., 2015 and references therein). Woolley et al. (2016) found that while species richness on continental shelves and upper slopes peaks in the tropics, deep-sea species reach their highest richness at mid-to-high latitudes, particularly across the boreal Atlantic Ocean. Therefore, higher free-living species richness in these regions may lead to greater parasite richness, as more diverse host communities provide a wider range of ecological niches for parasites. This would promote host-specific adaptations and parasite speciation, resulting in more diverse parasite assemblages. Nonetheless, many biotic and abiotic factors influence the richness of parasite communities and broad generalisations must be drawn carefully.

The results presented herein highlight the potential parasite biodiversity and host-parasite relationships still to be uncovered in deepwater marine ecosystems. By shedding light on these neglected components of ecosystems, the present study contributes to the development of the growing fields of ecological and environmental parasitology. The study of parasite communities evidences the close and complex relationships occurring between parasites, their hosts and the environment, and can make a significant contribution to unravelling the intricate dynamics at play in natural systems.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Andrea Higueruelo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Dallarés:** Conceptualization; Investigation; Methodology; Project administration; Resources; Supervision; Writing - review & editing. **Anna Soler-Membrives:** Conceptualization; Funding acquisition; Methodology; Resources; Software; Supervision; Writing - review & editing. **Bjoern C. Schaeffner:** Conceptualization; Investigation; Methodology; Project administration; Resources; Supervision; Writing - review & editing.

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CHAPTER 4.1

The Deep, the Dark, the Cozy: Description of Calicotyle carmenae n. sp. (Monogenea: Monocotylidae) from Icelandic deep water catsharks (Elasmobranchii: Pentanchidae)

Andrea Higueruelo, Sara Dallarés and Bjoern C. Schaeffner

Abstract

A new species of *Calicotyle* (Monogenea: Monocotylidae) is described from three deepwater catshark species (Elasmobranchii: Pentanchidae) collected off Iceland. *Calicotyle carmenae* n. sp. is characterised by a haptor with one central and seven peripheral loculi, two hamuli, and several hooklets, matching the morphological characteristics of the genus. It is further distinguished by a U-shaped double vagina, a male copulatory organ (MCO) that loops once, intercaecal vaginal pores and the absence of eye spots. A molecular phylogenetic analysis based on 28S ribosomal DNA sequences supports its placement within the genus and reveals distinct genetic differences to its congeners. In addition, the morphology and host-specificity of *C. carmenae* n. sp. are discussed, thereby contributing to a broader understanding of the ecological roles of *Calicotyle* species. The study also provides the first record of a species of *Calicotyle* from pentanchid sharks, highlighting the potential for further discoveries as research expands into different hosts and deeper, underexplored marine ecosystems.

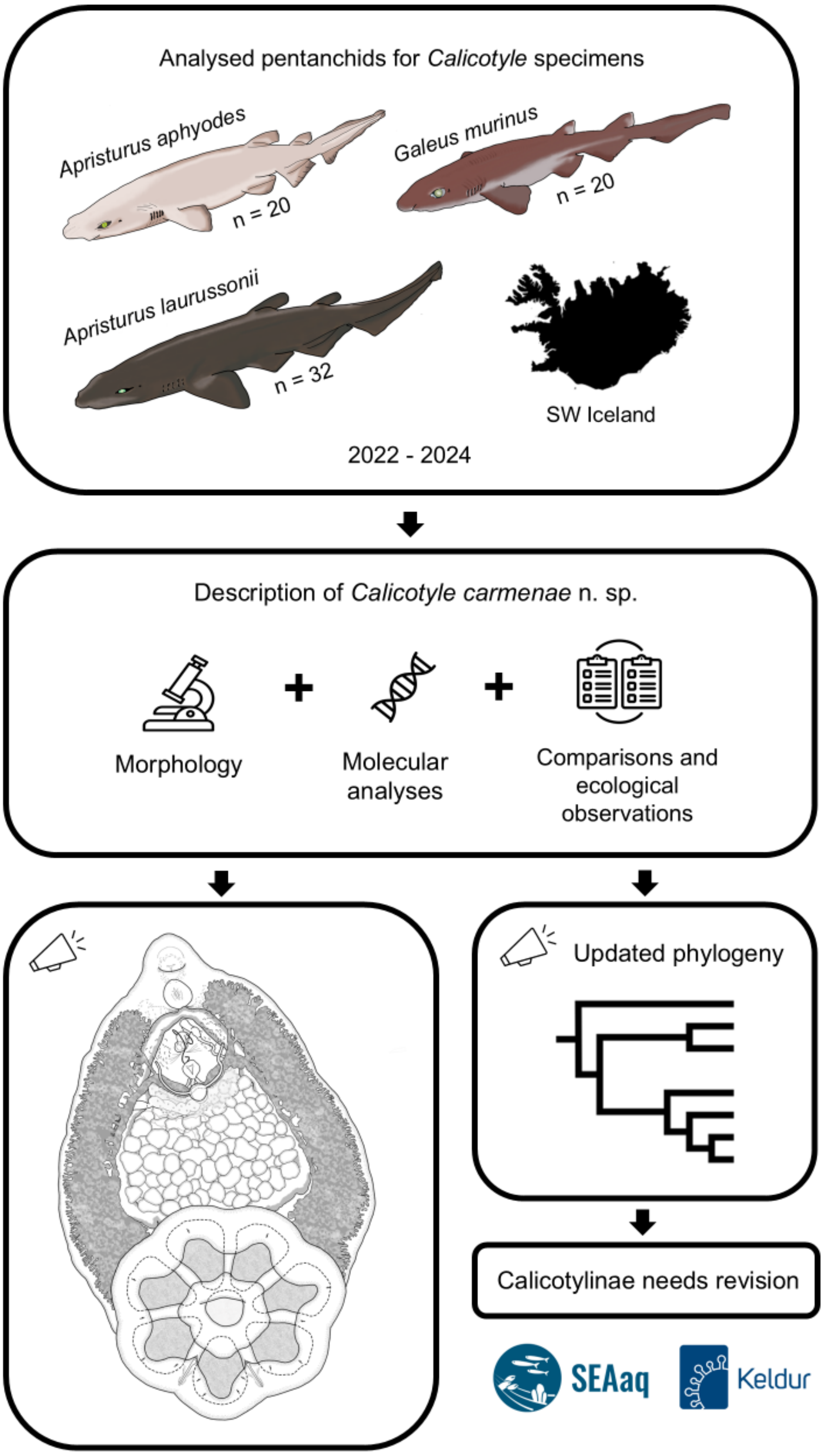
Keywords

Monogenea, elasmobranchs, Calicotyle, molecular phylogeny, deep sea, marine parasitology

Highlights

- A new species of monogenea*, Calicotyle carmenae*, is described from Icelandic pentanchids
- *Calicotyle* spp. may rely on the haptor for attachment more than previously thought.
- Integration of morphological and molecular techniques is recommended for describing monogeneans
- *Calicotyle* spp. seem to be family-specific rather than species-specific

Graphical abstract



1. Introduction

Monocotylidae Taschenberg, 1879 is a monogenean family that infects chondrichthyans (sharks, rays and chimaeras). The family has been object of taxonomic interest, since many species within the family are believed to await scientific discovery and description (Chisholm et al., 1995). At present, the Monocotylidae comprises eight subfamilies including the Calicotylinae Monticelli, 1903. Species of Calicotylinae can be distinguished by a variety of morphological features, such as the shape of the hamuli and the male copulatory organ, the direction of the vaginae, and the arrangement of the vitellarium. Morphological differentiation between individual species are subtle and, in certain cases, this has caused disagreements about the validity of certain taxa (Chisholm et al., 2001a). Although monogeneans are generally considered to possess a high level of host-specificity, some monocotylids, including species of *Calicotyle* Diesing, 1850 may be less host-specific, increasing the difficulties for species discriminations (Chisholm et al., 1997; Chisholm & Whittington, 1996; Whittington et al., 2000).

Calicotylinae contains two genera: *Dictyocotyle* Nybelin, 1941*,* a monotypic genus with *D. coeliaca* Nybelin, 1941 as the only species, and *Calicotyle* Diesing, 1850 (Chisholm et al., 2001b), which at present includes 21 valid species. Unlike most monogeneans, which are ectoparasites, species of *Calicotyle* typically infect the spiral valve, cloaca, rectum, rectal gland or oviducts of their hosts. This genus is distinguished from other monocotylid genera by the morphological features of the haptor, which presents one central and seven peripheral loculi and lacks haptoral accessory structures other than two hamuli and several hooklets (Chisholm et al., 1997).

Up until now, not a single species of *Calicotyle* has been reported from deepwater catsharks of the family Pentanchidae, despite the latter representing the most species-rich of all shark families. Some pentanchids are poorly known and, as fisheries and research expand to deeper waters, new species and new information regarding their biology is expected to be revealed (Ebert et al., 2021). Studies on parasites of deepwater catsharks from the northern Atlantic are particularly scarce, and a predominant portion of their parasite diversity (particularly endoparasitic helminths) are neglected.

Within pentanchid catsharks, the white ghost catshark, *Apristurus aphyodes* Nakaya & Stehmann, 1998, the Iceland catshark *Apristurus laurussonii* (Saemundsson, 1922), and the mouse catshark, *Galeus murinus* (Collett, 1904) are among the most frequent chondrichthyans present in Icelandic waters (NE Atlantic) (Jakobsdóttir et al., 2023). These three species are small-bodied (less than 80 cm) bottom dwellers distributed in the northern Atlantic Ocean (with *A.*

aphyodes and *G. murinus* having a more restricted NE Atlantic distribution) in depths of 380 to 2,060 m (Ebert et al., 2021). They are currently listed as “Least Concern” in the International Union for the Conservation of Nature’s (IUCN) Red List of Threatened Species, with a stable population trend (Finucci & Armstrong, 2024; Kulka et al., 2020; Rigby et al., 2024). However, only two parasites have been reported from *A. laurussonii* and *A. aphyodes*, namely the cestodes *Ditrachybothridium macrocephalum* Rees, 1959 and *Yamaguticestus kuchtai* Caira, Bueno & Jensen, 2021 (Bray & Olson, 2004; Caira et al., 2021), respectively, while none has been cited from *G. murinus*.

The present study provides the morphological description of a new species of *Calicotyle* recovered from these three pentanchids off Iceland. In addition, a phylogenetic framework is provided and patterns related to morphology and host-specificity of the genus are discussed.

2. Materials and methods

2.1. Collection of specimens

Twenty specimens of *A. aphyodes,* 20 *A. laurussonii* and 32 *G. murinus* were obtained between 2022 and 2024 during the Icelandic Autumn Groundfish Survey performed annually by the Marine and Freshwater Research Institute of Iceland (MFRI). Specimens were obtained by bottom trawling using the research vessels *Árni Friðriksson* (RE200) and *Breki*, at depths ranging from 700 to 1,300 m in Southwestern Icelandic waters in the North Atlantic Ocean.

A sub-sample of spiral valves of the three species was removed onboard and stored either in 95% ethanol or fixed with hot saline and later transferred to 4% buffered formalin for molecular and morphological identification purposes, respectively. The remaining individuals were frozen at -20°C until further parasitological examinations. In the laboratory, both frozen shark individuals and already fixed spiral intestines were examined for the presence of parasites and specimens of *Calicotyle* were stored in 70% ethanol. A total of 77 individuals were detected in the proximal spiral valve, attached to the mucosa of the rectal region. A single specimen was found in the body cavity. Infection parameters (prevalence and intensity) are provided only for the type-host (sensu Bush et al., 1997), *A. aphyodes*, for which the largest data set was available.

2.2. Morphological study

For morphological observations, monogeneans were stained either in haematoxylin or iron acetocarmine, dehydrated in a graded series of ethanol, cleared in clove oil and permanently mounted on microscope slides in Canada balsam. The haptor, which covered part of the body in ventral view, was detached from the body of few specimens prior to staining in order to reveal potential internal structures. For proper observations of haptoral features, both hamuli from three specimens recovered from *A. aphyodes* were detached and mounted in Canada balsam. Drawings were made with the aid of a drawing tube attached to an Olympus BH light microscope, and measurements obtained with a stage micrometer. All measurements are reported in micrometers as the range followed, in parentheses, by the mean ± standard deviation and the number of measurements taken (n). The descriptive part provides measurements from all specimens found in spiral valves of *A. aphyodes* which have been fixed with hot, almost boiling, saline prior to preservation in formalin.

Three additional specimens were examined using scanning electron microscopy (SEM). Specimens were dehydrated in a graded ethanol series, placed in hexamethyldisilizane (HMDS) and allowed to air dry. After dehydration, samples were coated with an alloy of gold (80%) and palladium (20%) at 5 nm thickness using an Emitech K550X sputter coater and subsequently examined with a SEM Zeiss Merlin high-resolution scanning electron microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

The terminology for morphological characteristics follows Chisholm et al. (1997). Type and voucher material has been deposited in the Naturhistorisches Museum (Vienna, Austria) (NM), the Natural History Museum (London, UK) (NHM) and in the Muséum d'Histoire Naturelle (Geneva, Switzerland) (MNHG).

2.3 Molecular and phylogenetic analysis

A specimen from each host was used for genomic DNA extraction. DNA extraction was performed using a "DNA QIA Cube HT" or with a "QIAgen DNA extraction kit following manufacturer's instructions. Nuclear large subunit ribosomal DNA (28S rDNA) was amplified through polymerase chain reaction (PCR) as described in Constenla et al. (2014) adjusted for Taqman Expression mastermix. Amplification primers used were either C1 (5'-ACCCGCTGAATTTAAGCAT-3') and D2 (5'-TGGTCCGTGTTTCAAGAC-3'), with internal primers Rob1 (5'-GTC CAA TAG CAA ACA AGT CCC G-3') and Rob2 (5'-CAC GYA CTR TTT ACT CTC-3') (Chisholm et al., 2001b; Hassouna et al., 1984), or LSU5 (5' -TAGGTCGACCCGCTGAAYTTAAGCA-3') and 1500R (5' -GCTATCCTGAGGGAAACTTCG-3') (Littlewood et al., 2000; Olson et al., 2003). Obtained PCR-products were analysed by capillary electrophoresis using a High-Resolution DNA kit in a Qiaxcel Advanced Instrument and viewed in the Qiaxcel ScreenGel (Qiagen) or analysed on RedGel-stained 1% TAE agarose gels. Sequencing of PCR products was performed by Genewiz-

Azenta or Macrogen Inc. using either the Sanger method or capillary electrophoresis, respectively. Obtained sequences were assembled either with the software Sequencher 5.4.6 from Gene Codes Corporation or with BioEdit 7.7.1 (Hall, 1999).

For phylogenetic analyses, the obtained sequences were aligned with the Muscle algorithm as implemented in MEGA v11 together with available sequences of *Calicotyle* and *Dictyocotyle* in GenBank that exceeded 900 base pairs (bp). *Empruthotrema orashken* Irigoitia, Braicovich, Rossin & Timi, 2019 (GenBank accession number MN190269) was used as an outgroup.

Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used for phylogenetic tree reconstruction. Prior to analyses the best-fit model of nucleotide substitution was selected with jModelTest v2.1.4 (Darriba et al., 2012) using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), respectively. The best-fitting model selected for the ML algorithm was GTR + G (nst = 6, rates = gamma, ngammacat = 4), and for BI it was TPM2uf + G (nst = 6, rates = gamma, ngammacat = 4). ML analyses were performed in PhyML 3.0 (Guindon et al., 2010) with a non-parametric bootstrap of 100 replicates. BI analysis was carried out with MrBayes 3.2.7 (Ronquist et al., 2012) through the CIPRES Science Gateway. In this case, Log likelihoods were estimated over 10,000,000 generations using Markov chain Monte Carlo searches on two simultaneous runs of four chains, sampling trees every 1,000 generations. The first 25% of the sampled trees were discarded as 'burn-in', and a consensus topology and nodal support estimated were calculated as posterior probability values (Huelsenbeck et al. 2001) from the remaining trees. Phylograms were edited with FigTree v.1.4.4 and Adobe Illustrator CS6. Pairwise genetic distance matrices ("uncorrected p-distance" and "number of differences" models) were calculated in MEGA v11.

3. Results

3.1. Phylogenetic analysis

The newly-obtained 28S rDNA sequences were 1,018 bp long for a specimen of *Calicotyle* ex *A. aphyodes*, and 1,167 bp long for specimens of *Calicotyle* ex *A. laurussonii* and *G. murinus*. The three sequences have been deposited in the GenBank database under accession numbers PX282454, PX282455 and PX282456.

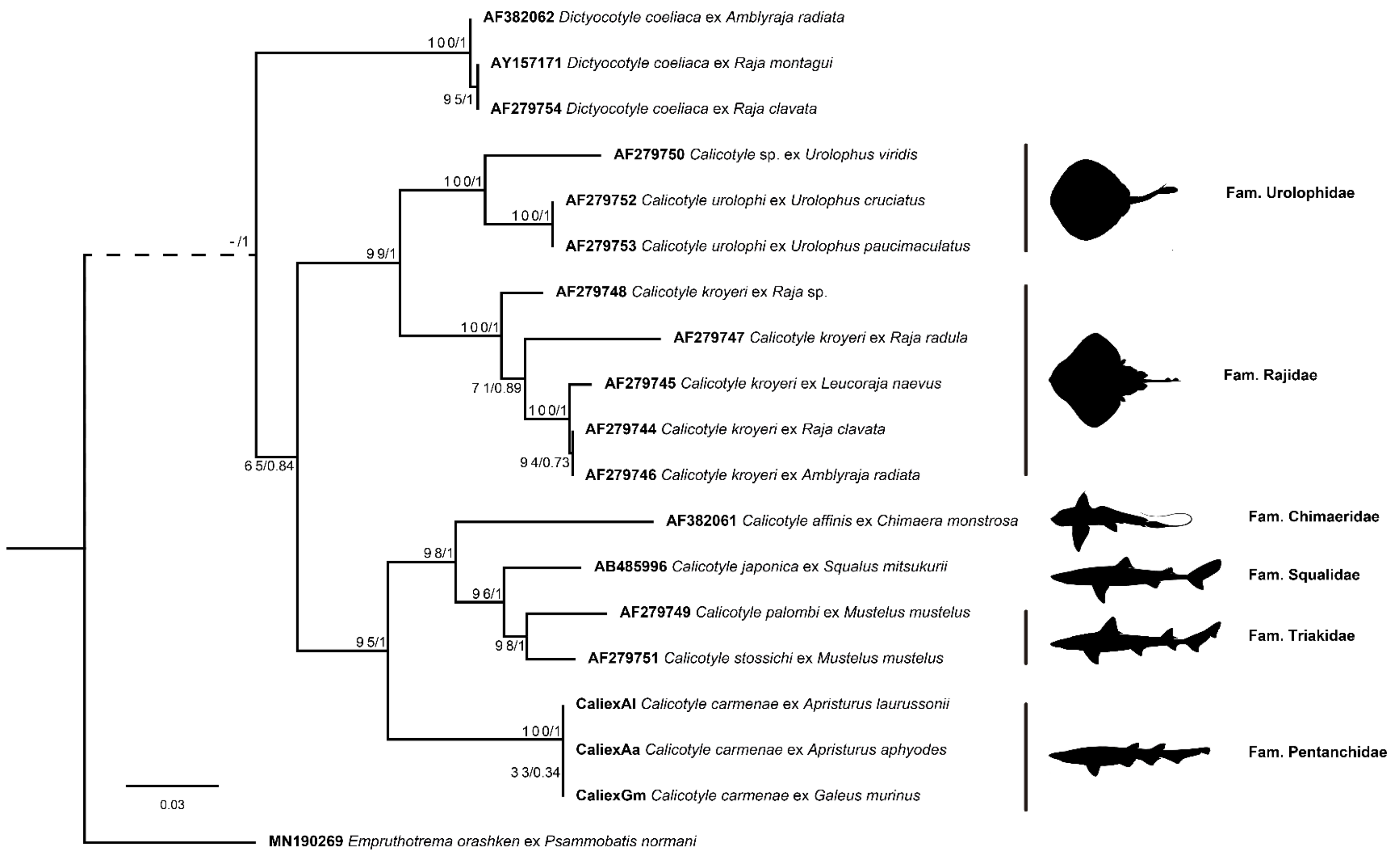
The resulting alignment was 1,016 bp in length. Newly-generated sequences were identical and showed the lowest genetic divergence (8.2%, 74 bp) from *Calicotyle japonica* Kitamura, Ogawa, Shimizu, Kurashima, Mano, Taniuchi & Hirose, 2010 infecting *Squalus mitsukurii* and the greatest divergence (11.1%, 99 bp) from *Calicotyle kroyeri* Diesing, 1850 infecting *Raja naveus* **(Table 1)**.

The newly-generated sequences formed a strongly-supported clade with other members of the genus infecting selachians and holocephalans, namely *C. affinis* Scott, 1911, *C. japonica*, *C. palombi* Euzet & Williams, 1960 and *C. stossichi* Braun, 1899, from which they differed by 8.2–9.1% (74–85 bp) **(Figure 1)**. This group of species of *Calicotyle* from selachians and holocephalans was sister to a clade including species of *Calicotyle* infecting batoids, from which the newly-generated sequences differed by 9.5–11.9% (84–99 bp). Collectively, sequences of *Calicotyle* infecting sharks and chimaeras differed by 9.5–12.6% (84–113 bp) from batoid-infecting congeners. Among the species of *Calicotyle* infecting batoids, there appear two clades with very strong support from both BI and ML analyses (99/1). One group infects batoids of the family Urolophidae while the other infects members of the family Rajidae. Sequences of *Dictyocotyle* form a separate clade differing from the *Calicotyle* sequences by 9.9–12.4% (88–111 bp) **(Table 1)**.

Table 1. Nucleotide genetic divergence for 28S rDNA among species of *Calicotyle* and *Dictyocotyle* included in the molecular phylogenetic analyses, including information on host species. Values below the diagonal are expressed as a percentage (p-distance) while values above the diagonal represent the number of bp differences.

	Species	Host	GenBank ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Calicotyle carmenae* n. sp.	*Apristurus aphyodes*	PX282454		0	0	100	100	99	85	84	84	77	97	81	86	96	96	99	96	74	154
2	*Calicotyle carmenae* n. sp.	*Apristurus laurussonii*	PX282455	0.0		0	100	100	99	85	84	84	77	97	81	86	96	96	99	96	74	154
3	*Calicotyle carmenae* n. sp.	*Galeus murinus*	PX282456	0.0	0.0		100	100	99	85	84	84	77	97	81	86	96	96	99	96	74	154
4	*Dictyocotyle coeliaca*	*Raja montagui*	AY157171	11.3	11.3	11.3		0	2	99	93	93	90	101	98	89	111	96	97	96	97	152
5	*Dictyocotyle coeliaca*	*Raja clavata*	AF279754	11.3	11.3	11.3	0.0		2	99	93	93	90	101	98	89	111	96	97	96	97	152
6	*Dictyocotyle coeliaca*	*Amblyraja radiata*	AF382062	11.2	11.2	11.2	0.2	0.2		98	92	92	88	100	96	88	109	95	96	95	95	151
7	*Calicotyle affinis*	*Chimaera monstrosa*	AF382061	9.1	9.1	9.1	11.2	11.2	11.0		96	96	67	107	71	101	104	111	113	111	70	162
8	*Calicotyle urolophi*	*Urolophus paucimaculatus*	AF279753	9.5	9.5	9.5	10.5	10.5	10.4	10.9		0	91	42	92	60	76	67	70	67	84	142
9	*Calicotyle urolophi*	*Urolophus cruciatus*	AF279752	9.5	9.5	9.5	10.5	10.5	10.4	10.9	0.0		91	42	92	60	76	67	70	67	84	142
10	*Calicotyle stossichi*	*Mustelus mustelus*	AF279751	8.4	8.4	8.4	10.2	10.2	9.9	7.2	10.4	10.4		99	33	94	100	100	104	100	36	155
11	*Calicotyle sp.*	*Urolophus viridis*	AF279750	11.0	11.0	11.0	11.4	11.4	11.3	12.2	4.7	4.7	11.3		99	67	83	72	75	72	90	151
12	*Calicotyle palombi*	*Mustelus mustelus*	AF279749	8.9	8.9	8.9	11.0	11.0	10.8	7.7	10.4	10.4	3.6	11.2		100	106	104	108	104	43	170
13	*Calicotyle kroyeri*	*Raja sp.*	AF279748	9.7	9.7	9.7	10.0	10.0	9.9	11.4	6.8	6.8	10.6	7.6	11.3		40	27	32	27	90	154
14	*Calicotyle kroyeri*	*Raja radula*	AF279747	10.8	10.8	10.8	12.4	12.4	12.2	11.7	8.6	8.6	11.3	9.4	12.0	4.5		42	45	42	103	151
15	*Calicotyle kroyeri*	*Amblyraja radiata*	AF279746	10.7	10.7	10.7	10.7	10.7	10.6	12.4	7.6	7.6	11.2	8.1	11.6	3.0	4.7		7	0	100	153
16	*Calicotyle kroyeri*	*Leucoraja naevus*	AF279745	11.1	11.1	11.1	10.8	10.8	10.7	12.6	7.9	7.9	11.7	8.5	12.1	3.6	5.0	0.8		7	104	158
17	*Calicotyle kroyeri*	*Raja clavata*	AF279744	10.7	10.7	10.7	10.7	10.7	10.6	12.4	7.6	7.6	11.2	8.1	11.6	3.0	4.7	0.00	0.8		100	153
18	*Calicotyle japonica*	*Squalus mitsukurii*	AB485996	8.2	8.2	8.2	10.9	10.9	10.7	7.6	9.5	9.5	3.9	10.2	4.7	10.2	11.6	11.1	11.6	11.1		165
19	*Empruthotrema orashken*	*Psammobatis normani*	MN190269	17.4	17.4	17.4	17.2	17.2	17.1	18.3	16.2	16.2	17.6	17.2	19.3	17.5	17.1	17.2	17.8	17.2	18.7	

Figure 1. Maximum likelihood (ML) and Bayesian inference (BI) consensus phylogenetic tree constructed using new partial 28S ribosomal DNA sequences of *Calicotyle carmenae* and retrieved sequences from GenBank. Outgroup*: Empruthotrema orashken*. Nodal support for BI and ML analyses are indicated as BI/ML. The scale bar indicates the expected number of substitutions per site. Host families parasitised are displayed as black figures.



3.2 Description

Family Monocotylidae Taschenberg, 1879

Subfamily Calicotylinae Monticelli, 1903

Genus *Calicotyle* Diesing, 1850

***Calicotyle carmenae* n. sp. (Figures 2–4)**

Type-host: White ghost catshark, *Apristurus aphyodes* Nakaya & Stehmann, 1998 (Carcharhiniformes: Pentanchidae).

Other hosts: Iceland catshark, A*pristurus laurussonii* (Saemundsson, 1922); mouse catshark *Galeus murinus* (Collett, 1904) (Carcharhiniformes: Pentanchidae).

Type-locality: Southwestern Icelandic waters (North Atlantic Ocean) at the edge of the Icelandic continental shelf, between the Reykjanes Ridge and the Denmark strait (63°4'48"N, 26°31'48"W – 65°15'36"N 28°9'36"W), at depths between 700 and 1,300 m **(Figure S1)**.

Site of infection: Rectum, spiral valve.

Prevalence and intensity of infection: Prevalence 30.4%, mean intensity 3.6 in *A. aphyodes*.

Type-material: Reference codes not yet available.

Representative DNA sequence: Partial sequences of the nuclear large subunit ribosomal RNA gene (28S rDNA), 1,018–1,168 bp in length, deposited in GenBank under accession numbers PX282454, PX282455, and PX282456.

Conservation assessment: Least concern [conservation assessment methodology for animal parasites (CAMAP) criterion 6].

Etymology: This species is dedicated to Carmen Fernández, mother to the first author (AH).

[Description based on specimens recovered from the type-host, *A. aphyodes*: 12 stained and mounted adult specimens (including the holotype and one hologenophore), three specimens examined with SEM, and dissected hamuli of three additional specimens. Metrical data of specimens recovered from *A. laurussonii* and *G. murinus* are provided in **Table S1**.]

Body including haptor 2,561–3,537 (3,146 ± 431, n = 4) in length, dorsoventrally flattened, occasionally anterior region bent towards haptor. Body pyriform, 1,902–3,049 (2,498 ± 492, n = 5) in length, greatest width at level of median region of testicular mass 1,902–2,244 (2,024 ±

190, n = 3), narrowing significantly at level of rounded cephalic region **(Figure 4A, B)**. Haptor flower-like in outline, 1,366–2,012 (1,555 ± 242, n = 6) in diameter, connected to main body by rather short peduncle, with slightly immersed central loculus and seven approximately equal, further immersed peripheral loculi, each delimited by muscular septa **(Figure 4D)**. Muscular rim of haptor folding inwards with an undulation, becoming wider at level of radial septa **(Figure 2A)**. Single pair of hamuli 211–227 (219 ± 6, n = 6) long, 59–72 (67 ± 6, n = 5) in maximum width, directed towards centre of haptor, provided with guard and relatively short blade **(Figure 2C)**, each at either side of posterior median loculus with tip expanding over marginal rim **(Figure 4E)**. Marginal hooklets 10–13 (11 ± 1, n = 5) long **(Figure 2D)**, located inside incurved margin of muscular rim, similarly distributed as in *C. kroyeri*. Haptor to body ratio 0.43–0.53 (0.48 ± 0.004, n = 4). Buccal cavity ventral, subterminal, not surrounded by sucker, encircled by several radial muscle fibres. Numerous small papillae surround edge of buccal cavity **(Figure 4H)**. Eye-spots not observed, likely absent. Pharynx bulbous, 148–249 (184 ± 27, n = 10) in diameter; oesophagus indistinct. Oesophageal glands in two groups at each side, located between inferior part of pharynx and beginning of vitellaria, with ducts leading towards posterior centre of pharynx **(Figure 2F)**. Intestinal caeca two in number, without diverticula, bifurcating from pharynx, extending posteriorly along internal margin of vitellarium, with slight medial turn at level of Mehlis' gland and second greater turn posterior to testicular mass before ending blindly in several lobes at medial level **(Figure 3)**.

Common genital pore opens ventrally, just anterior to oötype, forming an external bulbous structure when MCO is evaginated **(Figure 4C)**. Testicular mass follicular, with 61–72 (65 ± 6, n = 3) testes; testes 57–133 (101 ± 19, n = 27) in diameter, occupying intercaecal space, reaching from level of ovary to region anterior to folding of intestinal caeca, distributed in two continuously juxtaposed (partially interspersed) rows. Vas deferens emerging from anterosinistral portion of testicular mass, running approximately parallel to left caeca with some bends, and thickening posterior to caeca bifurcation to form the seminal vesicle. Seminal vesicle curved and elongate, maximum width 41–73 (52 ± 11, n = 10), turns posteriorly, narrowing before connecting to proximal portion of the ejaculatory bulb, 61–80 (71 ± 6, n = 10) long by 76–95 (89 ± 6, n = 10) wide, with two spherical internal chambers. Distal ejaculatory bulb elongates ventrally, connecting to funnel-shaped base of male copulatory organ (MCO). MCO sclerotised, 573–783 (678 ± 63, n = 10) long, emerging posteriorly for short distance, then recurving, passing distally to ejaculatory bulb before turning and extending posteriorly towards proximal ejaculatory bulb, terminating at level of oötype, where it bends in different forms, displaying

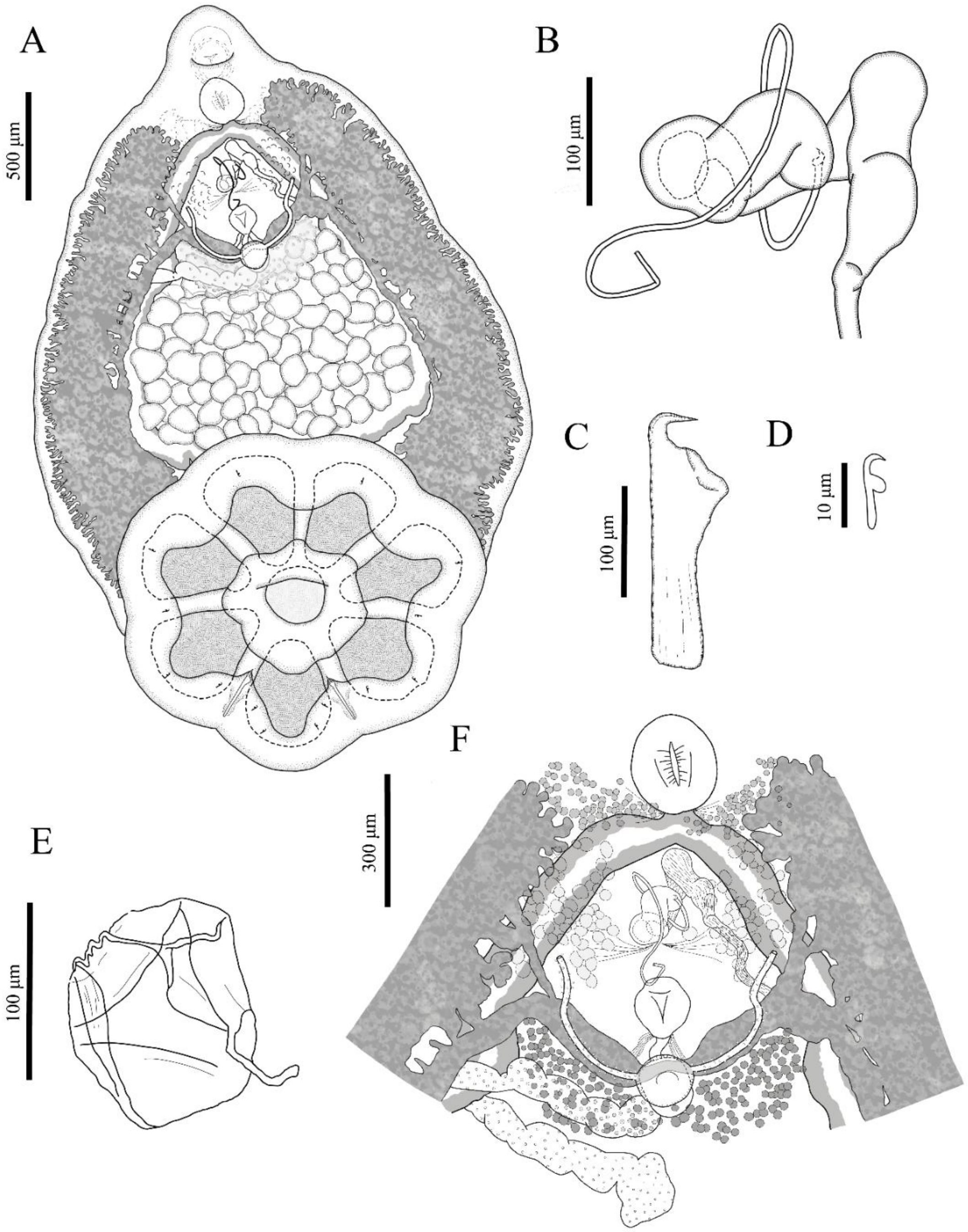


Figure 2. Line drawings of *Calicotyle carmenae* n. sp from *Apristurus aphyodes* off Iceland. **A**, holotype, entire view, hooklets not in scale; **B**, male terminal genitalia; **C**, hamulus, lateral view; **D**, hooklet, lateral view; **E,** tetrahedral egg, mostly collapsed; **F,** anterior body region with terminal genitalia, detailed ventral view.

variability **(Figure 2B; 4G)**. Ejaculatory duct not obvious. Prostatic gland cells weakly stained, larger than Mehlis' gland cells, observed between vitellaria at level of ejaculatory bulb, with ducts leading towards proximal ejaculatory bulb.

Ovary convoluted, 40–119 (67 ± 17, n = 30) in width, forming lobes, starting dorsally to fertilisation chamber, turning dorsally over right caecum, then heading towards medial region dorsal to testicular mass, ending blindly approximately in centre of body. Distal region of ovary enlarged, 67–171 (108 ± 28, n = 10) in width, containing small immature oocytes, while large mature oocytes are visible in proximal region of ovary. Vitellarium dendritic, occupying space between body margins and caeca, on each side of body from level of intestinal bifurcation to posterior end of body, not confluent posteriorly. Vitelline ducts dense, arising from inner medial margins of vitellarium, forming transverse vitelline ducts at upper half of body, anterior to ovary. Transverse vitelline ducts 171–405 (288 ± 51, n = 20) long, broader in central portion, 52–138 (94 ± 21, n = 20), before narrowing considerably at conjunction, at level of seminal receptacle. Fertilisation chamber oval, 57–133 (98 ± 25, n = 10) in diameter, dorsal to transverse vitelline ducts, connected to base of ootype through short duct. Ootype muscular, 110–171 (133 ± 21, n = 10) long by 112–150 (128 ± 13, n = 10) wide, with nearly triangular lumen and thick walls. Vaginae two in number, each 219–419 (317 ± 63, n = 14) long, U-shaped, uniform along its length, arising from seminal receptacle, located ventrally to conjunction of transversal vitelline duct, running approximately parallel to transverse vitelline ducts, turning anteriorly prior to reaching caeca and slightly twisting again outwards just before reaching vaginal pores. Small glands distributed along length of vaginas. Vaginal pores intercaecal (or slightly overlapping caeca), opening ventrally at level of common genital pore **(Figure 4C, F)**. Seminal receptacle 62–140 (98 ± 23, n = 10) long by 86–181 (114 ± 29, n = 10) wide, with short marginal striations. Transverse vitelline ducts normally obscuring junction of vagina and seminal receptacle. Mehlis' gland posterior to transverse vitelline ducts, overlapping ovary ventrally at right side, with two groups of collecting ducts connecting to base of ootype from both sides. Eggs tetrahedral, with short appendage, approximately 114 in diameter (n = 1) **(Figure 2E)**, present in 36% of specimens.

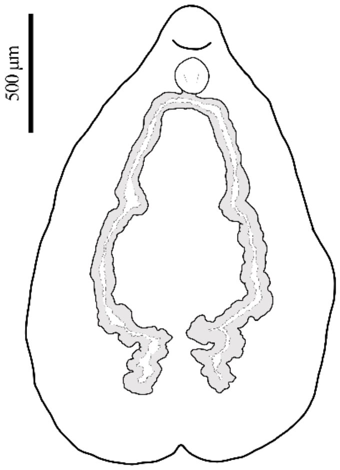


Figure 3. Line drawing of *Calicotyle carmenae* n. sp from *Apristurus aphyodes* off Iceland with the haptor removed and illustrating the path of the intestinal caeca.

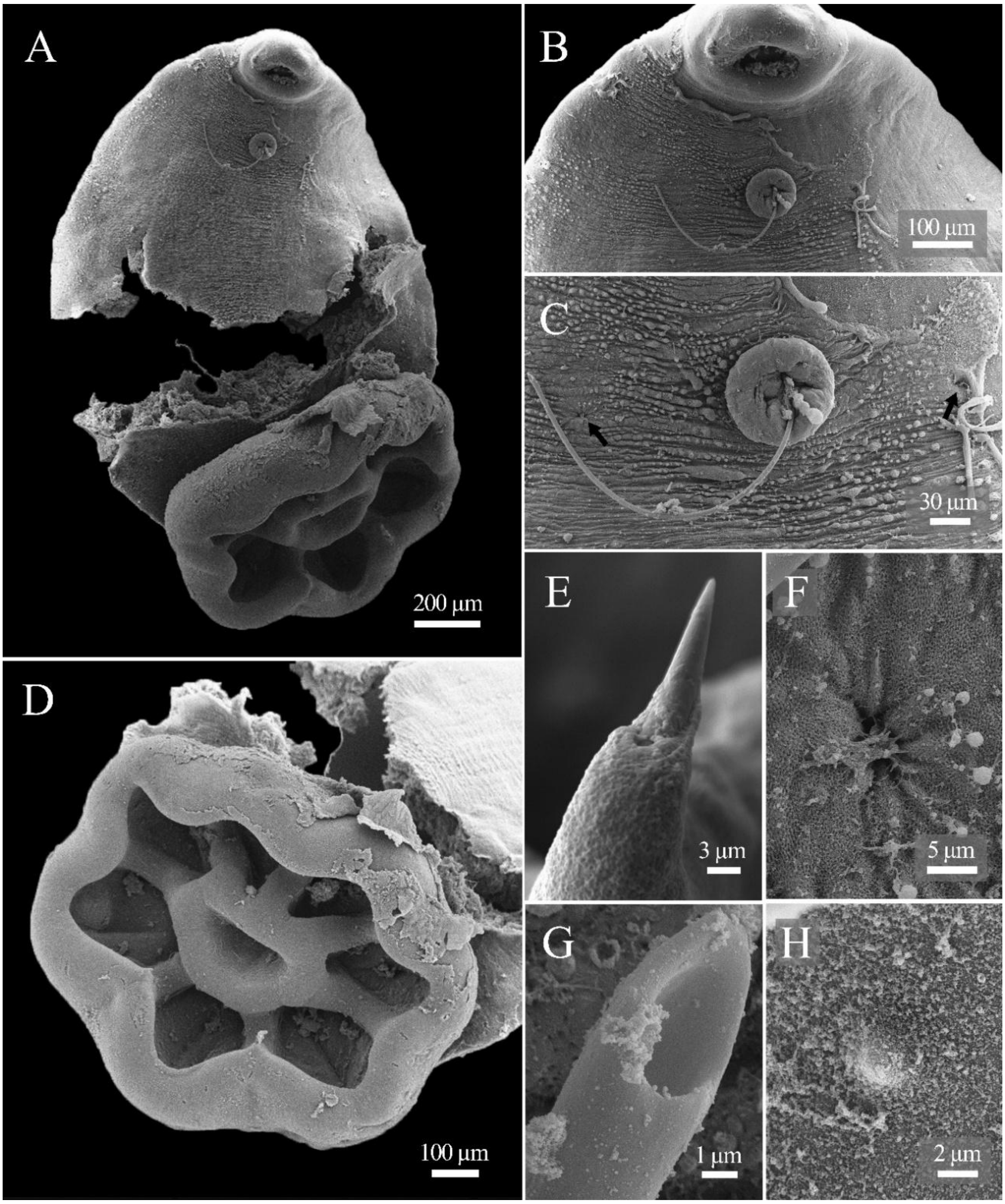


Figure 4. Scanning electron micrographs (SEM) of *Calicotyle carmenae* n. sp from *Apristurus aphyodes* off Iceland. **A**, whole specimen, ventral view; **B**, anterior region of body, ventral view; **C**, central region of body, ventral view, with male copulatory organ (MCO) everting from genital and vaginal pores (arrows); **D**, haptor; **E**, tip of haptor hamuli, close view; **F**, vaginal pore, close view; **G**, tip of MCO, close view; **H**, papillae from edge of buccal cavity, close view.

3.3 Remarks

Calicotyle carmenae has been described from the rectum of pentanchid catsharks inhabiting Icelandic waters. This constitutes the first record of a *Calicotyle* species in a pentanchid host, as well as the northernmost occurrence of the genus reported to date. The placement of *C. carmenae* in the genus *Calicotyle* is justified by the presence of a haptor with one central and seven peripheral loculi, two hamuli and several hooklets, a double vagina, an ejaculatory bulb with two spherical internal chambers and follicular testes (Chisholm et al., 1997).

Calicotyle carmenae is distinguished from all other valid species of *Calicotyle* by a unique combination of morphological characters. It possesses a MCO that loops once, a feature shared only with *C. asterii* (Szidat, 1970) Suriano, 1977, *C. australis*, *C. mitsukurii* Cordero, 1944, and *C. similis* (Szidat, 1972) Timofeeva, 1985 (Chisholm et al., 1997). However, C. carmenae has a U-shaped vagina, in contrast to the V-shaped vagina of *C. australis* and *C. mitsukurii*. The vaginal pores are intercaecal or slightly overlapping the intestinal caeca in *C. carmenae*, whereas in *C. similis* the vaginal pores are positioned lateral to the caeca. The lacking eye spots further differ *C. carmanae* from *C. asterii*. In the general body shape, *C. carmenae* closely resembles *C. urobati* Bullard & Overstreet, 2000 and *C. urolophi* Chisholm, Beverley-Burton & Last, 1991 by having a pyriform body with a large haptor (Bullard and Overstreet 2000; Chisholm et al. 1997). All three species are the only ones with a haptor/total body ratio larger than 0.4, however, they differ in internal structures such as the shape of the MCO, vaginal pores lateral to the caeca, or the presence of anterolateral glands or eyespots.

Unfortunately, all the eggs were found mostly collapsed, though their tetrahedral shape, in accordance with other congeneric species, could still be distinguished. A distinctive characteristic feature of *C. carmenae* is the flower-like appearance of the haptor, in contrast to the predominantly circular haptors described in other species of *Calicotyle* (e.g., Chisholm et al., 1997; Neifar et al., 2001; Ñacari et al., 2020).

Papillae are documented for the first time in species of *Calicotyle*, distributed around the buccal cavity. While they may occur in other members of the Calicotylinae, papillae have previously been reported only in *Dictyocotyle coelica*, where they occur not only around the buccal cavity but also around the genital and vaginal pores (Poddubnaya et al., 2015).

In taxonomic descriptions of species of *Calicotyle*, prostatic gland cells are rarely mentioned (e.g., Bullard and Overstreet 2000; Chisholm et al. 1997), and some authors have specifically noted the absence of “male accessory glands” in *C. australis* (Glennon et al. 2005; Whittington et al. 1989). It was not until transmission electron microscopy (TEM) studies of *C. affinis* that prostatic gland cells with ducts associated with the ejaculatory bulb were observed and described (Poddubnaya et al. 2018a). In the present study, these cells were observed in whole mounts and are illustrated here for the first time in a description of a species of *Calicotyle*.

This study also presents a SEM-based observation of an evaginated MCO. In this condition, the genital pore formed an external protruding bulbous structure, a condition which was not observed in individuals with non-evaginated MCO. Given that this marks the first record describing this condition, it remains unclear whether this it is specific to the species examined or if it may also occur in other monocotylids.

Measurements in the description are provided only for individuals parasiting *A. aphyodes*, as it was the only host from which enough specimens were heat-fixed. Nonetheless, it is worth noting that measurements from other host species reported in **Table S1** suggest potential intraspecific size variation linked to host size: smaller individuals were recorded in *G. murinus*, whereas larger specimens occurred in *A. laurussonii*, consistent with host size differences. However, the limited sample size and the fact that specimens from both *A. laurussonii* and *G. murinus* were not heat-fixed prior to preservation in formalin prevent definitive conclusions.

Interestingly, during the collection of specimens, some individuals were found firmly attached to the rectal wall, leaving clearly visible marks from the haptor fixation upon removal **(Figure 5)**.

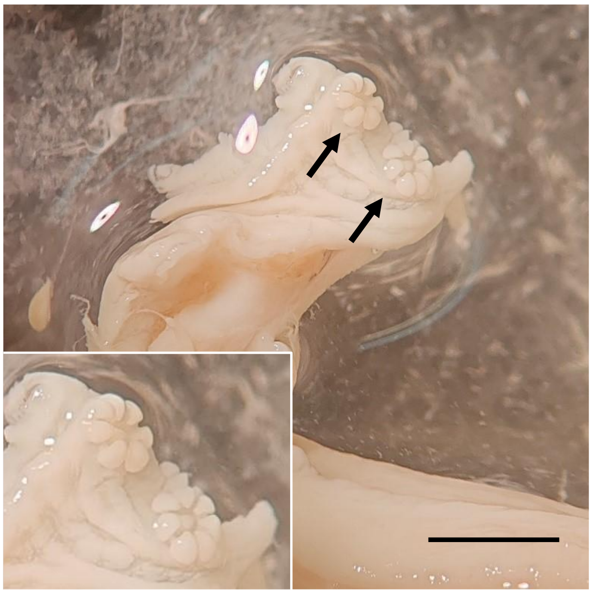


Figure 5. Haptoral marks (arrows) of *Calicotyle carmenae* n. sp. on the rectal mucosa of *Apristurus aphyodes*. In the lower-left, detail of haptoral marks.

4. Discussion

The present study integrates morphological observations with molecular techniques. Ribosomal DNA sequences have been used as a taxonomic tool since the beginning of molecular phylogenetic studies and are considered to be markers for species differentiation (Aiken et al., 2007). The large subunit ribosomal DNA (28S rDNA) has been widely applied in numerous studies of monocotylid monogeneans, where it provides adequate resolution for species discrimination (Bullard et al., 2021; Chisholm et al., 2001a, b; Fehlauer-Ale et al., 2011), unlike in other taxonomic groups such as cestodes, where this marker often fails to resolve closely related taxa (Littlewood et al., 2008). The combination of morphological and molecular data strengthens the validity of the *C. carmenae* and its taxonomic position following an integrative taxonomy approach.

Although the genus *Calicotyle* underwent a comprehensive revision by Chisholm et al. (1997), the absence of well-preserved specimens for certain taxa (*e.g.*, *C. inermis* Woolcock, 1936, *C. mitsukurii*, and *C. ramsayi* Robinson, 1961) and several gaps of knowledge in relation to biological traits (see Ñacari et al., 2020), combined with the subsequent description of new species incorporating additional morphological characters, has resulted in inconsistencies and a lack of standardisation in the characterisation of certain diagnostic features. These include the haptoral accessory structures, consisting of a pair of hamuli (absent only in in *C. japonica* and *C. inermis*) and hooklets, which have been (if observed) reported in varying numbers. The hooklets are very small and difficult to observe without damaging the haptor, particularly when their position within the muscular rim is perpendicular, in which case only the tip is visible. Given that haptoral accessory structures are generally a conserved feature among monocotylid genera (Chisholm et al., 1995), it is likely that species of *Calicotyle* possess a fixed number of hooklets, most likely 14, arranged symmetrically, as observed in *C. kroyeri* (Chisholm, 1997). Similarly, other morphological features of the haptor are often insufficiently described and characterised. The shape of the haptor is typically described as 'circular', rarely addressing or illustrating the morphology of the haptor muscular rims. Further research is needed to clarify whether two haptoral morphologies (*i.e.*, circular and flower-like, as observed in *C. carmenae*) occur within the genus and whether this represents a diagnostic trait among species of *Calicotyle*.

In the review of Chisholm et al. (1997), the female reproductive system has not been addressed in detail beyond the V- or U-shaped vaginae. For instance, the presence or absence of vaginal glands has not been addressed. These glands were observed in the present study and in additional species (*e.g.*, *C. californiensis*, *C. urobari*) (Bullard and Overstreet, 2000). Therefore, it remains speculative if these glands represent a unique synapomorphic feature within the Calicotylinae. In some species descriptions, such as the one of *C. carmenae*, two distinct chambers associated with the female reproductive system are reported: the first is situated at the point where the vaginae converge, and the second, positioned posteriorly, which connects the first chamber with the ootype and the ovary. If only a single chamber is described in previous species records, it is generally referred to as the seminal receptacle. However, in cases where two chambers have been described, the terminology becomes inconsistent. The first chamber is termed the “proximal region of the vaginae” in *C. californiensis* and *C. confusa* Kanaeva, Vodiasova, Ha, Uppe & Dmitrieva, 2025 (Bullard and Overstreet, 2000; Kanaeva et al., 2025), “vaginal chamber” in *C. cutmorei* Kritsky & Chisholm, 2020 (Kristky and Chisholm, 2020), and “seminal receptacle” in *C. affinis* (Poddubnaya et al., 2018b). In contrast, the second chamber, is referred to as the “seminal receptacle” in *C. cutmorei* and *C. californiensis* (Bullard and Overstreet, 2000; Kristky and Chisholm, 2020), “ootype” in *C. confusa* (Kanaeva et al., 2025) and “fertilisation chamber” in *C. affinis* (Poddubnaya et al., 2018b). Based on the anatomical arrangement of these chambers, the first chamber, at the convergence of the vaginae, may primarily function as a seminal receptacle, storing spermatozoa, as demonstrated by TEM observations in *C. affinis* (Poddubnaya et al., 2018b). The second chamber which connects the distal extremity of the ovary with the ootype and receives the ducts emanating from the seminal receptacle and the vitelline reservoir, likely serves as the site where oocyte fertilisation occurs. Therefore, as implemented in the present description, it might be more appropriate to follow the terminology proposed by Poddubnaya et al. (2018b), namely seminal receptacle and fertilisation chamber.

In line with these observations, in some species (e.g., *C. australis*, *C. confusa*, *C. kroyeri*, *C. urobati*, *C. urolophi*, *C. vicina* Neifar, Euzet & Ben Hassine, 2001), the vagina appears to comprise two differentiated regions, with the inner portion being thicker. These regions are usually referred to as the proximal and distal vagina, or as the muscular and seminal portions of the vagina (Bullard and Overstreet, 2000; Glennon et al., 2005; Kanaeva et al., 2025; Neifar et al., 2001). It is likely that this enlarged section corresponds to a species-specific modification of the seminal vesicle rather than being part of the vagina itself. However, this assumption requires further verification at the functional level. Detailed examination of the female reproductive system is important, as the features described above may represent diagnostic characters to distinguish closely related species.

Monogeneans are usually considered to exhibit high levels of host specificity (Whittington et al., 2000). However, in the case of *C. carmenae*, it was recorded from three host species, belonging to two genera of the same family and occurring within a relatively restricted geographical range. Our results are consistent with previous phylogenetic analyses, which suggest that species of *Calicotyle* tend to parasitise members of the same family, with hosts distributed within relatively small geographical areas (Chisholm et al, 2001a). Although *C. kroyeri* has been reported from 20 host species representing four families (i.e., Rajidae de Blainville, 1816; Anacanthobatidae von

Bonde & Swart, 1923; Narcinidae Gill, 1862; and Chimaeridae Rafinesque, 1815), across geographically distant areas (Chisholm *et al.*, 2001a; Kanaeva *et al.*, 2025), phylogenetic analyses by Chisholm *et al.* (2001a) indicate that many of these records may not refer to the same species. Instead, *C. kroyeri* appears to have served as a "dumping ground" for individuals found in the cloaca of various rajid species. Conversely, some species are presumed to be synonymous, subject to the examination of additional material. *Calicotyle affinis* is considered potentially synonymous with *C. australiensis* Rohde, Heap, Hayward & Graham, 1992, and *C. australis* Johnston, 1934 may be synonymous with *C. mitsukurii* and *C. vicina* (Chisholm et al., 1997; Glennon et al., 2005). Furthermore, *C. sjegi* Kusnetzova, 1970 is regarded as a *species inquirenda*, while *C. inermis* is considered a *species incerta sedis* (Chisholm et al., 1997).

The validity and resurrection of *Paracalicotyle* for specimens collected from triakid and carcharhinid sharks (i*.e.*, *C. stossichi*, *C. palombi*, *C. asterii*, *C. californiensis*, *C. cutmorei*) has been proposed by some authors based on their more elongated body shape and smaller haptor size of specimens (Bullard and Overstreet, 2000; Bullard et al., 2021). However, C. carmenae, which belongs to the same clade, has a pyryform body shape and possesses a large haptor more comparable to those of C. urobati and C. urolophi (both from rajid hosts).Although the molecular divergence between the two principal *Calicotyle* clades (9.5–12.6%) is similar to that observed between *Calicotyle* and *Dictyocotyle* (9.9–12.4%), there are no consistent morphological synapomorphies to justify erecting a separate genus based solely on host type (i.e., parasites infecting batoids versus those infecting selachians and holocephalans). Consequently, we concur with the position of Chisholm et al. (1997) in retaining the current generic classification, unless subsequent multidisciplinary revisions demonstrate robust morphological or molecular justification for generic separation.

It has been suggested that in *Calicotyle* spp., the absence of a marginal membrane and the presence of a thick haptoral rim have reduced the haptor's ability to maintain an effective suction seal, leading these species to rely more heavily on their hamuli for attachment (Chisholm et al., 1995). Chisholm & Whittington (1998) further argued that suction-based attachment may be less important for internal monocotylids, which are not exposed to strong water currents, compared with species inhabiting the nasal cavities or external surfaces of their hosts. Nonetheless, in the present study, several specimens were observed firmly attached to the host mucosa, leaving clear haptor attachment marks. Some species possess highly reduced hamuli, and the hooklets, located inside the marginal rim, are likely vestigial. Together, these observations suggest that *Calicotyle* spp. may rely on the haptor for fixation to a greater extent than previously thought. Overall, the inconsistencies and knowledge gaps identified in this study highlight the need for a comprehensive revision of the subfamily, involving re-examination of deposited material and the acquisition of new specimens and molecular data.

Cartilaginous fishes are among the most threatened marine vertebrates on this planet, with about one-third of species facing extinction (Dulvy et al., 2021; IUCN, 2025; Truter et al., 2025). Their parasites, particularly highly host-specific ones, often face equal or greater risk of co-extinction (Truter et al., 2025). Including these parasites in conservation assessments is critical for preserving ancient host–parasite systems that have co-evolved over millions of years (Caira & Jensen, 2017; Lymbery & Smit, 2023; Truter et al., 2025). Several parasite species of threatened host species have been assessed, such as three species of cestodes from the endangered white skate *Rostroraja alba* (Lacepède, 1803) (Van der Spuy et al., 2022), the monogenean *Dermopristis pterophila* Ingelbrecht, Morgan & Martin in Ingelbrecht, Morgan, Lear, Fazeldean, Lymbery, Norman & Martin, 2022 from the critically endangered longcomb sawfish *Pristis zijsron* Bleeker, 1851 (Ingelbrecht et al., 2022), and the copepods *Pandarus rhincodonicus* Norman, Newbound & Knott, 2000 from the endangered whale shark *Rhincodon typus* Smith, 1828 (Norman et al., 2021) and *Caligus furcisetifer* Redkar, Rangnekar & Murti, 1949 from the critically endangered common sawfish *Pristis pristis* (Linnaeus, 1758) (Morgan et al., 2010). Dallares et al. (2025) assessed the conservation status of *Carrassoniella sinuosiceps* (Williams, 1959) from the bluntnose sixgill shark, *Hexanchus griseus* (Bonnaterre, 1788) following the criteria specified in the conservation assessment methodology for animal parasites (CAMAP) by Kwak et al. (2020).

The assignment of *C. carmenae* to the "Least concern" category results from the application of criterion 6 of CAMAP, since required ecological and historical data for criteria 1–5 have not been available for the new species at present. *Calicotyle carmenae* infects at least three pentanchids, all of which are suspected to have stable population trends and currently assessed as "Least concern" in the IUCN Red List (Finucci & Armstrong, 2024; Kulka et al., 2020; Rigby et al., 2024). However, parasites, and monogeneans in particular, may be even more vulnerable than their hosts to anthropogenic disturbances (Gilbert & Avenant-Oldewage, 2021; Wood et al., 2010), and face a high risk of co-extinction (Koh et al., 2004), underscoring the importance of maintaining stable host populations to support parasite conservation. Continuous monitoring of *C. carmenae* infection levels according to CAMAP is therefore essential to accurately assess its conservation status over time.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

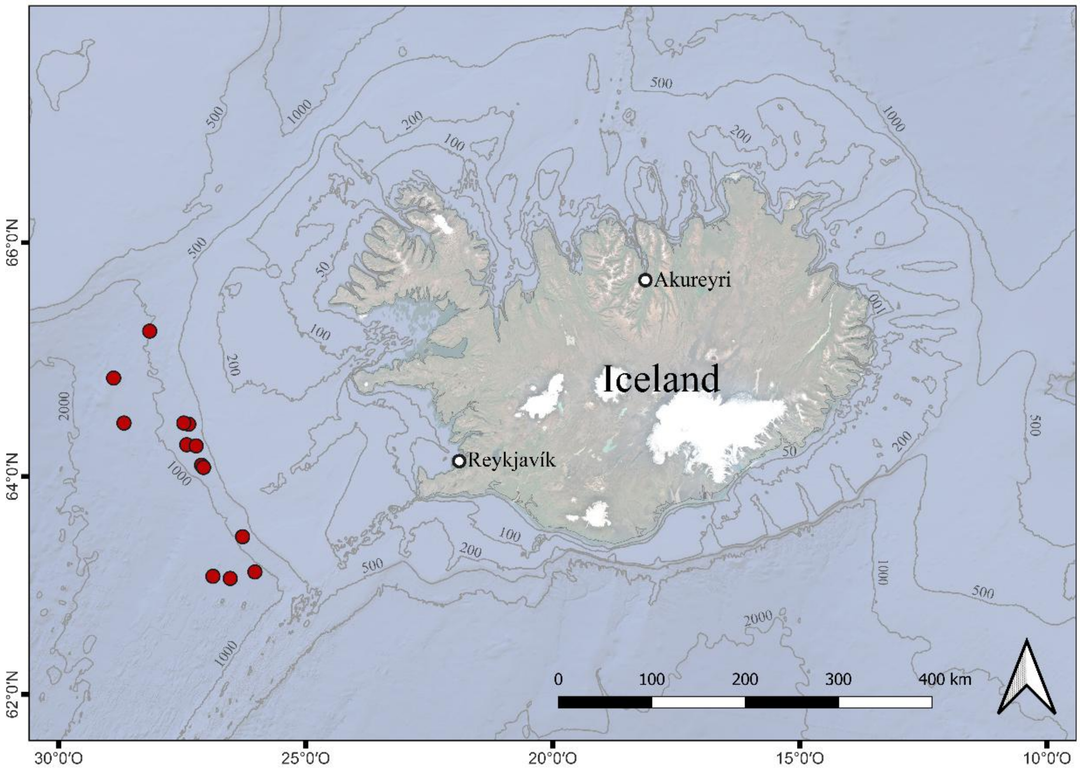

Figure S1. Map of the study area. Red dots indicate the sampling stations where the pentanchid sharks parasited with *Calicotyle carmenae* were collected.

Table S1. Metrical features of *Calicotyle carmenae* n. sp. ex *Apristurus laurussonii* and *Galeus murinus* captured off Iceland. Abbreviations: n, number of specimens measured; number of total measurements in parentheses. Measures in µm. Standard deviation (SD) provided for n ≥ 3.

	ex. Apristurus laurussonii					***ex. Galeus murinus***				
	n	**Mean**	**SD**	**Min**	**Max**	**n**	**Mean**	**SD**	**Min**	**Max**
Total body length	2	4451		4268	4634	1	2488		2488	2488
Body length (without haptor)	2	4024		3927	4122	2	1646		1415	1878
Body max. width	2	2622		2171	3073	6	1541	362	1098	2171
Pharynx diameter	3	228	42	200	276	5	184	21	160	217
Haptor diameter	3	1587	136	1506	1744	5	1183	200	963	1415
MCO length	2	845		839	851	6	648	75	556	785
Seminal vesicle width	2	41		37	46	6	52	10	39	66
Testes diameter	3 (9)	89	30	54	131	5 (14)	85	15	62	110
Proximal Ejaculatory bulb length	2	80		80	80	5	75	8	63	85
Proximal Ejaculatory bulb width	2	90		90	90	5	101	10	90	115
Oötype length	2	229		205	252	4	113	8	105	124
Oötype width	2	160		157	162	5	115	17	95	133
Seminal receptacle length	2	79		62	95	2	79		71	86
Seminal receptacle width	2	98		81	114	2	102		90	114
Ovary width at distal part	2	143		95	190	1	95		95	95
Ovary width	2 (9)	94	37	55	162	2 (5)	44	15	29	67
Vagina length right						1	300		300	300
Vagina length left						1	233		233	233
Vaginal chamber length	3	83	7	76	90	3	67	21	52	90
Vaginal chamber width	3	106	22	81	119	3	97	19	76	114
Vitelline reservoir right length						4	285	113	214	452
Vitelline reservoir right width	2	110		90	129	4	92	24	71	124
Vitelline reservoir left length						4	235	30	200	262
Vitelline reservoir left width	2	105		90	119	5	80	23	52	110
Ratio haptor/body	2	0.37		0.35	0.38	1	0.57			
MCO/TL	2	0.19		0.18	0.20	1	0.25			

GENERAL DISCUSSION

In the present thesis, a series of studies across various research disciplines have been conducted, in some cases using integrative approaches. Significant progress has been made in advancing insights across multiple topics related to catsharks and, in particular, *Scyliorhinus canicula*. As scientific techniques and knowledge continue to evolve, particularly in the context of global change, there is the need to continue to deepen our understanding of marine ecosystems. The following section discusses general aspects that have emerged throughout the thesis and outlines potential directions for future research where efforts should be focused.

1. Life history traits and reproduction of catsharks: the knowns and unknowns

Sharks exhibit the greatest diversity of reproductive modes among vertebrates, all of which require internal fertilisation, including various forms of oviparity and viviparity (Carrier et al., 2012; Ebert et al., 2021). The reproductive strategies of carcharhiniform sharks are more diverse than in any other chondrichthyan order (Nakaya et al., 2020).

In catsharks (families Scyliorhinidae and Pentanchidae), several strategies have been documented. These include short single oviparity (the ancestral mode of reproduction for catsharks), in which the egg case is deposited shortly after its formation; multiple oviparity, when females retain several eggs per oviduct, and the recently described sustained single oviparity, in which a single egg case is retained until the embryo has reached an advanced developmental stage before being laid. In addition, yolk-sac viviparity, including both single and multiple pregnancies, has been reported in a few pentanchid species **(Table 1)** (Nakaya et al., 2020).

However, a considerable knowledge gap persists regarding the reproductive modes of many catshark species. For example, information from Ebert et al. (2021) indicates that in over half of the species in this group (53.5%), reproduction is either uncertain, presumed or reported as oviparous without further specification, while in 18.5% of the species it remains entirely unknown.

Similarly, other information on life history traits, such as mating behaviour, length at maturity, egg case hatching time, reproductive cycle or sperm storage capacity, among others, is still missing for many species (Carrier et al., 2012; Ebert et al., 2006; Flammang et al., 2008). The limited biological knowledge on many catshark species, together with unknown distribution or population status, is reflected in their IUCN Red List status, where 19.5% are classified as Data

Deficient (DD), a higher proportion than the 13.9% recorded for chondrichthyans as a whole (IUCN, 2025).

Table 1. Different modes of reproduction reported in cathsarks. Modified from Nakaya et al. (2020).

Parity	Mode proposed	Definition	Family	Genus and/or species
OVIPARITY	Short single oviparity (syn. short, extended, external and simple oviparity)	One egg case per oviduct is kept for a short time, and is laid immediately before embryo develops	Pentanchidae	*Apristurus, Asymbolus, Figaro, Haploblepharus, Holohalaelurus, Parmaturus* *Bythaelurus bachi, B. canescens, B. dawsoni, B. naylori, B. vivaldi* *Galeus antilensis, G. arae, G. cadenati, G. eastmani, G. mincaronei, G. murinus, G. nipponensis, G.sauteri*
			Scyliorhinidae	*Atelomycterus, Cephaloscyllium* (excluding *C. sarawakensis* and *C. silasi*), *Poroderma, Schroederichthys, Scyliorhinus*
	Sustained single oviparity	One egg case per oviduct is retained for a long time, and is laid after embryo grows large	Scyliorhinidae	*Cephaloscyllium sarawakensis, C. silasi*
	Multiple oviparity (syn. retained oviparity)	Plural egg cases per oviduct are retained for a long time, and are laid after embryos grow large	Pentanchidae	*Halaelurus boesemani, H. buergeri, H. lineatus, H. maculosus, H. natalensis, H. quagga, H. sellus* *Galeus atlanticus, G. melastomus, G. piperatus*
VIVIPARITY	Yolk-sac viviparity (single pregnancy)	One embryo per uterus is retained until delivery	Pentanchidae	*Bythaelurus clevai, B. hispidus, B. lutarius, B. stewarti* *Cephalurus cephalus*
	Yolk-sac viviparity (multiple pregnancy)	Plural embryos per uterus are retained until delivery	Pentanchidae	*Galeus polli*

In order to integrate all published biological traits and population trends for sharks, rays, and chimaeras into a centralized database, the open-access initiative *Sharkipedia* was recently developed (Mull et al., 2022). *Sharkipedia* currently contains 4247 measurements of 59 traits, including measures related to length, age, growth, reproduction, demography and allometric relationships, covering a total of 178 species.

Considering that currently there are over 1200 species of extant chondrichthyans, much work is still to be done. The availability of these data is essential for properly conducting ecological risk assessments and for developing effective fisheries management strategies, conservation policies, and international agreements (Carrier et al., 2012; Cortés et al., 2015; Dulvy et al., 2021).

2. Importance of integrative assessment of a marine species for its commercialisation

Global change and fishing pressure are causing alterations in the structure of marine ecosystems and affecting fisheries, particularly at the local scale (MacNeil et al., 2010; Pauly et al., 1998; Piroddi et al., 2017). In the Mediterranean Sea, these impacts are intensified by its semi-enclosed nature, dense coastal populations, and intensive use of land and marine resources (see Introduction, section 3) (Micheli et al., 2013). In this context, some species are being negatively affected, while others may thrive, creating both challenges and new opportunities that require adaptive responses from fisheries (Ojea et al., 2020).

For instance, on the Catalan coast, an increase in warm-affinity species in the catch composition (*e.g.,* round sardinella *Sardinella aurita*, deep-water rose shrimp *Parapenaeus longirostris*) and a decrease of cold-affinity ones (*e.g.,* blue withing *Micromessistius poutassou*, Atlantic mackerel *Scomber scombrus*, European hake *Merluccius merluccius*, Norway lobster *Nephrops norvegicus,* sardine *Sardina pilchardus*) has been detected (Espasandín et al., 2025 and references therein). As for sharks, medium to large-sized species have declined in the region (Nuez et al., 2021) whereas catshark populations such as those of *Scyliorhinus canicula* and *Galeus melastomus* appear to be increasing (Finucci et al., 2021a, 2021b).

An increasingly common approach to studying wildlife and its changes is through the collection of information from non-scientist stakeholders who are in direct contact with wildlife, known as Local Ecological Knowledge (LEK) (Anadón et al., 2009). In marine ecosystems, scientific collaboration with local fishers is an important source of information, as they possess a high level of LEK regarding fish populations, including elasmobranchs, and changes in the fishing resources, being able to offer reliable insights into the status of commercial marine species (Barbato et al., 2021; Piñeiro-Corbeira et al., 2022; Zelli et al., 2025). Given their ecological and economic value, continuous monitoring of marine resources is essential to ensure their health, conservation, and long-term sustainability (Österblom et al., 2017).

To properly evaluate a species for its sustainable commercialisation, an integrative and holistic assessment is required, taking into account LEK, health status, biological traits (particularly reproductive biology), and ultimately its market potential and safe human consumption. Comprehensive studies in this direction should integrate a wide range of parameters, from general physiological indicators such as body condition indices, parasitological descriptors (with particular attention to zoonotic parasites), or nutritional profiles, to more specific indicators of stress and impact, including histological alterations, concentrations of heavy metals, ingestion of anthropogenic items (AIs), and enzymatic biomarkers (Carreras-Colom et al., 2022; Muns-Pujadas et al., 2025; Rodríguez-Romeu et al., 2022). In addition, it is also paramount that together with this, updated species-specific and region-specific biological data and reproductive parameters are considered to provide fishing guidelines and potential management measures, such as protected breeding areas, establishing a minimum length of catch or implementing closed seasons (Cochrane & Garcia, 2009; Lowerre-Barbieri et al., 2011).

In the present thesis, the significant proportion of discards represented by the small-spotted catshark (*S. canicula*) along the Catalan coast motivated a multidisciplinary assessment of the species, aiming to evaluate its potential for commercialisation in the region. Although no zoonotic parasites were detected, encysted larvae in the tail musculature reduces flesh quality. Furthermore, mercury (Hg) levels well above European Commission (EC) limits made its recommendation for consumption non-viable. The small-scale differences detected in several of the studied variables (*i.e.*, parasitological assemblages, AI ingestion, heavy metal concentrations), along with the observed decrease in length at maturity over time, highlighted the importance of monitoring a species across both temporal and geographic scales.

3. (Cat)Shark consumption in the Balearic Sea and worldwide

Up to 100 million sharks and rays are estimated to be killed annually in fisheries worldwide, with the global trade in their products generating more than US $4.1 billion (*ca.* €3.77 billion) between 2012 and 2019 (Niedermüller et al., 2021). This reflects their long history of exploitation, as sharks and rays have traditionally been harvested for their meat, their liver oil for cosmetics and health products, their fins as a cultural delicacy, their skin for leather and sandpaper, and even their jaws and teeth have been commercialised as curios and in previous times were used to make weapons (Jabado et al., 2024).

Accounting for about 22% of the global shark meat trade, the European Union (EU) stands as the primary supplier to Southeast and East Asian markets. From 2009 to 2019, Spain dominated world trade in fresh and frozen shark meat, having the most extensive trade network, involving 85 export and 65 import partners, exporting and importing by value of approximately US $536 and US $289 million, which accounted for 184,000 t and 136,000 t, respectively. In the case of ray meat, Spain is the fifth exporter by volume (Niedermüller et al., 2021). Most of Spain shark captures are obtained by the Atlantic fleet operating within Spain's exclusive economic zone (EEZ), in other countries, and in international waters (*i.e.*, high seas fisheries) (FAO, 2022a; Guallart et al., 2024).

By contrast, the production of Spanish fleets in the Mediterranean Sea is comparatively small, representing 8.3% of annual Spanish fishing landings and about 2% of the total Spanish shark and ray catches (Guallart et al., 2024). In fact, in the Mediterranean Sea as a whole, where small-scale fisheries predominate, chondrichthyan landings averaged 12,942 t between 2018 and 2020, representing only 1.7% of overall species landings (FAO, 2023). In the Spanish Mediterranean region, chondrichthyan landings fluctuated between 600 – 1000 t in the period between 2000 – 2020, with a modest annual economic value of the order of EUR 2 million (FAO, 2022b; Guallart et al., 2024).

Most elasmobranch species in the Mediterranean Sea are captured unintentionally as bycatch, yet they are sometimes landed and can frequently be found in local markets, often mislabelled or grouped under broad taxonomic categories (Colloca et al., 2025). Numerous studies have reported the mislabelling of shark products throughout the supply chain, many of which involved protected and threatened species, both globally (Hasan et al., 2023 and references therein) and in the Mediterranean Sea (Giagkazoglou et al., 2022; Giovos et al., 2021). Accurate identification of elasmobranch species is particularly important in the Mediterranean Sea, as over half of them (65%, 51 out of 79) are currently classified as threatened by the IUCN Red List (IUCN, 2025). The General Fisheries Commission for the Mediterranean (GFCM) reported the capture of approximately 25,300 specimens belonging to various conservation-priority species between 2008 and 2019, with longliners (drifting and set longlines) accounting for 55% of these catches (Carpentieri et al., 2021).

In the Balearic Sea, elasmobranchs are primarily sold for their meat, with livers marketed only occasionally and no established tradition of finning, although ray “finning”, the practice where only the fins of elasmobranchs are landed and the rest of the body is discarded at sea, has been observed (Barría & Colmenero). Pelagic shark catches are not notable in this region, and landings are predominantly composed of Rajiformes and catsharks (*S. canicula* and *G. melastomus*) which have low commercial value. Some species are landed eviscerated, missing certain parts, or skinned, as is typically the case for catsharks, which makes accurate identification difficult (Barría & Colmenero, 2019; Guallart et al., 2024). In fact, Barría & Colmenero (2019) identified 27 species of sharks and rays landed along the Catalan coast, including some threatened with extinction, whereas official statistics (Direcció General de Pesca i Afers Marítims, Generalitat de Catalunya) reported only 18 species. Moreover, landings are often recorded under broad taxonomic categories, with up to 55% of ray landings coded simply as “*Raja* sp.”. In addition to mislabelling, many consumers unknowingly consume shark meat, as often fail to recognize that certain common or scientific names refer to sharks (Carabelli et al., 2025). For example, the small-spotted catshark can be translated as “gat”, “pintarroja”, “petite roussette” or “gattuccio”, while the blackmouth catshark is commonly termed “moixina”, “bocanegra”, “pristiure à bouche noire” or “boccanera” (FishBase, 2025), losing the connection to the word “shark”.

The lack of transparency and knowledge regarding elasmobranch consumption is particularly problematic given that many elasmobranchs, as long-lived predatory species, often exhibit high levels of certain pollutants, such as heavy metals and persistent organic pollutants (POPs), which undergo bioaccumulation and biomagnification processes (Alves et al., 2022; Tiktak et al., 2020).

Mercury (Hg) is of major concern, as in its methylated form (methylmercury, MeHg) readily accumulates in muscle tissue (*i.e.*, the edible part) and concentrations exceeding regulatory limits have been documented in multiple shark species across various regions (Hasan et al., 2023; Sandheinrich & Wiener, 2011). Notably, the high methylation potential of the Mediterranean Sea contributes to elevated concentrations in elasmobranchs from this basin (Capodiferro et al., 2022; Giovos et al., 2022; Storelli et al., 2022). Furthermore, as demonstrated in the present thesis, demersal species such as catsharks, which show exponential accumulation patterns, are especially prone to Hg accumulation (see Chapter 3).

Given that the vast majority of sharks landed on the Catalan coast are demersal (Barría & Colmenero, 2019), this is a matter of special concern, as these species may exhibit Hg levels exceeding European Commission limits (EC, 2023), as has already been observed in *S. canicula* and *G. melastomus*. This surely needs to be addressed in future studies in order to ensure consumer safety.

Furthermore, although the burden of zoonotic parasites, such as Anisakid nematodes, appears to be lower in species from the Mediterranean basin (including catsharks) compared to Atlantic species (see Chapter 4), the presence of encysted parasites in the flesh can still compromise the quality and market value of catshark meat. As in the present thesis, some studies have documented the frequent occurrence of encysted *Grillotia adenoplusia* larvae in *S. canicula* and *G. melastomus* muscle tissue from the Mediterranean Sea (Dallarés et al., 2017a; Dallarés et al., 2017b; Santoro et al., 2021, 2022). Parasite loads were particularly high in *G. melastomus* from the Tyrrhenian Sea, with a mean of 181.65 larvae per individual, reaching up to 1,421 in a single specimen (Santoro et al., 2021).

The correct traceability and transparency in elasmobranchs supply chains, from capture to final sale, are essential to ensure both the sustainability of shark fisheries and consumer health (Hasan et al., 2023; Niedermüller et al., 2021).

4. *Scyliorhinus canicula* and other catsharks as biomonitors

In the introduction of the present thesis the concepts of biomonitor and bioindicator species are outlined, together with the main criteria used for their selection. This section focuses specifically on the use of *S. canicula* and other catshark species as biomonitors, particularly in relation to the ingestion of anthropogenic items (AIs) and the accumulation of heavy metals.

Scyliorhinus canicula and *Galeus melastomus* have been identified by several studies as appropriate sentinel species for assessing seafloor debris ingestion at small spatial scales, in accordance with the monitoring requirements established under the Marine Strategy Framework Directive (MSFD) for European waters (Alomar & Deudero, 2017; Fossi et al., 2018; Acevedo-Quilis et al., 2024; Sbrana et al., 2022; Valente et al., 2019). Due to its broader depth distribution, reaching up to 2,000 m (Ebert et al., 2021), *G. melastomus* has been considered especially appropriate for assessing AIs ingestion within deep-sea ecosystems (Sbrana et al., 2022).

In line with the present thesis, *S. canicula* has recently been identified as a potential biomonitor for heavy metal pollution in marine environments. Their egg-cases have likewise been suggested as sensitive bioindicators for Cd and Pb accumulation (Molera Arribas, 2025).

Several characteristics support the suitability of these catsharks as biomonitors. The knowledge on their biology and ecology is satisfactory, they are widely distributed throughout the Mediterranean and Atlantic Ocean, classified as Least Concern on the IUCN Red List, of limited commercial value, and frequently captured in fisheries, making their sampling cost-effective (Finucci et al., 2021a, 2021b; Fossi et al., 2018; Molera Arribas, 2025). Some of their ecological traits (*e.g.*, benthic lifestyle, opportunistic feeding behaviour and relatively high trophic position and long lifespans) make them prone to the ingestion of AIs from sediments and to the accumulation of heavy metals (Fossi et al., 2018; Molera Arribas, 2025; Valente et al., 2019). Due to their non-migratory behaviour and restricted movement range (Ramírez-Amaro et al., 2018), *S. canicula* can serve as a reliable bioindicator of local pollution, capable of reflecting environmental contamination even with small sample sizes (N ≈ 30) (Fossi et al., 2018; Molera Arribas, 2025; Valente et al., 2019). In particular, the use of *S. canicula* females should be prioritised in monitoring studies to more accurately represent local pollutant levels, since they are known to exhibit a higher philopatric behaviour than males (Gubili et al., 2014; Molera Arribas, 2025).

Additionally, extensive background data exist on debris ingestion and heavy metal concentrations in these species, providing a solid baseline for monitoring studies. For instance, the earliest documented cases of AIs ingestion involved individuals of *S. canicula* and *G. melastomus* collected in 2013 and 2010, respectively (Anastasopoulou et al., 2013; Neves et al., 2015), with numerous subsequent studies conducted throughout the Mediterranean and Atlantic Ocean (see Chapter 2, Table 4 for *S. canicula* records). Similarly, heavy metal concentrations have been well documented for both species throughout their distribution range, with some of the earliest records dating back to individuals collected in 1984–1985 (Hornung et al., 1993; Vas, 1991) (see Chapter 3, Table 4 for a review of Hg concentrations in *S. canicula*).

Nevertheless, the representativeness and comparability of the data largely depend on the standardisation and reproducibility of the monitoring process (Savoca et al., 2025; Wesch et al., 2016). Several considerations should therefore be taken into account for future monitoring studies. First, species metadata should be systematically reported, including specimen length, weight, sex, reproductive stage and the geographic coordinates of collection (Hanke et al., 2013).

As with any scientific procedure, monitoring studies must adhere to quality assurance and quality control (QA/QC) protocols to support the validity of the data obtained, making further comparisons reliable.

With respect to methodologies for assessing AIs ingestion in marine biota, most studies employ digestion techniques, typically using KOH, NaOH or H_2O_2, whereas visual inspection is employed in slightly fewer than one third of cases (Vanavermaete et al., 2024). However, certain digestion methods can degrade natural cellulose and semi-synthetic fibres (Athey & Erdle, 2022; Dehaut et al., 2016), thereby reducing the comparability of results. Since fibres are the predominant type of debris found in biota and cellulose is frequently reported (Marmara et al., 2023; Rodríguez Romeu, 2022), visual inspection protocols should be prioritised when the aim is to characterise the total amount of AIs ingested. When employing visual inspection, it is advisable to present the AI ingestion data separately for synthetic and naturally derived items. Because fibres can shatter within the digestive tract, reporting the total length of ingested fibres is also recommended (TLAI). Furthermore, the entire gastrointestinal tract should be examined rather than only the stomach, which is particularly important in elasmobranchs, since AIs can accumulate in higher abundances in the spiral valve (Valente et al., 2019).

For AI ingestion analyses, it is essential to minimise background contamination, particularly airborne contamination, throughout all steps of the process. This can be accomplished through various measures, including processing samples within a laminar flow cabinet or in an isolation device, rinsing all equipment and work surfaces with filtered distilled water, avoiding plastic tools during sample handling, wearing cotton laboratory coats and nitrile gloves, and implementing appropriate procedural controls, among others (Rodríguez Romeu, 2022; Wesch et al., 2016, 2017).

For the determination of heavy metal concentrations in biota, several analytical techniques are employed (Berto et al., 2020; El Hosry et al., 2023). Among these, CV–AAS is widely recommended for mercury, whereas ICP–MS is preferred for other metals (Berto et al., 2020; Ferreira et al., 2015). Whenever possible, the use of these techniques should be prioritised for better standardisation. In addition, to improve comparability across studies and with the maximum concentrations established by the Regulation 2023/915 for Hg, Pb, and Cd (EC, 2023), results should be reported on a wet-weight basis (Berto et al., 2020), preferably in parts per million (ppm); if expressed on a dry-weight basis, the percentage of water content in the samples should also be provided. Finally, environmental monitoring of trace metals should standardise the selection of target tissues based on tissue-specific accumulation patterns; while muscle tissue is suitable for the quantification of Hg, other organs such as liver should be targeted for the analyses of Cd and Pb (Guidance Document No. 25 European Commission, 2010).

In the case of heavy metals, analyses should be conducted in accordance with the criteria and standards established by Commission Directive 2009/90/EC. Validation of the obtained results requires the implementation of both internal and external quality control measures, such as the inclusion of procedural blanks, the use of certified reference materials, and regular participation in laboratory intercomparison exercises. The limits of detection (LOD) and limits of quantification (LOQ) should be acceptable and reported (EC, 2009).

Beyond the quantification of AIs and heavy metals, future investigations could also explore the potential of catsharks as biomonitors for contaminants of emerging concern (CECs), including persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), chlordane (CHLs), and dichlorodiphenyltrichloroethane (DDT), for which elasmobranchs have already been identified as promising candidates (Alves et al., 2022; Bezerra et al., 2019). In addition, research in the Global South, where data remain scarce, is needed to fill existing knowledge gaps and improve the understanding of global trends (Diarra & Prasad, 2021; Savoca et al., 2025). Frequently captured catsharks, such as the Izak Catshark *Holohalaelurus regani* (Gilchrist, 1922) in Namibia and South Africa or the Chilean Catshark *Schroederichthys chilensis* (Guichenot, 1848) in the Southeast Pacific Ocean (Dulvy et al., 2020; Pollom et al., 2020), could potentially serve as biomonitors in such studies.

5. Parasites as bioindicators and biomonitors

In recent decades, the field of marine parasitology has experienced a growing interest in the use of parasites as biological and ecological indicators of their hosts, due to their high species diversity, diverse life history strategies, and specific environmental requirements (Dallarés et al., 2014; Locke et al., 2013; Sasal et al., 2007; Sures et al., 2025; Williams et al., 1992).

In order for parasites and their communities to serve as effective bioindicators, accurate species identification at the lowest taxonomic level possible is fundamental, although it can be sometimes challenging (Cribb et al., 2025) and requires specific taxonomic knowledge. On the one hand, different species with limited morphological differentiation, especially at larval stages, may be erroneously grouped together, resulting in cases of cryptic diversity (Nadler & De Len, 2011; Perkins et al., 2011; Stout et al., 2024). Conversely, synonymous species may be mistakenly described as separate taxa for several reasons, including unrecognised morphological plasticity or descriptions based on poorly preserved material or on a limited number of specimens (Cribb et al., 2025). Consequently, integrative approaches combining both molecular and morphological techniques for parasite identification are essential to provide robust taxonomic baseline data to support subsequent parasitological outcomes (Cribb et al., 2025; Nadler & De Len, 2011; Perkins et al., 2011; Stout et al., 2024).

In the present thesis, it has been demonstrated that, even with relatively small sample sizes, parasites can provide valuable insights into multiple aspects of the biology and ecology of their hosts, including trophic interactions, dietary patterns, evolutionary history, habitat preferences or population connectivity (Timi, 2025). For poorly studied species with limited available information, such is the case of Icelandic pentanchids and numerous catsharks, the use of parasites can be particularly useful to infer some of these aspects and to detect differences at small spatial scales (Gérard et al., 2022).

Additionally, parasites have proven to be effective bioindicators of environmental changes and anthropogenic impacts due to their sensitivity to ecological disturbances. Changes in parasite communities can reflect habitat alterations, changes in environmental conditions, pollution levels, and overall ecosystem health, making them valuable tools for monitoring marine ecosystems (Lafferty, 1997; Suret et al., 2025; Vidal-Martínez et al., 2010). As an example, larval cestode *Grillotia adenoplusia* (Pintner, 1903) Palm, 2004, commonly found in demersal sharks, including the catsharks *G. melastomus* and *S. canicula*, has recently been proposed as a sentinel species for monitoring anthropogenic disturbances in the Mediterranean Sea, such as food-webs stability, environmental pollution and environmental parameters (*i.e.*, sea temperature, salinity) (Palomba et al., 2023).

The use of parasites as biomonitors of pollutants has been gaining scientific attention in recent years (Sures et al., 2017, 2025; Williams & Mackenzie, 2003). In particular, cestodes and their plerocercoids are known to accumulate heavy metals, including Hg, Cd, and Pb, at much higher levels than host tissues (de Buron et al., 2009; Sures et al., 2017). For example, a study found that the cestode *Paraorygmatobothrium* sp. contained Cd and Pb at concentrations 445 and 438 times higher, respectively, than those measured in its host, the shark *Carcharhinus dussumieri* (Müller & Henle, 1839) (Malek et al., 2007).

Considering the capacity of cestodes to accumulate pollutants and the high abundance of *Grillotia* spp. in Mediterranean catsharks (Santoro et al., 2021), the accidental consumption of parasite-infected flesh may represent an additional route of human exposure to heavy metals. Given the already concerning Hg concentrations in catsharks and other benthic species from the Mediterranean Sea (Chapter 3), studies analysing heavy metal concentrations in musculature-encysted parasites are essential to further evaluate potential human health implications.

Finally, this thesis highlights the substantial biodiversity yet to be described, particularly among metazoan parasites of poorly studied species or underexplored habitats such as the deep sea. The study of this group of organisms advances fundamental scientific knowledge and greatly contributes to the characterization of global biodiversity.

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CONCLUSIONS

1. In *Scyliorhinus canicula*, five reproductive stages in females and three in males were characterised based on macroscopic examination, and further supported by histological analysis, body size patterns, and somatic index variations **(Ch. 1)**.

2. Sexual maturity in *S. canicula* was associated with a significant decrease in the hepatosomatic index, supporting the liver's key role in energy storage and as a resource provider for reproductive purposes, particularly through lipid mobilization for vitellogenesis, spermatogenesis, and related processes **(Ch. 1)**.

3. Liver colour variability in *S. canicula* was linked to lipid content and reflected reproductive rather than nutritional status, indicating its potential use as a visual indicator of reproductive investment **(Ch. 1 and 2)**.

4. The year-round presence of mature males and egg-laying females, stable gonadosomatic index values and frequent sperm occurrence in the oviducal glands (likely indicative of sperm storage) supports the conclusion that *S. canicula* has a continuous reproductive cycle **(Ch. 1)**.

5. The size at maturity (i.e. length at 50% maturity, L50) of *S. canicula* is decreasingin the Balearic Sea compared to previous estimates, thus shifting towards earlier maturity. Available data for the same species from other Mediterranean and Atlantic regions suggest that a similar pattern may be occurring at a larger spatial scale **(Ch. 1)**.

6. Nearly nine out of ten *S. canicula* analysed from the Balearic Sea (87%) ingested anthropogenic items (AIs), with a mean of 5.47 fibres per individual. However, when cellulosic fibres are excluded to allow comparison with digestion-based studies, ingestion values align with previous findings in the Mediterranean, suggesting that digestion methods may significantly underestimate AIs ingestion, while visual inspection provides more representative estimates of AIs loads **(Ch. 2)**.

7. The observed variability of AIs ingestion in *S. canicula* at multiple geographic scales, together with its higher ingestion rates relative to other marine species, its benthic nature, intermediate trophic level, wide distribution, and abundance, highlights its strong potential as a biomonitor of marine AIs contamination **(Ch. 2)**.

8. The population of *S. canicula* in the Catalan Coast does not seem to be negatively affected by any major pathology nor by AIs ingestion and trace metal concentrations **(Ch. 2)**.

9. Interspecific variability in muscular Hg concentration among marine organisms appears to be strongly influenced by their habitat use, which can be as significant as trophic position, with benthic species showing greater accumulation potential **(Ch. 3)**.

10. Bioaccumulation of Hg in muscle tissue of catsharks (*i.e., S. canicula* and *Galeus melastomus*) increases exponentially with shark length **(Ch. 3)**.

11. The presence of AIs in *S. canicula* does not pose a risk for human consumption, the occurrence of encysted *G. adenoplusia* larvae in the muscle may diminish the flesh quality and the levels of Hg make it not recommended for consumption. Individuals longer than 37.03 cm have a 50% probability of exceeding the European Commission (EC) consumption limits for Hg, a length slightly below the reported length at maturity (37.9 and 37.2 cm of females and males, respectively); making it impossible to establish a suitable size range for its consumption without risking the inclusion of immature individuals **(Ch. 1, 2 and 3)**.

12. The high Mediterranean methylation potential results in high Hg levels in marine species. In the NW Mediterranean, Hg levels in benthic species raise consumer health concerns, with a single portion of *S. canicula* or *G. melastomus* exceeding the Tolerable Weekly Intake (TWI) set by EFSA. Like tuna, benthic fish from the Catalan coast should be consumed in moderation, especially by pregnant women and children. There is a need for stricter monitoring in the region and further research into other commercially important benthic species, including elasmobranchs **(Ch. 3)**.

13. *Scyliorhinus canicula* is an ideal sentinel species for monitoring heavy metals due to its wide distribution, high abundance, limited mobility, and strong bioaccumulation capacity. Considering its bioaccumulation capacity muscle tissue is suitable for Hg monitoring, whereas the liver represents a more appropriate target organ for monitoring Pb and Cd **(Ch. 2 and 3)**.

14. For the first time, the metazoan parasite communities of the three most common pentanchid species in Iceland, *Apristurus aphyodes*, *Apristurus laurussonii*, and *Galeus murinus*, have been comprehensively characterised, revealing 15 distinct parasite taxa and resulting in 27 new parasite-host records **(Ch. 4)**.

15. A new monogenean species has been described, namely *Calicotyle carmenae* n. sp., and other parasite taxa identified in Icelandic pentanchids may also represent species new to science, highlighting the potentially hidden parasite diversity yet to be uncovered in deepwater marine ecosystems **(Ch. 4 and 4.1)**.

16. Although some hosts exhibited significant associations with specific parasites, eight parasite taxa (representing eight out of nine commonly found parasites) were detected in more than one host species, indicating similar feeding habits, ecological niches, and trophic positions among these sympatric species **(Ch. 4)**.

17. Parasite communities of *S. canicula* and *G. melastomus* off the Catalan coast are characterised by low average richness and diversity, and high species dominance, whereas North Atlantic pentanchids exhibit lower dominance and higher species richness and diversity, revealing differences in parasite community structures between Mediterranean and North Atlantic sharks **(Ch. 2 and 4)**.

18. Variations in ecological and biological traits, such as feeding behaviour, prey availability, body size, and habitat preference, can influence the composition and structure of parasite assemblages in catsharks, even at small spatial scales, being indicative of host biology and ecology **(Ch. 2 and 4)**.