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

Andrea Higueruelo [a], Maria Constenla [a], Francesc Padrós [a], Paula Sánchez-Marín [b], Maite Carrassón [a], Anna Soler-Membrives [a,*], Sara Dallarés [a]

[a] *Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain*
[b] *Centro Oceanográfico de Vigo, Instituto Español de Oceanografía (IEO-CSIC), Subida a Radio Faro, s/n, 36390 Vigo, Spain*

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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.

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

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[*] Corresponding author.
E-mail address: anna.soler@uab.cat (A. Soler-Membrives).

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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.

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 hm^3/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

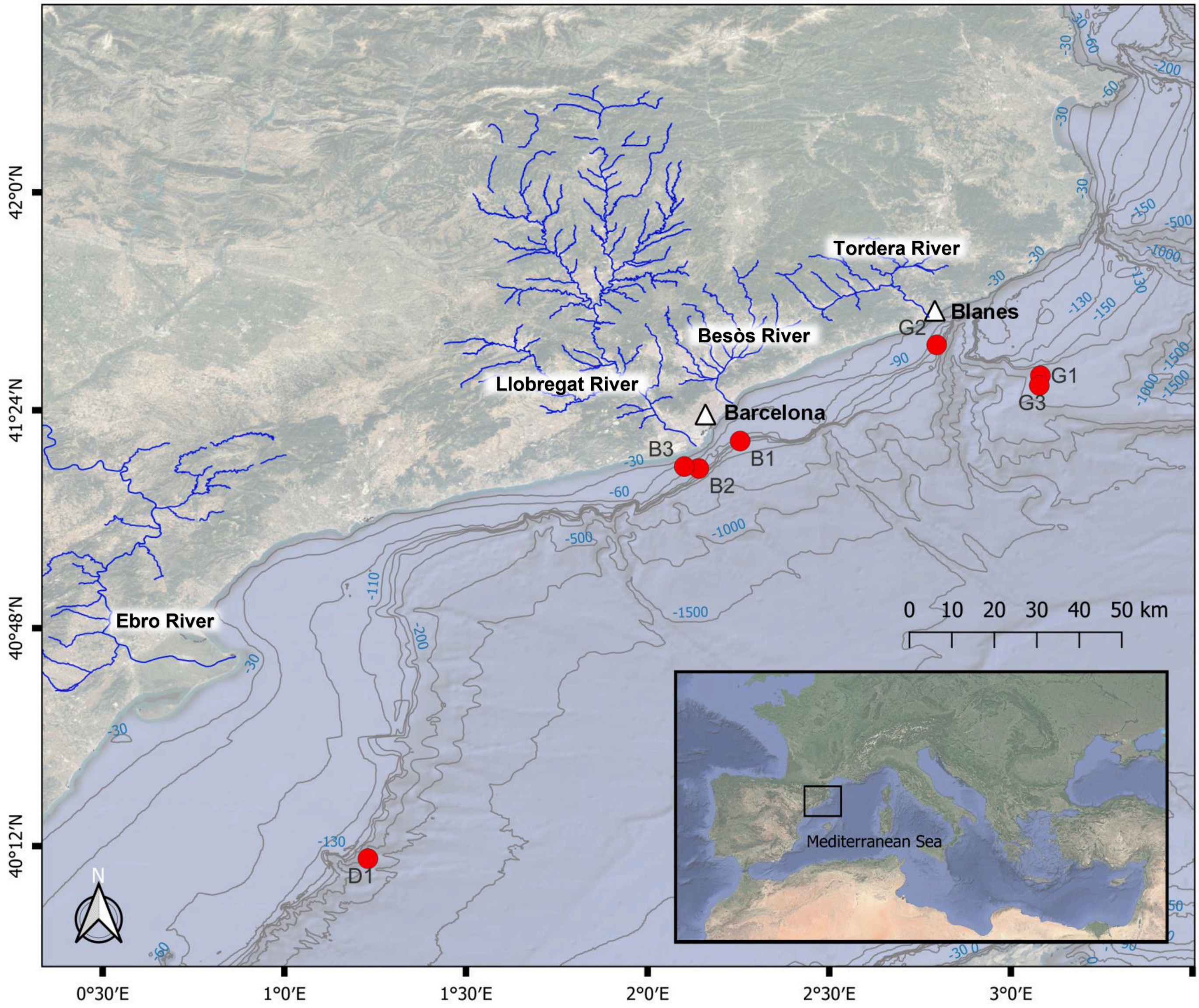

Fig. 1. Map of the sampling area. Red dots indicate sampling sites and white triangles the nearby important cities. From north to south, Blanes (G1, G2 and G3), Barcelona (B1, B2 and B3) and Ebro Delta (D1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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

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. In bold, mean values for the global area (Catalan Coast).

Locality	Blanes	Barcelona	Ebro Delta	Catalan Coast
Depth (m)	111–473	61–88	229	**–473**
n	19	22	20	**61**
Biometric data and condition indices				
TL (cm)	33.91 (5.05)	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**

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 quantitation (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.

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

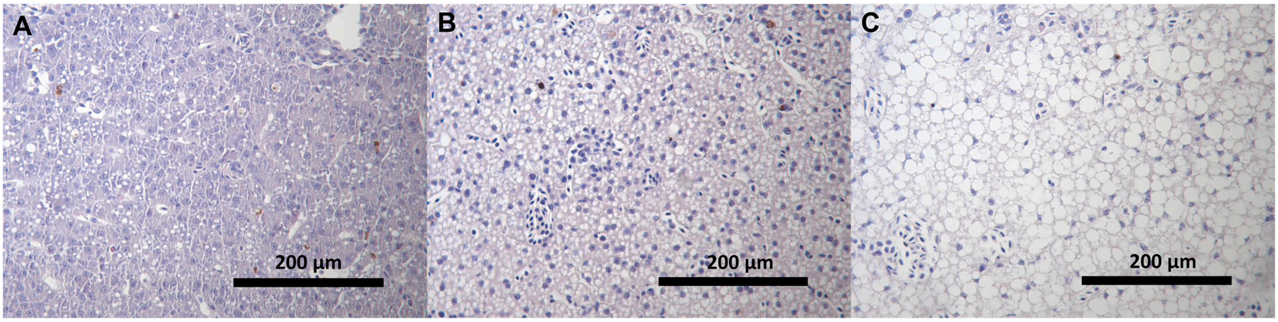

Fig. 2. Categories of liver lipid deposition of *Scyliorhinus canicula* (20×). A) Low lipidic deposition, B) Medium lipidic deposition, C) High lipidic deposition.

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 / (α × 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.

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, $\chi^2 = 28.72$, $p < 0.001$; Fig. 3A).

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).

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, $\chi^2 = 7.77$ 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, $W = 315$, $p = 0.003$).

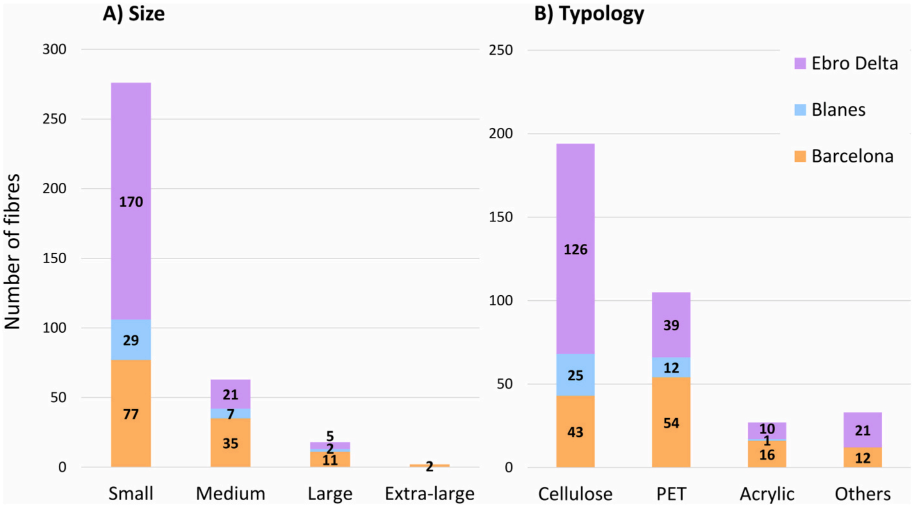

Fig. 3. Differences in Size (A) and Typology (polymeric composition) (B) of fibres found in the digestive tracts of *Scyliorhinus canicula* captured off three localities of the Catalan Coast. The category "Others" includes unknown composition, PP (polypropylene), PA (polyamide) and wool.

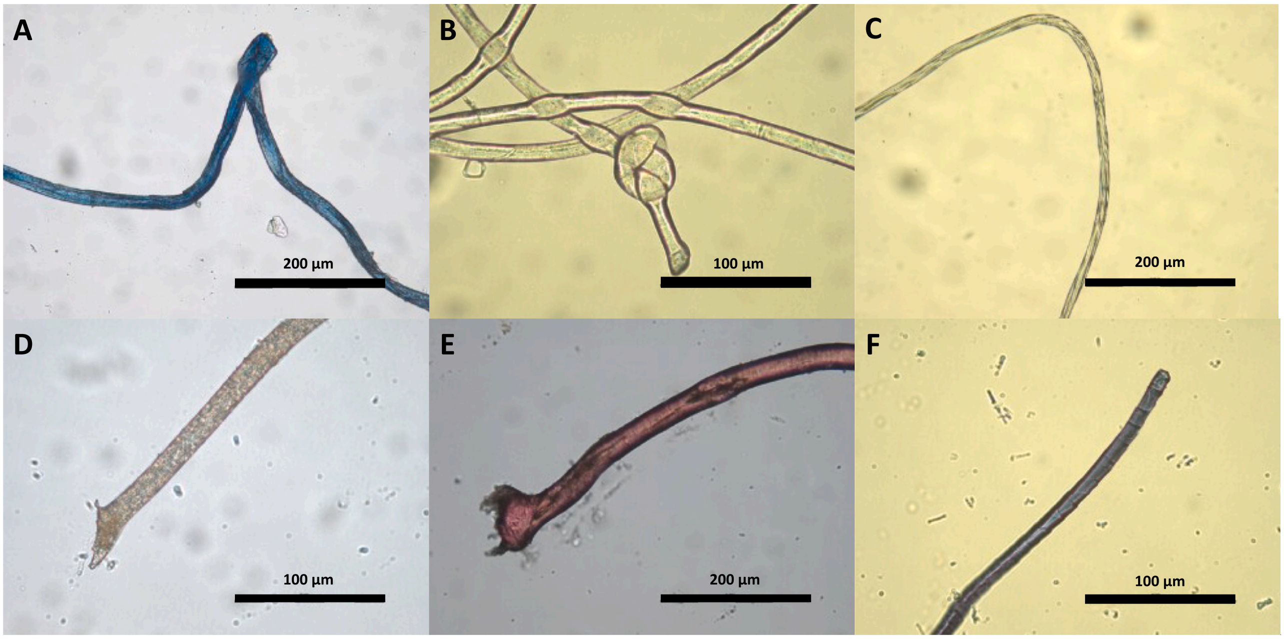

Fig. 4. Representative images of the six different polymeric categories of fibres found in the digestive tracts of *Scyliorhinus canicula.* A) Cellulose, B) PET (polyethylene terephthalate), C) Acrylic (polyacrylonitrile), D) PA (polyamide), E) PP (polypropylene), F) Wool.

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.

			Blanes		Barcelona		Ebro Delta	
Parasite taxa		Site of infection	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}	

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 – P) and the mean diversity index (H) ($R_s = -0.98$, $p < 0.001$), 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.

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).

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

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.In bold, mean values for the global area (Catalan Coast).

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)**

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).

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 $Rs < 0.65$).

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-

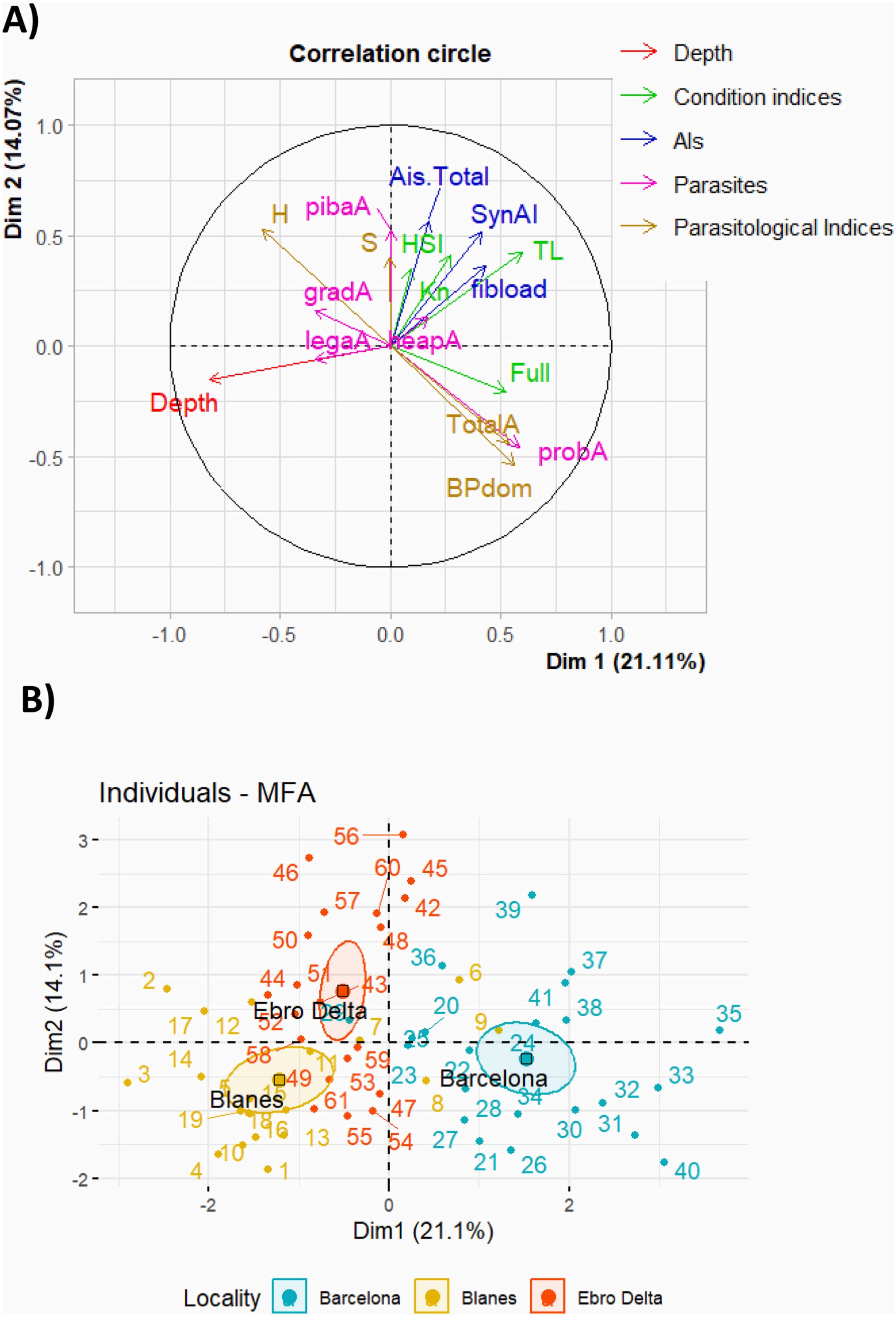

Fig. 5. Multiple factor analysis (MFA) among samples of *Scyliorhinus canicula* caught off three different areas of the Catalan coast. A) MFA among body condition indices (total length (TL); Le Cren relative condition index (Kn); stomach fullness (Fullness); hepatosomatic index (HSI)), abundance of anthropogenic 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.

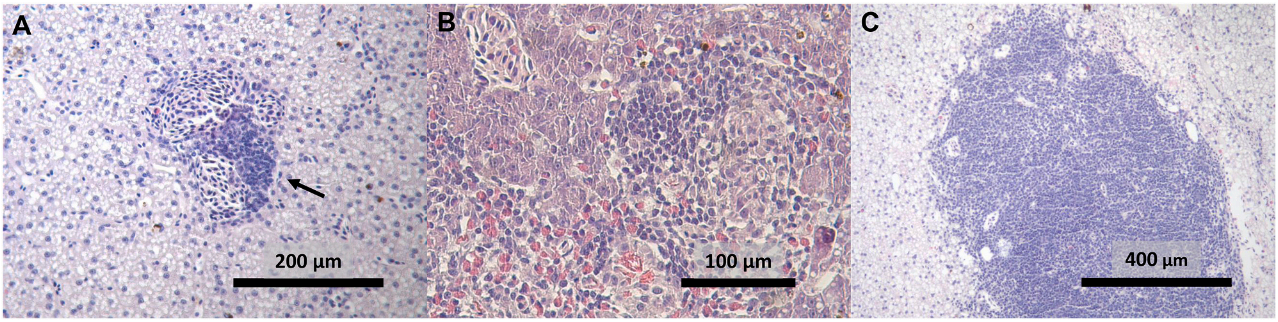

Fig. 6. Liver histological results of *Scyliorhinus canicula* captured off the Catalan coast. A) Inflammatory foci associated to a blood vessel (arrow) B) High presence of eosinophilic granular cells within a blood vessel C) Large inflammatory focus.

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.

Still, *S. canicula* from the present study area, together with those of other Mediterranean regions, are generally characterized 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 characterize 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

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)	68.42 %	Visual inspection	Present study
			Syn = 0.68 (0.89)	Syn = 47.37 %		
	Barcelona	20	5.7 (4.1)	90.91 %		
			Syn = 3.68 (3.06)	Syn = 86.36 %		
	Ebro Delta	22	7.2 (5.8)	100 %		
			Syn = 2.74 (2.62)	Syn = 80 %		
	Catalan Coast	**61**	**5 (4.7)**	**86.89 %**		
			Syn = 2.43 (2.70)	**Syn = 72.13 %**		

the dominance of fibre-shaped items of the marine environmental microlitter 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 found 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 (Gajić 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 indeices. 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; Gajić 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; Gajić 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 Gajić 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). *S. 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 ($2.86 \pm 2.01\ \mu g \cdot g^{-1}$ 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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