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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 analyzing 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.

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

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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 hm^3/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 hm^3/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).

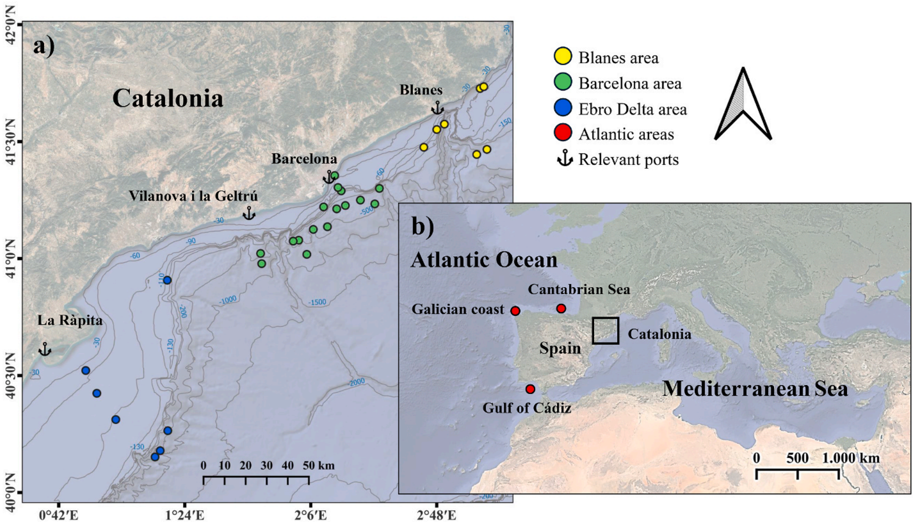

Fig. 1. Map of the study area. Sampling points from three different areas off the Catalan coast and additional sampling points from the Atlantic Ocean.

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 $_{juvenile}$	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 $_{juvenile}$	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 $_{adult}$	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 $_{adult}$	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 HNO_3 (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). Exceptionally, 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 × 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 · 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 · 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: $P = 1/(1+\exp(-\beta 0+\beta 1^*X))$, 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 ($R^2 = 0.62$ 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.

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 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; $\chi^2 = 70.64$, $p < 0.001$) 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

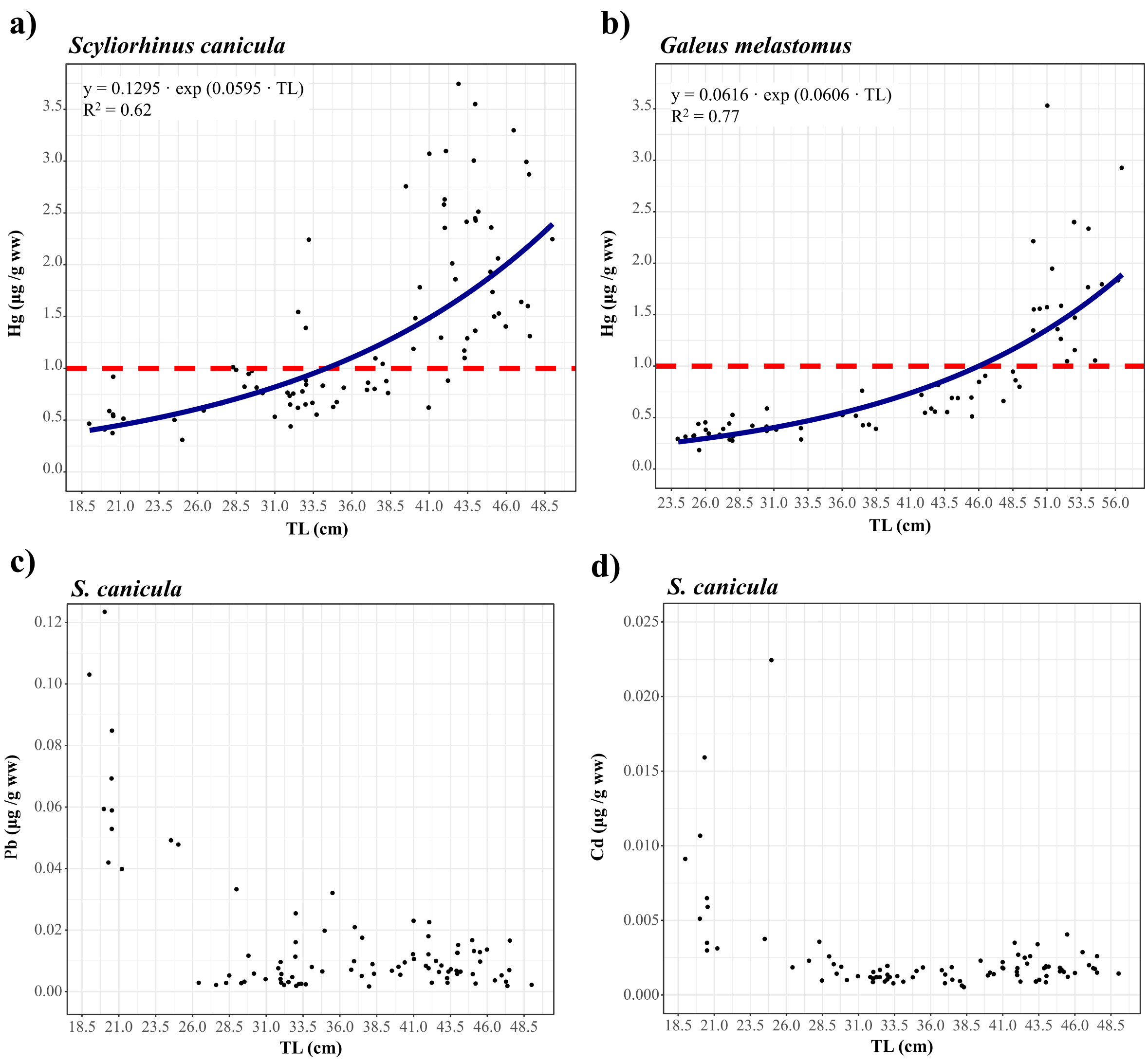

Fig. 2. Correlation plots between trace metals muscle concentrations and total length of sharks. Correlation of mercury (Hg) concentration in muscle tissue of a) *Scyliorhinus canicula* and b) *Galeus melastomus* with total length (TL; cm). Correlation of c) lead (Pb) and d) cadmium (Cd) concentrations 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.

displaying the lowest concentrations (Table 2). Contrary, Pb concentrations did not differ among Spanish study areas (K-W; $\chi^2 = 4.74$, $p = 0.19$). 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; $\chi^2 = 29.36$, $p < 0.001$) (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).

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 ($R^2 = 0.73$). Similarly, for *G. melastomus,* individuals larger than 49.48 and 49.57 cm had a 50 % and 95 % probability of exceeding the limit ($R^2 = 1$).

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

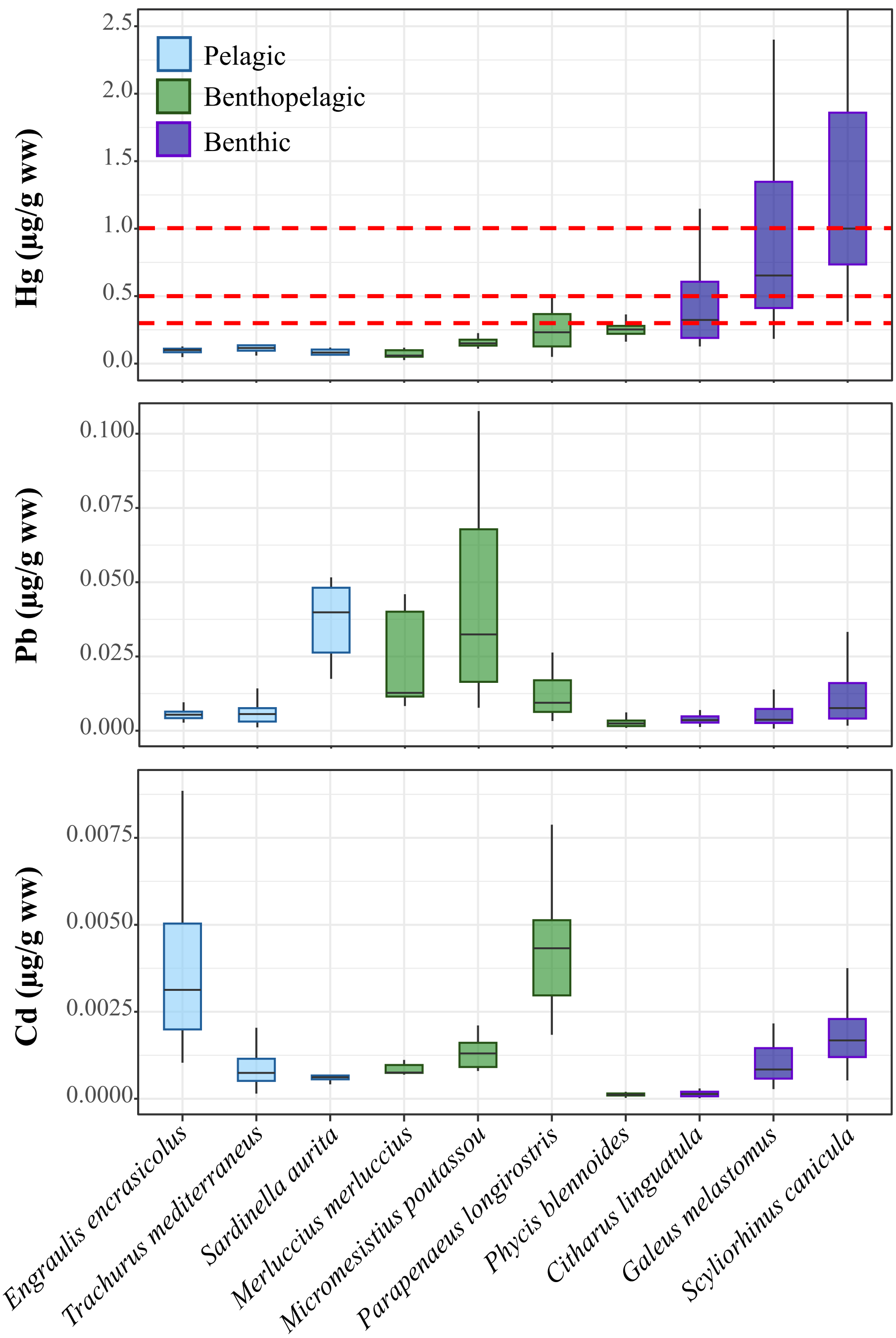

Fig. 3. Boxplots displaying concentrations of mercury (Hg), lead (Pb) and cadmium (Cd) in the different target species, grouped according to their habitat distribution. All values are given in µg/g · wet weight (ww). Red dashed lines indicate the maximum permitted Hg limits for consumption established 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.

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; 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

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)

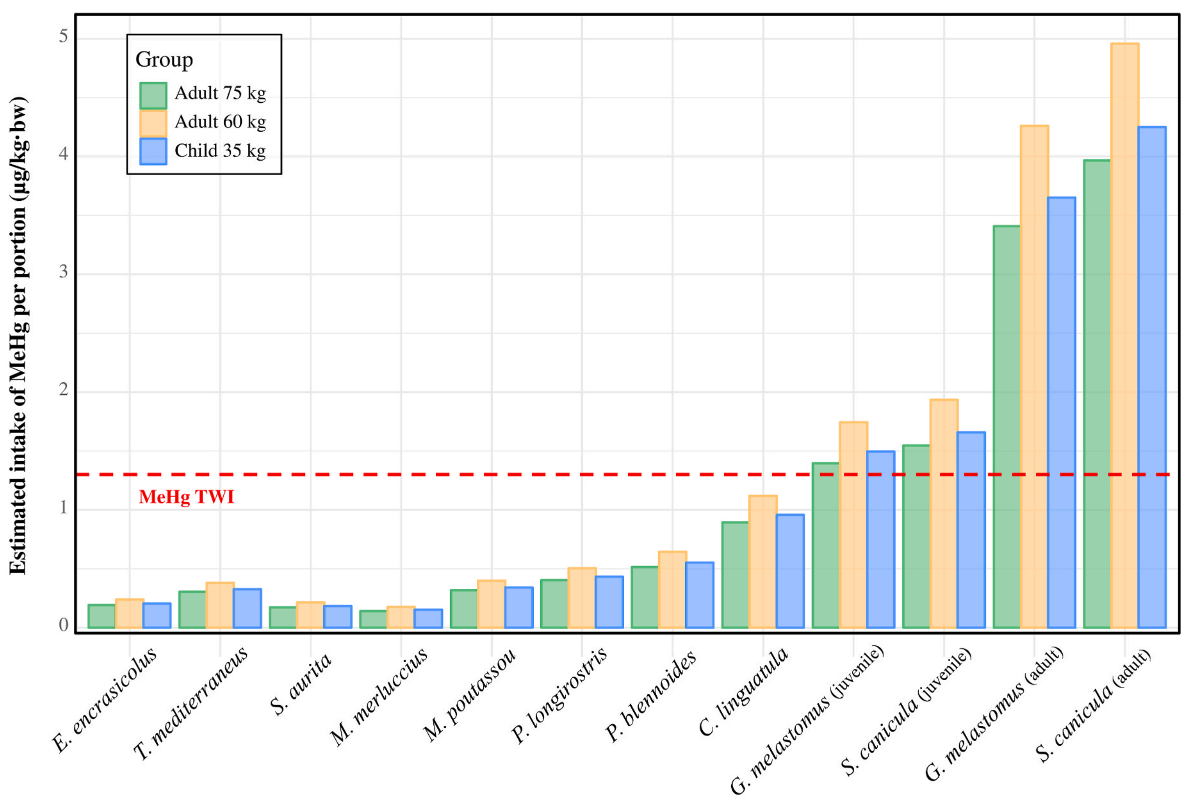

Fig. 4. Estimated methyl mercury (MeHg) intake per portion (corresponding to 150 g for adults and 75 g for children) of the different analysed species from off the Catalan coast, expressed in μg/kg · body weight (bw) depending on the consumer weight (an adult of 75 or 60 kg, or a child of 35 kg). In 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).

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 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

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).

Species	Estimated intake of MeHg per portion (µg/kg·bw)			% TWI 1 portion a month			1 portion a week			3 portions a week		
	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**

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 (Belivermiş 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., 2005; 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 Cadiz (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

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)
Gulf of Cádiz	33.12 ± 1.91 (30–35.8)	24	0.35 ± 0.08 (0.21–0.56)	2013	Present study

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 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 Méndez-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 to 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 exemples include the red shrimp (*Aristeus antennatus* Risso, 1816), Norway lobster (*Nephrops norvegicus* Linnaeus, 1758), black-bellied 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 commmercialization. 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 Sánchez-Marín:** Writing – review & editing, Methodology, Investigation. **Laura Muns-Pujadas:** Writing – review & editing, Methodology. **Maria Constenla:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Sara Dallarés:** 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.122022.

Data availability

Data will be made available on request.

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