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# Characterization of metal contaminants in the Critically Endangered angelshark (*Squatina squatina*): A first ecotoxicological insight

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ABSTRACT

Elasmobranchs are particularly susceptible to heavy metal bioaccumulation due to their apex predator status, high trophic level and limited metabolic detoxification capacity. This poses significant risks to Critically Endangered angelshark (*Squatina squatina)* populations in contaminated habitats. This study quantified 21 trace elements and heavy metals in liver and muscle tissues from 24 stranded *S. squatina* in the Canary Islands, Spain, using cold vapor atomic absorption spectrophotometry (CV-AAS) and inductively coupled plasma optical emission spectrophotometry (ICP-OES). No statistically significant correlations were found between metal concentrations and size, probably due to the limited size range (104.31 ± 18.29 cm TL). Sex also did not affect metal concentrations in the assessed tissues. Liver samples exhibited significantly higher metal concentrations than muscle, consistent with the detoxification and storage functions of this organ. Geographical comparisons revealed significantly elevated concentrations of mercury (Hg; liver: 0.997 ± 1.467 mg/kg ww, muscle: 0.835 ± 0.533 mg/kg ww) and cadmium (Cd; liver: 2.09 ± 1.76 mg/kg ww, muscle: 0.841 ± 1.54 mg/kg ww) in the Canary Islands population compared to other Squatinidae species around the world. These differences are likely driven by a combination of dietary composition, habitat characteristics, volcanic activity and anthropogenic factors. Although toxicological thresholds for elasmobranchs remain undefined, elevated pollutant levels may impair growth, reproduction, and juvenile development, threatening long-term population viability. Establishing baseline contaminant thresholds is crucial for assessing ecotoxicological risks and informing targeted conservation efforts for this species.

## 1. Introduction

Coastal ecosystems worldwide are exposed to numerous direct and indirect anthropogenic stressors, primarily due to the discharge of wastewater and chemical products, driving serious and often irreversible changes (Krishna et al., 2025). These activities severely threaten marine ecosystems by harming aquatic flora and fauna, disrupting trophic networks, and reducing biodiversity (Krishna et al., 2025). An imbalance of heavy metal and trace element concentrations could impact coastal environments, with their persistence and non-biodegradable nature exacerbating ecological damage (de Luna Beraldo et al., 2023; Kim et al., 2019; Tiktak et al., 2020). Contamination arises from both human activities and natural processes, with natural element levels influencing regional patterns and accumulation in marine life (Kravchenko et al., 2014; Lozano-Bilbao et al., 2024).

A wide range of metals, including iron (Fe), copper (Cu), and zinc

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(Zn), are commonly found in marine organisms and play vital roles in biological functions (Carrasco-Puig et al., 2024; Consales and Marsili, 2021; Khawar et al., 2024). However, toxic metals such as mercury (Hg), cadmium (Cd), and lead (Pb) pose significant health risks, disrupting metabolic processes and accumulating in organisms over time(Baby et al., 2011; Cherfi et al., 2014). Macronutrients such as sodium (Na) and potassium (K) are essential for proper metabolism and osmoregulation in sharks (Brooks et al., 2012; Manire et al., 2001). Changes in environmental conditions can affect these mechanisms, and elevated concentrations may indicate stress (Brooks et al., 2012; Manire et al., 2001).

Elasmobranchs are highly susceptible to heavy metal accumulation due to their apex predator status, high trophic position, and slow metabolic elimination rates (Consales and Marsili, 2021; Cortés, 1999; Dulvy et al., 2021; Ferretti et al., 2010). These species primarily acquire trace elements through their diet, leading to significant metal concentrations in different tissues, which may impair physiological functions and disrupt ecosystem dynamics (Carrasco-Puig et al., 2024; Díaz-Delgado et al., 2024; Lozano-Bilbao et al., 2020). Studies have documented the presence of Hg (Díaz-Delgado et al., 2024; Storelli et al., 2005), Cd (Bustamante et al., 1998; Carrasco-Puig et al., 2024) and Pb (Carrasco-Puig et al., 2024; Martins et al., 2021; van Hees and Ebert, 2017) in multiple shark species, with concentrations varying based on tissue type, geographic location and maturation state (Hauser-Davis et al., 2021; Roubie et al., 2024; Tiktak et al., 2020; Türkmen et al., 2013). Research has demonstrated that metal toxicity can compromise systemic health and osmoregulatory functions in various shark species, including the tiger shark (*Galeocerdo Cuvier*), blacktip shark (*Carcharhinus limbatus*) and piked dogfish (*Squalus acanthias*) (Eyckmans et al., 2013; Wosnick et al., 2021). Additionally, hepatic alterations, as indicated by oxidative stress markers, have been observed in different shark species like blue shark (*Prionace glauca*) and mako shark (*Isurus oxyrinchus*) (Barrera-García et al., 2013; Vélez-Alavez et al., 2013).

Recent studies show that the Canary Islands have the highest shark richness in the Macaronesia region, hosting 56 of the 78 recorded species; however, 56 % of these species are facing extinction according to IUCN criteria (Varela et al., 2025). *S. squatina*, classified as Critically Endangered (CR) by the IUCN Red List of Threatened Species (Morey et al., 2019), belongs to one of the world's most threatened shark and ray families, Squatinidae (Dulvy et al., 2014; Kyne et al., 2020). The Canary Islands are an important stronghold for this species (Mead et al., 2023; Meyers et al., 2017). While a recovery plan aiming to protect and restore *S. squatina* in the Canary Islands is currently underway, the species faces threats from habitat destruction, fishing, and pollution, which have yet to be quantified (Jiménez-Alvarado et al., 2020).

Metal contamination poses a particular concern in the Canary Islands due to high anthropogenic use of the marine environment. Several factors have been identified as contributing to this contamination, including underwater outfalls (Lozano-Bilbao et al., 2021b), deposits from aquaculture cages (Xie et al., 2020), agriculture and improper waste management (Barton et al., 1998; Pascual-Fernández et al., 2018), oil spills, plastic waste, and other human-driven impacts (Lozano-Bilbao et al., 2024). Research on metal contamination in the Canary Islands has been carried out in several marine groups, including benthic algae (Lozano et al., 2003), barnacles (Lozano-Bilbao et al., 2021a), anemones (Lozano-Bilbao et al., 2020) and deep-sea sharks (Lozano-Bilbao et al., 2018). However, many vulnerable marine organisms, including coastal elasmobranch species, remain understudied and data poor.

There is limited information regarding pollution levels in *S. squatina* populations, particularly in the Canary Islands, where no prior studies have been conducted. The only available study on *S. squatina* reports metal concentrations in the liver of a single specimen from Cardigan Bay (UK) (Morris et al., 1989). Across the wider Squatinidae family, pollution and ecotoxicology studies have been carried out on *S. californica* in the Gulf of California ($n = 94$) and Tomales Bay (USA) ($n = 18$) (Escobar-Sánchez et al., 2016; Gassel et al., 2004), *S. guggenheim* in Southeastern Brazil (n = 9) (Martins et al., 2021), *S. argentina* in Southern Brazil ($n = 2$) (Kütter et al., 2009), *S. dumeril* in the Northwestern Atlantic (n = 1) (Greig et al., 1975), and *S. aculeata* in the North-Eastern Mediterranean ($n = 1$) (Turan et al., 2021). Despite these efforts, there remains a significant gap in ecotoxicological data for *S. squatina*, particularly in the eastern Atlantic.

The present study aims to analyse the concentrations of toxic heavy metals (Al, Cd, Hg, and Pb), trace and essential metals (B, Ba, Co, Cu, Cr, Fe, Li, Mn, Mo, Ni, Sr, V, and Zn), and macroelements (Ca, K, Mg, and Na) in the white muscle and liver tissues of *S. squatina* in the Canary Islands. We evaluated the influence of biological factors, including body length, weight, sex and life stage (juvenile, adult), on metal concentrations in tissues, and compared metal concentrations across the different *S. squatina* management units present in the Canary Islands (Meyers et al., 2024). Concentrations from other species in the Squatinidae family were compared with those analysed in this study to assess species-specific differences and possible biogeographic variation.

## 2. Material and methods

### 2.1. Study area

The Canary Islands are a Spanish archipelago in the North Atlantic Ocean, and part of the Macaronesia region. Located approximately 100 km off the northwest coast of Africa at the nearest point, the archipelago comprises eight main islands distributed over 500 km from east to west: La Graciosa, Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma, and El Hierro (Fig. 1) (Fernández-Palacios and Whittaker, 2008). This study focuses on the central and eastern islands, where *S. squatina* is more frequently reported (Meyers et al., 2017), and samples were collected from Tenerife, Fuerteventura, Lanzarote, and La Graciosa.

### 2.2. Sample collection

All samples were collected from 24 stranded individuals between 2017 and 2023 (Tenerife ($n = 4$), Fuerteventura (n = 1), Lanzarote ($n = 18$), and La Graciosa (n = 1)) (Fig. 1). The specimens were recovered by the competent authorities, including the Civil Guard and Environmental Agents, and managed by the Angel Shark Project (ASP, https://angelsharkproject.com/) under the relevant permits. Researchers from the Universidad de Las Palmas de Gran Canaria (ULPGC) at the Institute for Animal Health and Food Security (IUSA) determined causes of death to be either anthropogenic or natural.

For each *S. squatina* specimen, weight, length and sex were recorded. In cases where decomposition prevented data collection, the sex was recorded as undetermined. The period each *S. squatina* remained on the beach before collection is unknown. After collection, *S. squatina* were preserved at −80 °C to maintain tissue integrity and chemical composition. The preservation period never exceeded one month prior to necropsy. During necropsy, samples were manually collected using nitrile gloves and non-metallic tools to prevent contamination, and approximately 10 g of white muscle and liver tissue were extracted from each specimen and preserved at −18 °C to prevent chemical degradation.

All samples were collected with permits from the Ministry of Environment and Ecological Transition of Spain and the Canary Island Government. Animal handling and sampling methods were assessed and approved by animal ethical committees at Universidad de Las Palmas de Gran Canaria.

### 2.3. Sample analysis

*S. squatina* muscle and liver samples were processed through incineration to determine the concentration of Al, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V and Zn (Lozano-Bilbao et al., 2021b, 2020). Samples were placed in porcelain crucibles, dried in an

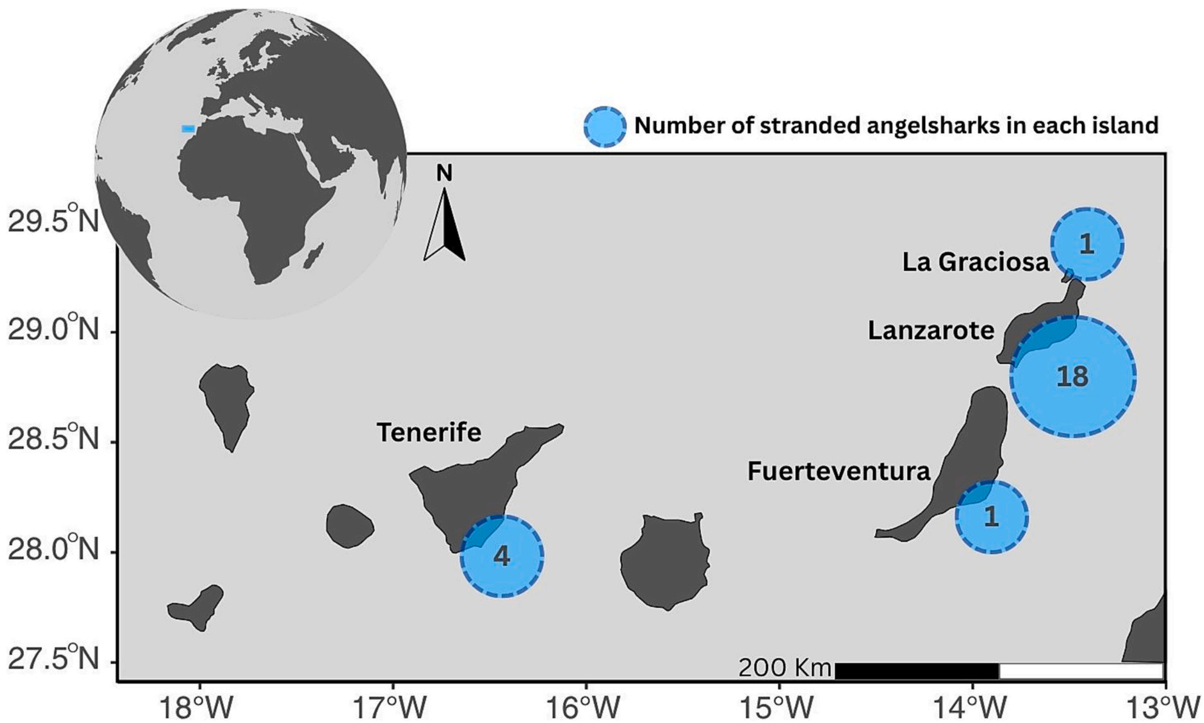


**Fig. 1.** Study area and sample collection, with the number of stranded *Squatina squatina* sampled from each island.

oven for 24 h at 80 °C and then incinerated in a furnace. The furnace was programmed to gradually reach 450 °C ± 25 °C over 24 h, and this temperature was maintained for an additional 24 h until greyish ashes were obtained. 65 % $HNO_3$ was then added to the samples and evaporated using a heating plate at 80 °C. Once treated, a re-incineration was conducted in the furnace for 24 h. Once white ashes were obtained, each of the samples was filtered with 1.5 % $HNO_3$ in flasks and made up to 25 mL with distilled water. The metal content in each sample was determined using inductively coupled plasma optical emission spectrophotometry (ICP-OES) (Lozano-Bilbao et al., 2021b).

For the Hg determination, 1 g of muscle and liver sample was processed with acid digestion pumps equipped with a Parr Instrument Teflon sample vessel, with 4 mL of 65 % $HNO_3$ and 2 mL of 30 % $H_2O_2$. The samples were then subjected to microwave digestion using a temperature program consisting of a 10-min ramp to 70 °C, followed by a 5-min hold at this temperature. This was followed by a 20-min ramp to 180 °C, followed by a 10-min hold at this final temperature. After digestion, samples were transferred to 10 mL volumetric flasks and diluted with 1.5 % $HNO_3$. The mercury concentration was then determined using cold vapor atomic absorption spectrophotometry (CV-AAS) (Franco-Fuentes et al., 2023). Quality control measures for mercury analysis included the use of certified reference materials traceable to NIST, recovery tests with spiked samples at 0.06, 0.5, and 2 mg/kg to verify accuracy and confirm the LOQ (0.06 mg/kg), duplicate sample analysis to ensure precision, periodic instrument checks with a multi-element standard, and full documentation to ensure traceability.

### 2.4. Statistical analysis

All statistical analysis was carried out in R Statistical Software v4.4.2 (Team, 2019), and figures were produced in R and Adobe Illustrator (2024). Descriptive statistical analyses were performed to calculate the mean, standard deviation, minimum, and maximum values of the data obtained in the study. For each dataset, normality was assessed using the Shapiro-Wilk test, and heteroscedasticity was evaluated using Levene's test. Radial plots were represented with the mean and with Min-Max normalization (0–1) to simplify their visualization.

Nested dataset designs were employed with the mixed factors "sex" (male, female), "Management Units" (Tenerife, East-Islands), according to the ones defined by (Meyers et al., 2024) "tissue" (liver, muscle) and "age" (newborn <30.5 cm, adult). As the datasets exhibited non-normal distributions, non-parametric Kruskal-Wallis tests were conducted with Benjamini-Hochberg (BH) adjustment, followed by Dunn's post hoc test. For graphical representation, outliers were removed using the Interquartile Range (IQR) method to simplify and stylize their visualization. While outliers were excluded from plots to reduce distortion and enhance clarity, they were retained in the analysis to preserve data integrity without assuming any distribution.

The Spearman rank correlation coefficient (rs) was used to assess correlations between element concentrations and biometric measurements, such as total length (cm), weight (kg), sex (male or female), and sampling area with a BH method for adjusting the *p*-values. Additionally, a principal component analysis (PCA) was performed to evaluate the distribution and correlation patterns of element concentrations.

For all analyses, statistical significance levels were set to p-value (P) $< 0.05$.

## 3. Results

Of the 24 *S. squatina* analysed in this study, there were 13 males and 11 females. Elevated heavy metal concentration was not identified as the primary cause of death during any of the necropsies. Total length (TL) varied from 30.5 to 120.5 cm and weight varied from 6.5 to 14 Kg (Table 1).

### 3.1. Small scale differences

Specimens were obtained from two management units, as identified in Meyers et al., 2024; the first unit included individuals from Tenerife, and the second unit comprised individuals from Fuerteventura, Lanzarote, and La Graciosa, collectively referred to as the Eastern Islands. Results revealed no clear spatial clustering of individuals based on geographic origin (Fig. 2) and the ordination plot shows considerable overlap between samples from Tenerife and the Eastern Islands.

**Table 1**
Total length (cm) and weight (kg) of *S. squatina* stranded in Canary Islands: number of stranded *S. squatina* (n), mean (MEAN) and Standard Deviation (SD). Data from two management units, Tenerife and Eastern-Islands (Fuerteventura, Lanzarote and La Graciosa), are shown separately. TL and weight data were not recorded for all the stranded *S. squatina* due to the preservation state.

| | | TL (cm) | Weight (Kg) |
|---|---|---|---|
| Canary Islands | Total (n = 24) | *n* = 22 | n = 20 |
| | MEAN ± SD | 104.31 ± 18.29 | 11.36 ± 1.81 |
| | Min-Max | 30.5–120.5 | 6.5–14 |
| | Males (*n* = 13) | *n* = 12 | n = 13 |
| | MEAN ± SD | 109.58 ± 3.93 | 10.57 ± 1.86 |
| | Min-Max | 104.1–117 | 6.5–13 |
| | Females (*n* = 11) | *n* = 10 | n = 9 |
| | MEAN ± SD | 99.49 ± 26.92 | 10.57 ± 1.86 |
| | Min-Max | 30.5–120.5 | 11.5–14 |
| Tenerife | Total (n = 4) | n = 4 | *n* = 3 |
| | MEAN ± SD | 85,65 ± 36.859 | 10.43 ± 3.68 |
| | Min-Max | 30.5–107.2 | 6.5–13.8 |
| Eastern-Islands | Total (n = 20) | *n* = 18 | *n* = 17 |
| | MEAN ± SD | 107.74 ± 19,76 | 11.71 ± 1.39 |
| | Min-Max | 30.5–120.5 | 9–13.13 |

Variation in metal concentrations appeared to be more strongly associated with tissue type, with liver samples showing greater influence from elements such as Cd, Mo, Al, and Zn. As no significant differences in metal concentrations were observed between the two local management units, all samples were pooled and treated as a single unit for subsequent analyses.

### 3.2. Concentrations of trace and macro elements

The concentrations of heavy metals, trace and macro elements detected in the samples of *S. squatina* are presented in Table 2. Element concentration levels in this study were not affected by sex, but a significant difference was observed in two cases: liver Mg (444.00 ± 289.71 mg/kg w.w. males and 586.76 ± 308.66 mg/kg w.w. in females) and white muscle Ba (0.09 ± 0.08 mg/kg w.w. males and 0.18 ± 0.14 mg/kg w.w. in females), where concentrations were significantly higher in females than males in both cases. Concentrations were not associated with pregnancy or other factors, consistent with findings from other studies where Mg and boron B concentrations showed no sex-related differences (Baró-Camarasa et al., 2023; Bosch et al., 2013).

The mean concentrations of the elements in white muscle were ranked in the following order from highest to lowest: Na > K > Mg > Ca > Fe > Zn > Al > Li > Sr > Cu > Cd > B > Mn > Cr > V > Ni > Mo > Pb > Co > Ba. In the liver, the element concentration sequence differs slightly: Na > K > Mg > Fe > Ca > Zn > Al > Li > Cu > Cd > Sr > Hg > Mn > V > Cr > B > Ba > Co > Mo > Ni > Pb. Na exhibited the highest concentrations in samples of both white muscle (2986.01 ± 1335.324 mg/kg w.w.) and liver (3952.09 ± 1576.02 mg/kg w.w.). High concentrations of other trace and macro elements included Mn (456.45 ± 253.04 mg/kg w.w.) and Hg (0.835 ± 0.53 mg/kg w.w.) in the white muscle, Fe in the liver (469.18 ± 502.27 mg/kg w.w.), Zn in both liver (24.92 ± 0.53 mg/kg w.w.) and white muscle (16.31 ± 37.07 mg/kg w.w.).and.

Concentrations of most elements were higher in liver samples than white muscle samples, except for K, Ni and B (Fig. 3). Mo, Zn, Cd, Pb, Fe, Co, V, Cu, Sr, and Na concentrations were all significantly higher in liver samples, while only B concentrations were significantly higher in white muscle samples (Fig. 4, Table 2). Specifically, Zn, Cd, and Pb concentrations in the liver were 1.50, 2.48, and 1.43 times higher, respectively, compared to white muscle, whereas B concentration in white muscle was 3.86 times higher than in the liver.

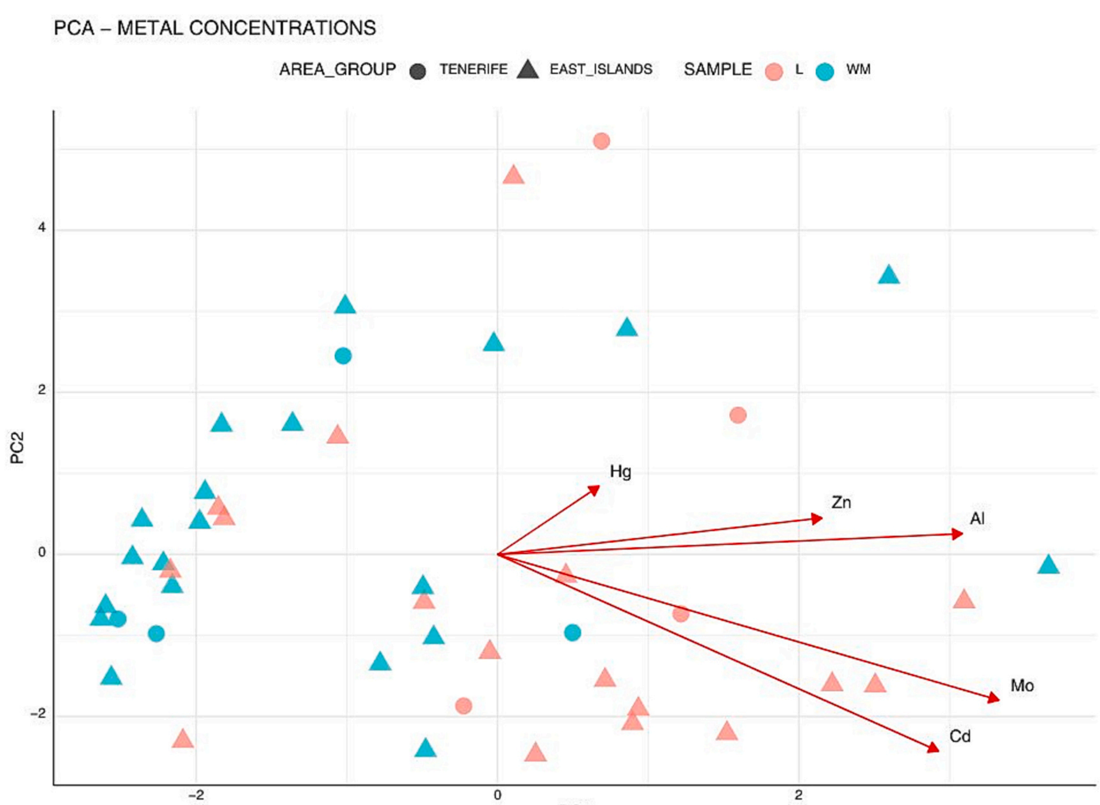


**Fig. 2.** Principal Component Analysis (PCA) of stranded *Squatina squatina* individuals. Sample shapes indicate the management units: Tenerife and the Eastern Islands (Fuerteventura, Lanzarote, and La Graciosa). Sample colours represent tissue type: Liver (L, blue) and White Muscle (WM, light red). The five most influential elements are represented by red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Element concentrations (mg/kg w.w.) in the liver and white muscle of *S. squatina* from the Canary Islands, presented for the total sample, males, and females. Values are given as mean ± standard deviation (SD). Detection limits (mg/L) for each element are also reported.

| | | Canary Islands | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total | | Males | | Females | |
| Elements | Detection limits (mg/L) | Liver | White Muscle | Liver | White Muscle | Liver | White Muscle |
| Hg | 0.000168 | 0.997 ± 1.467 | 0.835 ± 0.533 | 0.46 ± 0.37 | 0.73 ± 0.33 | 1.65 ± 1.93 | 1.07 ± 0.78 |
| Cd | 0.0007 | 2.09 ± 1.76 | 0.841 ± 1.54 | 1.86 ± 1.46 | 0.81 ± 1.74 | 2.18 ± 2.14 | 1.27 ± 1.87 |
| Pb | 0.0009 | 0.023 ± 0.015 | 0.016 ± 0.020 | 0.024 ± 0.014 | 0.011 ± 0.008 | 0.021 ± 0.017 | 0.021 ± 0.027 |
| Al | 0.005 | 13.91 ± 17.92 | 7.43 ± 8.10 | 8.75 ± 6.46 | 7.51 ± 10.27 | 19.33 ± 24.40 | 8.28 ± 5.70 |
| **Zn** | 0.0027 | 24.92 ± 15.05 | 16.31 ± 37.07 | 20.36 ± 11.35 | 9.64 ± 11.89 | 28.58 ± 18.40 | 25.40 ± 51.21 |
| Mo | 0.0016 | 0.071 ± 0.069 | 0.021 ± 0.035 | 0.055 ± 0.039 | 0.024 ± 0.044 | 0.083 ± 0.092 | 0.023 ± 0.026 |
| Co | 0.001 | 0.080 ± 0.061 | 0.020 ± 0.032 | 0.068 ± 0.052 | 0.024 ± 0.038 | 0.089 ± 0.072 | 0.026 ± 0.040 |
| Ni | 0.0009 | 0.046 ± 0.044 | 0.064 ± 0.073 | 0.033 ± 0.016 | 0.077 ± 0.094 | 0.060 ± 0.060 | 0.046 ± 0.038 |
| Fe | 0.004 | 469.18 ± 502.27 | 88.15 ± 166.16 | 412.99 ± 338.01 | 65.70 ± 134.58 | 493.83 ± 658.95 | 133.48 ± 202.29 |
| B | 0.008 | 0.122 ± 0.290 | 0.471 ± 0.785 | 0.094 ± 0.212 | 0.41 ± 0.77 | 0.16 ± 0.36 | 0.49 ± 0.81 |
| Mn | 0.0008 | 0.633 ± 0.454 | 0.419 ± 0.330 | 0.56 ± 0.31 | 0.48 ± 0.42 | 0.70 ± 0.58 | 0.34 ± 0.17 |
| Cr | 0.001 | 0.141 ± 0.108 | 0.133 ± 0.116 | 0.11 ± 0.061 | 0.15 ± 0.15 | 0.18 ± 0.14 | 0.11 ± 0.041 |
| V | 0.0014 | 0.191 ± 0.200 | 0.043 ± 0.090 | 0.15 ± 0.11 | 0.052 ± 0.114 | 0.18 ± 0.15 | 0.053 ± 0.085 |
| Ca | 1.629 | 323.92 ± 247.01 | 282.42 ± 348.95 | 298.31 ± 217.72 | 324.42 ± 470.62 | 351.35 ± 275.26 | 244.59 ± 100.55 |
| Cu | 0.003 | 2.22 ± 1.99 | 1.10 ± 0.77 | 1.89 ± 1.43 | 1.07 ± 0.72 | 2.55 ± 2.45 | 1.12 ± 0.83 |
| Mg | 1.580 | 492.74 ± 296.00 | 456.45 ± 252.04 | 444.00 ± 289.71 | 424.60 ± 208.95 | 586.76 ± 308.66 | 516.60 ± 297.77 |
| Sr | 0.003 | 1.83 ± 1.49 | 1.29 ± 1.89 | 1.89 ± 1.73 | 1.53 ± 2.52 | 1.75 ± 1.15 | 1.16 ± 0.81 |
| Ba | 0.0006 | 0.127 ± 0.119 | 0.125 ± 0.110 | 0.078 ± 0.043 | 0.09 ± 0.08 | 0.18 ± 0.15 | 0.18 ± 0.14 |
| Li | 0.013 | 3.10 ± 3.27 | 2.46 ± 1.98 | 2.41 ± 1.63 | 2.19 ± 1.96 | 4.42 ± 4.57 | 2.72 ± 1.97 |
| K | 1.764 | 2498.87 ± 1002.92 | 2654.714 ± 1513.06 | 2266.068 ± 830.9132 | 2475.309 ± 690.6823 | 2801.524 ± 1165.3851 | 2866.738 ± 2145.1852 |
| Na | 2.221 | 3952.09 ± 1576.02 | 2986.01 ± 1335.34 | 3898.62 ± 1640.97 | 3175.01 ± 1565.94 | 4136.60 ± 1539.48 | 3036.92 ± 1365.22 |

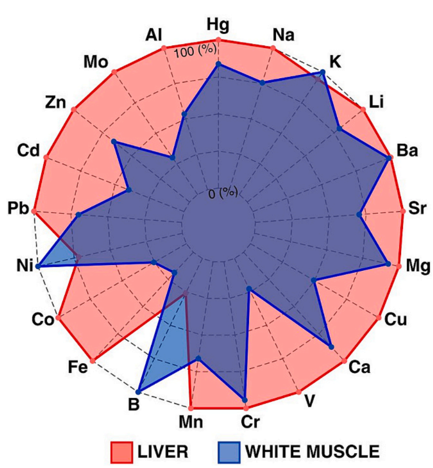


**Fig. 3.** Comparison between *Squatina squatina* liver and white muscle from Canary Islands, metal concentration represented with a Min-Max normalization for each metal.

The analysis revealed no statistically significant correlations between element concentrations and biometric parameters (Fig. 5). Similarly, no significant differences in element concentrations were found between adults and newborns ($\leq$30.5 cm, $n$ = 2). However, strong positive inter-element correlations were observed. In liver tissue, Co was strongly positively correlated with Mo, Zn and Cd, Mo correlated positively with Cd, Zn and V, and Zn was positively correlated with Cd. In white muscle tissue, significant positive correlations were found between Zn and both Mo and Cu, between Ni and Cr, and between V and Co. A single negative correlation was observed between Hg and Cr in white muscle tissue.

### *3.3. Comparison with other Squatinidae species*

Metal concentrations in *S. squatina* from the Canary Islands ($N$ = 24) were compared with available data for other *Squatina* species worldwide (Table 3). In liver tissue, Cd concentrations in Canary Islands specimens (2.09 ± 1.76 mg/kg w.w.) were approximately nine times higher than those reported for *S. guggenheim* from southern Brazil (0.229 ± 0.214 mg/kg w.w) (Martins et al., 2021), and 34 times higher than those observed in *S. squatina* from Cardigan Bay, United Kingdom (0.06 mg/kg w.w.) (Morris et al., 1989). In white muscle, Cd concentrations (0.841 ± 1.54 mg/kg w.w.) were also elevated in the Canary Islands specimens compared to *S. guggenheim* (0.161 ± 0.041 mg/kg w.w.) (Martins et al., 2021). Cr concentrations in liver tissue were substantially higher in the Canary Islands specimens (0.141 ± 0.108 mg/kg w.w.) compared to *S. squatina* from the UK (<0.6 mg/kg w.w.)(Morris et al., 1989) and *S. guggenheim* from Brazil (0.077 ± 0.061 mg/kg w.w.) (Martins et al., 2021). However, in muscle tissue, *S. guggenheim* from southern Brazil exhibited Cr concentrations (0,327 ± 2.77 mg/kg w.w.)(Martins et al., 2021) approximately three times higher than those observed in the Canary Islands population.

Pb concentrations in both liver (0.023 ± 0.015 mg/kg w.w.) and muscle (0.016 ± 0.020 mg/kg w.w.) were higher in *S. guggenheim (*Martins et al., 2021*)* and *S. squatina* from Cardigan Bay (Morris et al., 1989)than in the Canary Islands specimens. Cu concentrations in *S. aculeata* from the northeastern Mediterranean (Turan et al., 2021) were > 200 times lower in muscle and 55 times lower in liver, compared to *S. squatina* from the Canary Islands (2.22 ± 1.99 and 1.10 ± 0.77 mg/kg w.w., respectively). Cu levels in *S. squatina* from Cardigan Bay (2.1 mg/kg w.w.) (Morris et al., 1989)and *S. guggenheim* (2.79 ± 2.08 mg/kg w.w.) (Martins et al., 2021) were comparable to those observed in the Canary Islands population.

In both tissues, Fe concentrations were higher in the Canary Islands *S. squatina* (469.18 ± 502.27 mg/kg w.w. liver and 88.15 ± 166.16 mg/kg w.w. muscle) than in *S. aculeata* (Turan et al., 2021) and *S. guggenheim (*Martins et al., 2021*)*. While Zn concentrations in muscle tissue were similar between *S. aculeata* (17.81 ± 1.89 mg/kg w.w.) (Turan et al., 2021) and Canary Islands specimens, Zn levels in liver tissue were markedly higher in *S. aculeata (*Turan et al., 2021*)*. A similar pattern was observed for Mn, with *S. squatina* from the Canary Islands exhibiting substantially higher concentrations in both tissues (0.633 ± 0.454 and 0.419 ± 0.330 mg/kg w.w.) compared to *S. aculeata* (0.03 ± 0.01 and 0.04 ± 0.00 mg/kg w.w.) (Turan et al., 2021). Regarding Hg,

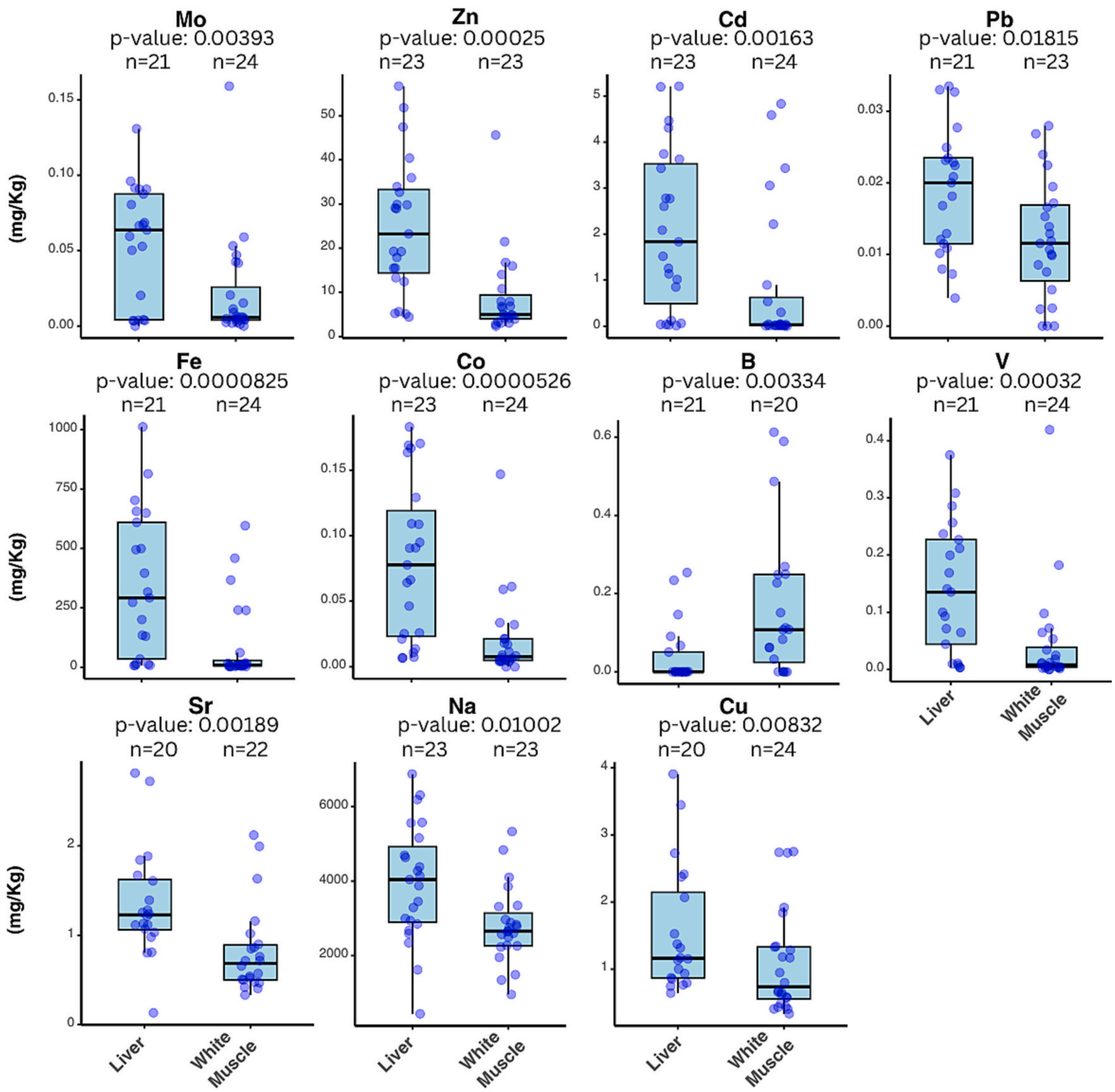


**Fig. 4.** Comparison between *Squatina squatina* Liver (L, $n$ = 23) and White Muscle (WM, $n$ = 24), only represented metals with a significant difference between tissues. Graphic stylized with outliers' exclusion.

the highest concentration in muscle tissue was reported for *S. aculeata* from the northeastern Mediterranean (19.9942 mg/kg w.w.) (Turan et al., 2021). In all other species reviewed, including *S. guggenheim*, *S. californica*, *S. dumeril*, and *S. argentina (*Escobar-Sánchez et al., 2016*;* Gassel et al., 2004*;* Greig et al., 1975*;* Kütter et al., 2009*;* Martins et al., 2021*;* Morris et al., 1989*)*, muscle Hg concentrations ranged between 0.02 and 0.47 mg/kg w.w., all lower than those observed in *S. squatina* from the Canary Islands (0.835 ± 0.533 mg/kg w.w.). Furthermore, the liver Hg concentration in the Canary Islands specimens (0.997 ± 1.467 mg/kg w.w.) was the highest among the compared species.

## 4. Discussion

This study highlights notable variability in metal concentrations among *S. squatina* individuals sampled from the Canary Islands, providing new insights into contaminant accumulation in this Critically Endangered species. Variability may reflect the heterogeneous distribution of metals within specimens, influenced by multiple biological and environmental factors. Given *S. squatina*'s long lifespan and high trophic level, such variability was expected, as the species is likely to bioaccumulate and biomagnify trace elements over time through both dietary intake and environmental exposure (Consales and Marsili, 2021). Analyzing larger individuals and a broader size range would provide a better understanding of bioaccumulation dynamics within the population. Similar patterns have been reported in other large shark species, including *Carcharhinus albimarginatus*, *Carcharhinus brachyurus*, and *Galeocerdo cuvier*, where metal levels increase with age and size due to prolonged exposure (Endo et al., 2016, 2008; Kim et al., 2019). However, the relatively narrow size range of sampled individuals (104.31 ± 18.29 cm TL), combined with the low number of juveniles (<30.5 cm TL), limited our ability to detect significant correlations between size and trace element concentrations.

Individual traits such as age, sex and behaviour may also influence contaminant profiles. Although behavioural sexual trends have been observed in *S. squatina* in La Graciosa, where males tend to occupy deeper waters and exhibit greater movement (Mead et al., 2023), no significant sex-based differences in metal concentrations were detected. This contrasts with other elasmobranchs such as *Prionace glauca* and *Alopias pelagicus*, where spatial and dietary sexual segregation influences metal accumulation (Álvaro-Berlanga et al., 2021).

Genetic studies have identified three distinct management units for *S. squatina* in the Canary Islands: Tenerife, Gran Canaria, and the Eastern Islands (Fuerteventura, Lanzarote, and La Graciosa) (Meyers et al., 2024). Despite differences in sample sizes (Tenerife: $n$ = 4; Eastern

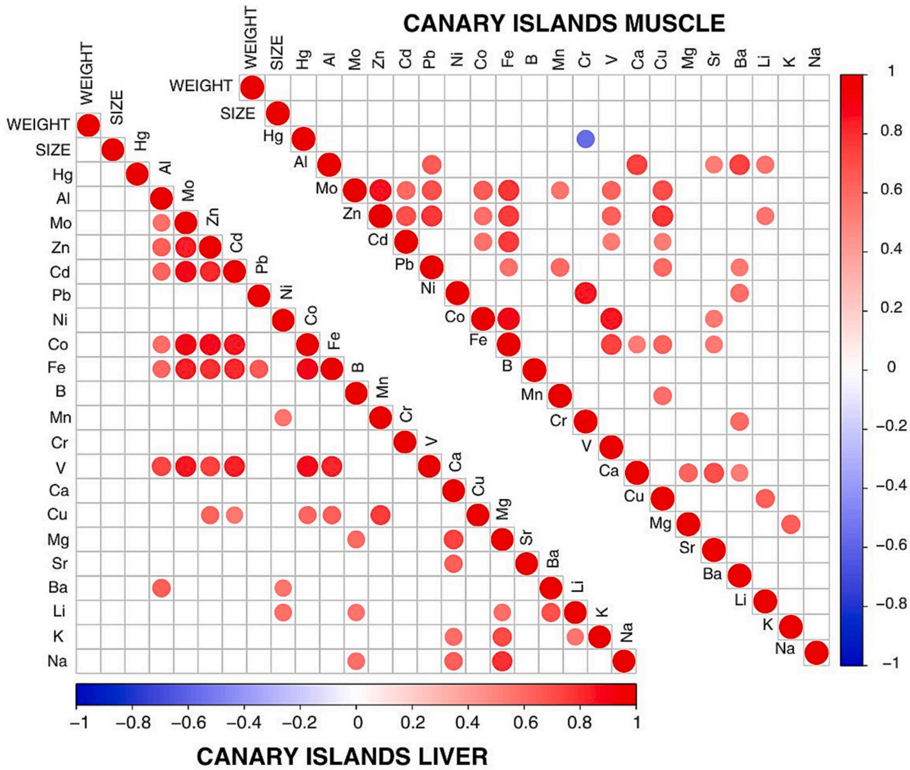


**Fig. 5.** Spearman correlation analysis between element concentrations and biometric parameters (Weight in Kg and Total Length in cm). Only statistically significant correlations are shown. Positive correlations are indicated in red and negative correlations in blue, with colour intensity representing the strength of the correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 3**
Comparison of trace metal concentrations (mg/kg w.w.) in Squatinidae family from previous articles. Not all metals were analysed in all the previous studies found.

| Species | Tissues | Location | Cd | Cr | Pb | Hg | Cu | Fe | Zn | Mn | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Squatina squatina* ($n = 24$) | Liver | Canary Islands | 2.09 ± 1.76 | 0.141 ± 0.108 | 0.023 ± 0.015 | 0.997 ± 1.467 | 2.22 ± 1.99 | 469.18 ± 502.27 | 24.92 ± 15.05 | 0.633 ± 0.454 | Present study |
| *Squatina squatina* ($n = 24$) | Muscle | Canary Islands | 0.841 ± 1.54 | 0.133 ± 0.116 | 0.016 ± 0.020 | 0.835 ± 0.533 | 1.10 ± 0.77 | 88.15 ± 166.16 | 16.31 ± 37.07 | 0.419 ± 0.330 | Present study |
| *Squatina aculeata* ($n = 1$) | Muscle | North-Eastern Mediterranean | – | – | – | 19.99 ± 1,61 | 0.01 ± 0.00 | 26.00 ± 8.24 | 10.85 ± 1.72 | 0.03 ± 0.01 | Turan et al., 2021 |
| *Squatina aculeata* ($n = 1$) | Liver | North-Eastern Mediterranean | – | – | – | 0.4 ± 0.04 | 0.02 ± 0.00 | 50.57 ± 9.02 | 17.81 ± 1.89 | 0.04 ± 0.00 | Turan et al., 2021 |
| *Squatina squatina* (n = 1) | Liver | Cardigan Bay (UK) | 0.06 | <0.6 | 0.7 | 0.07 | 2.1 | – | – | – | Morris et al., 1989 |
| *Squatina dumeril* (n = 1) | Muscle | North-western Atlantic | – | – | – | 0.08 | – | – | – | – | Greig et al., 1975 |
| *Squatina argentina* (n = 2) | Muscle | Southern Brazilian | – | – | – | 0.304 | – | – | – | – | Kütter et al., 2009 |
| *Squatina californica* ($n = 18$) | Muscle | Tomales Bay | – | – | – | 0.47 ± 0.15 | – | – | – | – | Gassel et al., 2004 |
| *Squatina guggenheim* ($n = 9$) | Liver | Southeastern Brazil | 0.229 ± 0.214 | 0.077 ± 0.061 | 0.026 ± 0.049 | 0.02 ± 0.01 | 2.79 ± 2.08 | 61.11 ± 23.29 | – | – | Martins et al., 2021 |
| *Squatina guggenheim* (n = 9) | Muscle | Southeastern Brazil | 0.161 ± 0.041 | 0,327 ± 2.77 | 0.222 ± 0.164 | 0.02 ± 0.05 | 1.02 ± 0.66 | 42.20 ± 11.98 | – | – | Martins et al., 2021 |
| *Squatina californica* ($n = 94$) | Muscle | Southern Gulf California | – | – | – | 0.24 ± 0.27 | – | – | – | – | Escobar-Sánchez et al., 2016 |

Islands: $n = 20$), no significant spatial variation in metal concentrations was detected across these regions or at finer geographic scales. This is somewhat surprising given the geophysical and oceanographic variation present across the Canary Islands (e.g. temperature gradients, volcanic activity), which would be expected to influence contaminant profiles. The findings of this study suggest that, for the purpose of contaminant analysis, *S. squatina* populations in the Canary Islands can be treated as a single management unit.

Tissue-specific differences in metal concentrations were consistent with physiological expectations. The liver, being the primary organ for xenobiotic metabolism and detoxification, showed higher concentrations of most trace metals compared to muscle, which reflects slower, long-term accumulation (Van der Oost et al., 2003). This pattern has been widely observed in elasmobranchs species such as *Carcharhinus* sp., *P. glauca* y *Scyliorhinus canicula* (Gilbert et al., 2015; Hauser-Davis et al., 2021; Storelli et al., 2005). In the current study, trace element distribution was metal-specific. Fe and Cr were particularly elevated in hepatic tissue. Fe is essential for haemoglobin production and enzymatic function, and its accumulation may reflect both physiological demand and dietary intake (Roubie et al., 2024). Crpresence in the liver is likely due to both its affinity for metallothionein's and environmental inputs such as industrial discharge or volcanic emissions (Oana, 2006; Roubie et al., 2024). The major seawater cations - Na, Mg, Ca and K - were found in high concentrations, especially in the liver, consistent with their physiological abundance and uptake in marine organisms (Turoczy et al., 2000). Interestingly, potassium (K) was particularly elevated, though the mechanisms behind this remain unclear. Iron levels were also markedly high in both tissues, consistent with other studies on sharks and rays (Barrera-García et al., 2013; Hornung et al., 1993; Martínez-Ayala et al., 2022; Türkmen et al., 2013), potentially linked to hepatic blood supply and haemoglobin content.

Interspecific differences within the Squatinidae family suggest regulatory and ecological mechanisms influencing metal accumulation (Turoczy et al., 2000). Elevated Cd and Hg levels in some *S. squatina* individuals likely reflect both anthropogenic contaminations from maritime traffic and coastal development (Barton et al., 1998; Lozano-Bilbao et al., 2021a), and natural inputs from volcanic activity (Rodríguez Martín et al., 2013). As observed in the Azores Islands, and possibly throughout the Macaronesian region, the volcanic origin of the archipelago, along with deep-sea and shallow-water hydrothermal activity, contributes to the release of heavy metals such as cadmium (Cd), leading to bioaccumulation of this element in local species (Torres et al., 2023). Dietary composition is a critical factor, with cephalopods known to be rich in Cd (Bustamante et al., 2002, 1998). The primarily ichthyophagous diet of *S. squatina*, with only occasional cephalopod consumption (Narvaez, 2013), likely explains the relatively low hepatic Cd concentrations ($2.09 \pm 1.76$ mg·kg$^{-1}$) when compared to other shark species such as *Mustelus schmitti* ($5.62 \pm 1.65$ mg·kg$^{-1}$), *Halaeulurus bivius* ($7.95 \pm 1.78$ mg·kg$^{-1}$), and *Somniosus pacificus* ($2.64 \pm 0.35$ mg·kg$^{-1}$) (Marcovecchio et al., 1988; McMeans et al., 2007). Regional dietary differences may also contribute to geographic variation in metal profiles; for example, in the Canary Islands, stomach content analyses show a greater reliance on cephalopods, with males consuming approximately 94 % bony fish and 4.8 % cephalopods, and females consuming 84 % bony fish and 14.4 % cephalopods (Narvaez, 2013). Being the *Sepia officinalis* the most common cephalopod prey in Canary Islands angelsharks (Narvaez, 2013). This contrasts with the predominantly fish-based diet of *S. squatina* in Cardigan Bay (Ellis et al., 2021). *S. guggenheim* diet consists primarily of bony fish (89.7 %) and lower proportions of crustaceans (4.8 %) and molluscs (4.4 %) (Vögler et al., 2003), and exhibits lower Cd concentrations. Comparing these results with other commercial shark species such as *Galeus melastomus*, *Mustelus asterias*, *Squalus acanthias*, and *Scyliorhinus canicula*, whose diets are primarily based on invertebrates and rely less than 5 % on cephalopod species, it is evident that cadmium (Cd) concentrations are much lower than those found in *S. squatina* in the Canary Islands (Domi et al., 2005; Ellis et al., 1996) Comparison of the present study with heavy metal studies on deep-water sharks in the Canary Islands indicate that Cd concentrations are higher in *S. squatina.* Again, this could be explained by dietary variation between deep-water and coastal species, with the diet of deep-sea sharks primarily consisting of benthic small fishes rather than cephalopods or crustaceans (Dunn et al., 2010). Examining the only available heavy metal analysis of sharks conducted in the Canary Islands, specifically in deep-sea species, and accounting for ecological differences, the Cd concentrations in *Centrophorus cryptacanthus* ($0.03873 \pm 0.02878$ mg/kg), *Centroscymnus coelolepis* ($0.08044 \pm 0.07070$ mg/kg), *Centrophorus uyato* ($0.01258 \pm 0.00772$ mg/kg), *Centrophorus granulosus* ($0.04380 \pm 0.02729$ mg/kg), *Deania histricosa* ($0.02525 \pm 0.02281$ mg/kg), *Deania profundorum* ($0.01245 \pm 0.03268$ mg/kg), and *Centrophorus squamosus*($0.12036 \pm 0.09280$ mg/kg) are relatively low compared to those observed in the angelshark population (Lozano-Bilbao et al., 2018).

Regarding Ni and Cu, the observed patterns align with ecological traits. Ni tends to be more prevalent in pelagic species, while Cu is typically higher in demersal species like *S. squatina* (Vas, 1991). In this study, hepatic Cu concentrations were nearly 50 times greater than those of Ni. The liver's filtering role may explain the higher bioaccumulation of most metals (Sures and Reimann, 2003). However, Ni and B were more concentrated in muscle than liver tissues, and some metals showed no significant tissue differentiation. Cu is an essential trace metal but can become toxic at high concentrations. It induces metallothionein production, which binds and regulates metals such as Zn and Cu (Adel et al., 2018). Elevated Cu in *S. squatina* may be linked to the consumption of hemocyanin-rich prey such as molluscs and crustaceans (Vas, 1991). This dietary link is further supported by comparisons with *S. guggenheim*, which consumes fewer molluscs and crustaceans and shows correspondingly lower Cu concentrations (Vögler et al., 2003). Excess Cu exposure is known to cause adverse physiological effects, including impaired locomotion and mortality in fish (De Boeck et al., 2010).

Finally, interspecific comparisons of mercury levels reveal that *S. squatina* in the Canary Islands exhibit higher concentrations than *S. guggenheim* (Brazil) and *S. dumeril* (Northeast Atlantic) but comparable levels to those of *S. californica* (Northern California) and *S. aculeata* (Mediterranean). In contrast, *S. argentina* exhibited lower muscle Hg levels than *S. squatina* in the present study. These differences may relate to ecological and individual factors such as trophic level, habitat, body size, and age (Rumbold et al., 2014). Interestingly, the Hg concentrations observed in *S. squatina* are more comparable to those found in batoids, likely a consequence of shared demersal lifestyles and close association with benthic sediments, where Hg tends to accumulate via anthropogenic sources (Gagnon et al., 1997).

Currently, there are no established toxicological thresholds defining safe pollutant concentrations in elasmobranchs (Tiktak et al., 2020). Determining natural background levels in elasmobranch species provides a relative measure to distinguish between naturally occurring concentrations and those influenced by anthropogenic activities and facilitates quantification of key threats such as pollution. Existing research indicates that contaminants such as Hg and Cd can disrupt reproductive physiology, potentially impairing reproductive success (Beaudry et al., 2015; Gilbert et al., 2015; Olin et al., 2014). Elasmobranchs are also known to engage in maternal offloading, a process through which pregnant females transfer accumulated pollutants to their offspring. This transfer may negatively impact embryonic development and postnatal health (Beaudry et al., 2015; Bezerra et al., 2019; Gilbert et al., 2015; Olin et al., 2014; van Hees and Ebert, 2017; Weijs et al., 2015). The elevated levels of Hg and Cd detected in *S. squatina* individuals in this study raise significant concerns about potential sublethal or even population-level impacts on this Critically Endangered species. Given the vulnerability of *Squatina* spp. (Dulvy et al., 2014) establishing species- or family-specific baseline contaminant thresholds, irrespective of geographic variation, is essential. It is highly recommended that further studies investigate the physiological effects of environmental contaminants on elasmobranchs to better predict the impact of these threats, particularly as these species are already facing additional stressors such as overfishing, habitat loss, and climate change (Dulvy et al., 2021; Tiktak et al., 2020; Mead et al., 2023). Such benchmarks would facilitate improved ecological risk assessment and environmental impact evaluation, thereby informing more targeted and effective conservation strategies.

## 5. Conclusions

This study is the first to analyse trace elements and heavy metals in the muscle and liver of *S. squatina* in the Canary Islands. The findings indicate that sex does not influence metal concentrations in either tissue. However, due to the limited size range of sampled individuals, no significant correlations were observed between metal concentrations and biometric measurements. Expanding the sample size to include a broader range of size and age classes would allow to generate a more accurate understanding of bioaccumulation dynamics. Tissue-specific differences were consistent with physiological function, with the liver containing significantly higher concentrations of Mo, Zn, Cd, Pb, Fe, Co, V, Cu, Sr and Na when compared to muscle tissue. Additionally, *S. squatina* individuals from the Canary Islands showed higher concentrations of Cd, Cu, Fe, Zn, and Mn compared to other Squatinidae species. These differences are likely influenced by a combination of dietary preferences, habitat characteristics, volcanic activity, and anthropogenic inputs, suggesting that metal accumulation is driven by region-specific variables. Sample collection remains limited due to strict legal protections of this Critically Endangered species, restricting sampling to stranded individuals and thereby limiting sample size and condition. Given these constraints, further research is essential to evaluate the ecological and physiological impacts of pollutant exposure on *S. squatina*. The elevated levels of Hg and Cd observed in Canary Islands specimens, exceeding those reported in other Squatinidae species, underscore the urgency of establishing species- or family-specific contaminant thresholds. This baseline is an important factor for assessing environmental risk, informing conservation priorities, and developing effective mitigation strategies to protect the health and long-term viability of this imperilled elasmobranch.

## CRediT authorship contribution statement

**Tomas Bañeras:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **David Jiménez-Alvarado:** Writing – review & editing, Supervision, Resources, Conceptualization. **Eva K.M. Meyers:** Writing – original draft, Supervision, Resources. **Hector Toledo-Padilla:** Writing – review & editing. **Arturo Hardisson:** Writing – review & editing, Resources. **Dailos Gonzalez-Weller:** Methodology, Investigation. **Joanna Barker:** Writing – review & editing. **Lucy R. Mead:** Writing – review & editing, Investigation, Conceptualization. **Ana Espino-Ruano:** Writing – review & editing, Investigation. **Ayoze Castro-Alonso:** Writing – review & editing, Resources, Investigation, Funding acquisition. **María José-Caballero:** Writing – review & editing, Resources, Methodology, Investigation. **Ángel Gutiérrez:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation.

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## Declaration of competing interest

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## Data availability

Data will be made available on request.

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