Heavy metal and trace element concentrations in the blood of scalloped hammerhead sharks (*Sphyrna lewini*) from La Paz Bay, México

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Abstract:

Sharks are particularly susceptible to bioaccumulation due to their life history characteristics and trophic position within marine ecosystems. Despite this, studies of bioaccumulation cover only a small proportion of extant species. In this study we report concentrations of trace elements and heavy metals in blood samples of *S. lewini* for the first time. We report high concentrations of several trace elements and heavy metals, with concentrations of some elements exceeding the limit determined safe for human consumption. High elemental concentrations may reflect biochemical differences between blood plasma and other tissues; however, they may also be symptomatic of high levels of exposure triggered by anthropogenic activities. We also provide evidence of elemental accumulation through ontogeny, the nature of which differs from that previously reported. Ultimately, this baseline study increases our understanding of interspecific and intraspecific variation in bioaccumulation and ecotoxicology in elasmobranchs which may prove important in ensuring adequate management.

Key words: Elasmobranchii, bioaccumulation, turnover rate, blood plasma, conservation

Introduction:

Pollution and bioaccumulation are two of the most significant anthropogenic factors affecting marine communities (Todd et al., 2010; Ley-Quiñónez et al., 2013; Boldrocchi et al., 2020). Whilst lethal affects have been reported in a number of taxa (Martin and Holdich, 1986) sub-lethal bioaccumulation of both elemental and organic contaminants is arguably an issue of even greater importance, given both the potential for trophic magnification (Gelsleichter et al., 2006; Gelsleichter & Walker, 2010; Storelli & Marcotrigiano, 2001) and difficulties in determining the true extent of bioaccumulation within any given species (Burkhard et al., 2012; Caurant et al., 1999; Rodríguez-Romero et al., 2021). Chronic, or intermittent exposure to these contaminants can significantly affect physiology, cellular biology, and behavior of living organisms (Gelsleichter et al., 2006; Jepson et al., 2005; Thophon et al., 2004; Ylitalo et al., 2005;), with these effects varying between and within species (Boldrocchi et al., 2019). Crucially, these effects can have pervasive and unpredictable long-term consequences for ecological communities as a result of interactions between taxa and the biogeochemical components of their environment (Moiseenko, 2017). Moreover, the chemical behavior of trace elements is likely to change in the face of anthropogenic climate change (Alves et al., 2023; Cabral et al., 2019; Cao et al., 2015; Eagles-Smith et al., 2014; Rodríguez-Romero et al., 2021;) and thus there is no guarantee that previous investigations of trace element bioaccumulation are representative of contemporaneous trends, or those that may be observed in the future. It should also be noted that marine bioaccumulation represents a potential health hazard for human populations (Akhbarizadeh et al., 2018; Cara et al., 2022), particularly in coastal communities relying on artisanal fisheries for a substantial proportion of their diet (Cartamil et al., 2011; Galvan-Magaña et al., 2013; Storelli et al., 2003; Vàzquez-Hurtado et al., 2010;). For these reasons, establishing the extent to which bioaccumulation is affecting marine organisms (at all trophic positions and in all ecosystems) is of critical importance.

Elasmobranchs (sharks, rays, and skates) are particularly susceptible to bioaccumulation as a result of their *k-selected* life-history parameters (Castro, 1993; Mull et al., 2012). Many elasmobranch taxa also occupy higher trophic levels (Maz-Courrau et al., 2011; Wosnick et al., 2021), a factor which has been shown to influence the bioaccumulation of many marine pollutants (Gelsleichter and Walker, 2010; Maz-Courrau et al., 2011; Pancaldi et al., 2019; Storelli et al., 1998, 2003). Bioaccumulation, its potential biological consequences, and putative adaptations to counteract them have been studied in several elasmobranch taxa (Escobar-Sánchez et al., 2010; Pancaldi et al., 2021; Wosnick et al., 2021), however such studies only account for a relatively small proportion of total elasmobranch diversity (Lee et al., 2015; Mull et al., 2012). Assessing the nature and potential consequences of trace element bioaccumulation in elasmobranch taxa at the population level is important given their intrinsic vulnerability to anthropogenic stressors such as overfishing (Dulvy et al., 2017; Walker, 1998), and the potential ecosystem-wide consequences of predator declines (Palkovacs et al., 2011; Parsons, 1992; Polis et al., 2000).

The Scalloped Hammerhead shark (*Sphyrna lewini*) is a large-bodied, migratory carcharhiniform shark, distributed worldwide in tropical and temperate oceans, where they inhabit both coastal and pelagic zones at depths of up to 275 meters (Compagno, 2011; Gulak et al., 2015; Moore & Gates, 2015). *S. lewini* is known to be in decline globally (Hayes et al., 2009), and has been listed as critically endangered by the International Union for the Conservation of Nature (IUCN) since 2018, predominantly due to overexploitation in longline fisheries (Aldana-Moreno et al., 2020) and low rebound potential (Pancoureau et al., 2018, 2021). Heavy metal and trace element concentrations have been studied previously in the liver and muscle tissues of *S. lewini*, however these tissues are thought to have a slow turnover rate relative to blood plasma, such that heavy metal and trace element concentrations do not necessarily reflect the current biogeochemical environment at the location in question (Kim et al., 2012; Whitehead et al., 2020). Such studies generally report heavy metal and trace element concentrations lower than those which would present risk to human consumers (Bergés-Tiznado et al., 2015, 2021; Ruelas-Inzunza et al., 2020). Some biological consequences marine pollution for *S. lewini* have also been considered (Boswell, 2015), although the extent to which these results are indicative of *S. lewini* populations globally remains poorly constrained. In this study we report concentrations of trace elements and heavy metals in blood samples of *S. lewini* for the first time. We consider the concentration of various trace elements and heavy metals in isolation (including implications for human health and ecology), as well as potential relationships between elemental concentrations and key life history parameters (size and sex). Finally, we consider the extent to which *S. lewini* may have evolved detoxification mechanisms to avoid deleterious consequences of bioaccumulation. This study will increase our understanding of interspecific and intraspecific variation in bioaccumulation and ecotoxicology in elasmobranchs. We consider potential ecological and biological consequences of marine pollution in this system and suggest reasonable directions for future work and conservation action.

MATERIALS AND METHODS

Ethics statement:

Data collection and analysis procedures in this study complied with national animal welfare laws; guidelines and policies and was authorised by Mexican wildlife authorities under the permit PPF/DGOPA-024/20 provided by the Comisión Nacional de Acuacultura y Pesca (CONAPESCA). Participants of this study neither promoted nor encouraged the harvesting of sharks and all samples were collected with the consent of the artisanal fishing communities.

Sample collection:

Blood samples were obtained from a total of 126 *S. lewini* individuals between December 1st 2020 and February 8th 2021 at the El Saladito fish camp on the west coast of La Paz Bay, Baja California Sur, Mexico (Figure 1). The highest number of individuals were registered in the month of December (n=103) accounting for a total of 81.7% of the sampled individuals, followed by February (n=13) with 10.3% and January (n=10) with 7.9%. Approximately 5 ml of whole blood was collected directly from the heart of each shark immediately after landing and stored in sterilized tubes at –40°C for further analyses. In addition, the following biological information was collected from each individual: total body length, measured from the tip of the snout to the apex of the upper lobe of the caudal fin (TL), and sex, determined by the presence or absence of the male intromittent organs (claspers). All sharks examined within this study were juvenile, as they were smaller than the estimated size at maturity for the species (Compagno, 2005). Size distribution ranged from 81 cm to a maximum size of 166 cm in TL, with a mean size of 108.9 cm ± 17.3 cm. Two size classes were defined based on TL with sharks < 100 cm considered ‘immature’, whereas individuals with TL ranging between 100 and 180 cm were considered ‘subadult’.

Sample analysis:

To remove excess moisture, blood samples were lyophilized using a LABCONCO freeze dry system at a constant temperature of -50°C for a total of 48 hrs at the Centro Interdisciplinario de Ciencias Marinas (CICIMAR) in La Paz, Mexico. Upon completion of the drying process, all blood samples were ground into a homogeneous powder using agate mortar and pestle, following the approach of Escobar-Sanchez et al. (2010). Subsequent analyses were performed in the university of Milano-Bicocca, Milan, Italy. Samples were hot concentrated by acid digestion in closed containers in specifically designed mineralizer Anton Paar Multiwave 5000 microwave ovens. Approximately 0.3 g of each sample was then digested in Teflon vials with 5 mL of Nitric Acid - HNO_3 (65%) and 1 mL of Hydrogen Peroxide - H_2O_2 (40%). Following acid digestion, each sample was transferred to a 50 mL plastic tube and brought to 20 mL by adding 14 mL of Milli Q water, in order to lower the acid concentration and avoid compromise of the instrumental reading. Trace elements analyses of Chromium (Cr), Cadmium (Cd), Copper (Cu), Iron (Fe), Nickel (Ni), Selenium (Se), and Zinc (Zn) were performed using an Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Optima 7000 DV PerkinElmer) with the Software control WinLab32, following the protocol of Pancaldi et al. (2019). Lead (Pb) and Arsenic (As) were analyzed with the Atomic Absorption Spectrophotometry with Graphite Furnace (GFAAS) PerkinElmer Analyst 600, while Mercury (Hg) was analyzed using AMA254 (Advanced Mercury Analyzer), using approximately 50 mg of each sample inside the furnace. The concentration of the analyte in the sample solution is expressed in ppb (μg/L) using the formula

$$C = (A - b) * V * (d/m)$$

where:

C is the concentration of the analyte in the sample, expressed in μg/L (ppb); A is the analyte concentration in the sample solution expressed in μg/L (ppb) obtained from the calibration curve by instrumental reading; b is the reference blank samples for control; V is the total extracting volume (20 mL); d is the dilution factor; and m the mass of the sample. All concentrations obtained from the analyses were expressed as μg/g (= ppm; = mg/kg) unless stated otherwise.

Statistical analyses:

Raw data obtained from the analyses were visualized cleaned before all analyses. Results have been reported as mean ± standard deviation, unless stated otherwise. Elemental concentrations were compared to those permitted for human consumption by the Food and Drug Administration and the World Health Organization (FAO Legal Notice No 66/2003). The assumption of normality was violated when performing Kolmogorov–Smirnov tests upon the resulting dataset; therefore, nonparametric statistical tests (Mann Whitney and Spearman's rho) were used to compare the heavy metal and trace elements concentrations between size classes and sexes. The ratio of Selenium (Se) to Mercury (Hg) was evaluated to investigate potential detoxification mechanisms operating in *S. lewini* (Ralston *et al.*, 2007; Ralston & Raymond, 2010). All statistical analyses were performed using IBM SPSS 28 Software (IBM SPSS 28, New York, US). Whilst trace element concentrations were established for all 126 individuals, heavy metal concentrations were only investigated in 55 individuals due to an insufficient volume of blood being collected from sampled sharks.

RESULTS

Elemental concentrations:

Besides Iron (Fe), Arsenic (As) was clearly the element with the highest concentration (16.82 ug/g), followed by Nickel (Ni) and Zinc (Zn) with concentration of 5.7 and 4.63 ug/g respectively (Figure 2). Iron (Fe) was expected to be the element present in greatest abundance due to its fundamental biological role in blood. Lead (Pb) was excluded from statistical analyses as it was present in concentrations below the detection limit of the Atomic Absorption Spectrophotometer and Graphite Furnace (GFAAS; dl = 0.05 μg/L).

Cadmium (Cd) concentration was higher than the edible limit set by the FAO in 40 of the 55 individuals tested, while Selenium (Se) in 18 of the 55 individuals tested (Table 1). Moreover, a single individual was found with Mercury (Hg) concentration over the edible limit set by the FAO (Table 1). No other elements were found to occur with concentrations above those considered to be edible by the FAO.

Elemental concentrations and size/sex:

Analysis of mean elemental concentrations utilizing both Mann-Whitney and Spearman's Rho tests revealed a statistically significant difference between immature and subadult individuals for the elements Iron (Fe; Mann-Whitney test $p = 0.017$; Spearman's rho Test, Fe: $\rho = 0.330$, $p = 0.016$; Figure 3a) and Chromium (Cr; Mann-Whitney test, $p = 0.016$; Spearman's rho Test, Cr: $\rho = 0.327$, $p = 0.015$; Figure 3a, Table 2). Whilst the concentration of all elements other than Nickel (Ni) appeared to be higher in premature than immature individuals, no additional significant differences between life stages were found ($p > 0.05$). The Spearman's Rho test was also used to test for a relationship between elemental concentration and TL. Significant positive correlations with TL were found for Zinc (Zn) ($p=0.012$), Iron (Fe) ($p=0.013$), Chromium (Cr) ($p<0.001$) and Copper (Cu) ($p=0.02$), whereas no significant relationship was recovered between TL and the concentration of any other element (Table 3). Whilst the concentrations of all elements appeared to be higher in males than females, neither Mann-Whitney nor Spearman's rho tests recovered evidence of significant relationships between sex and elemental concentration ($p > 0.05$, Figure.3b).

Se: Hg ratio:

The molar ratio between Se and Hg could be calculated for only 34 individuals as in the remaining individuals selenium (Se) concentration was below the detection limit of the apparatus used. Se:Hg ratio was found to be greater than 1 in all but one individual, as the concentration of selenium (Se) exceeded that of mercury (Hg). The lowest ratio recorded was 0.55:1, whereas the greatest was 91.98:1 (Figure 4). No relationship between Se:Hg ratio and TL was found (Mann-Whitney test, $p > 0.05$).

DISCUSSION

Elemental concentrations in *S. lewini*: implications for ecology and human health

This study is the first assessment of heavy metals and trace elements concentrations from *S. lewini* blood plasma. Iron (Fe) was found to be the trace element in greatest abundance (Table 1), with a mean concentration comparable to that found in other elasmobranch taxa (Boldrocchi et al., 2019; Merly et al., 2019). This result was entirely expected given the vital role of Fe in blood plasma as a component of hemoglobin (Wells, 1999).

Besides iron, arsenic (As) was the element found in greatest abundance (Table 1), mirroring studies in other elasmobranchs (Merly et al., 2019). Intriguingly, arsenic levels were substantially higher in the blood plasma than those found in muscle tissues of *S. lewini* (Bergés-Tiznado et al., 2021; Boldrocchi et al., 2019), where arsenic would be expected to concentrate. Hypothetically such a difference could result purely from differential local arsenic abundance and consequently differential exposure, however similar relationships between blood and muscle tissue concentrations of arsenic have also been reported in *Carcharodon carcharias* (Merly et al., 2019). Exposure to arsenic in aquatic environments can have a range of damaging physiological consequences even if only a relatively small proportion of arsenic is found in a toxic form (Dringen et al., 2016; Greani et al., 2017), and for this reason we suggest that both

S. lewini and *C. carcharias* may possess arsenic detoxification mechanisms In the event that no such detoxification mechanism exists, the reported blood plasma arsenic (As) concentrations in both taxa would be extremely concerning and suggest that urgent modifications to local management plans are required. Selenium and zinc have both been shown to provide protection against arsenic toxicity in fishes (Roy and Battacharya, 2006; Zeng et al., 2005), however neither of these elements were present in particularly high concentrations (Table 1). Nickel (Ni) exhibited a similar trend, present at a concentration similar to that found in *C. carcharias* (Table 1; Merly et al., 2019) and significantly higher than that found in *S. lewini* muscle tissue (Boldrocchi et al., 2019). Whilst considered a potentially toxic element in many taxa (Blewett and Leonard, 2017), there is mounting evidence that Ni may be essential for fishes (Chowdhury et al., 2008; Pyle and Coutoure, 2011), and thus it remains to be seen whether arsenic and nickel blood plasma concentrations are driven by common underlying processes or not.

Whilst some elements were found in far higher concentrations than expected based on studies utilizing muscle and liver tissues, others did not differ noticeably: copper and cadmium concentrations (Table 1) were found to be comparable to those reported by in *S. lewini* muscle tissue (Powell and Powell, 2001; Ruelas-Inzunza et al., 2020). Selenium (Se) is an intrinsic component of the central nervous system (Steinbrenner and Sies, 2013); however it can be toxic when present at high levels (Berges-Tiznado et al., 2015; Peterson et al., 2009; Ruelas-Inzunza et al., 2020). The Se levels in *S. lewini* blood plasma were found to be low (Table 1) in comparison with both other *S. lewini* tissues (Boldrocchi et al., 2019) and *C. carcharius* blood plasma (Merly et al., 2019). Mercury (Hg) concentration was also far lower than expected on the basis of existing studies (Berges-Tiznado et al., 2015; Merly et al., 2019). Given the toxic effects of both mercury and selenium at high concentrations (Escobar-Sánchez et al., 2010, 2011; Lemly, 2002) one could suggest that these results have positive implications for *S. lewini* population-level health. However, this study utilized exclusively juvenile individuals, and thus the trophic position of said individuals (and consequently the extent to which biomagnification has occurred) is likely to be lower than in adult individuals utilized in other studies (Cerutti-Pereyra et al., 2022; Estupiñán-Montaño et al., 2021).

Whilst the absolute concentration of selenium was lower than expected in *S. lewini* blood plasma, the Se:Hg ratio was found to be relatively high in most sampled individuals (Figure 4). This result is consistent with previous studies addressing mercury accumulation in *S. lewini* (Berges-Tiznado et al., 2015; Pancaldi et al., 2019; Ruelas-Inzunza et al., 2020), and suggests that Se is indeed performing a role in detoxification of Hg. This protective effect is thought to be multifaceted, with competitive inhibition, formation of Hg-Se complexes and increase in glutathione peroxidase activity (which inhibits oxidative damage by Hg) all thought to contribute to the redistribution, detoxification, and excretion of mercury (Ralston et al., 2007; Pancaldi et al., 2019; Raymond and Ralston, 2020).

Several correlations between the concentrations of different elements were detected: namely, iron concentration was positively correlated to zinc and selenium concentrations, a trend which has also been reported in *C. carcharias* (Merly et al., 2019) and other taxa (Schmitt et al., 2007). Such correlations are thought to occur due to significant interplay between the regulatory mechanisms governing the usage of these metals (Ehrensburger and Bird, 2011). It has been reported that high levels of iron and zinc could potentially offset the toxic effects of arsenic by replacement (Merly et al., 2019; Wang et al., 2020), however we are unable to verify or refute such claims based on our results.

Generally, our results demonstrate that trace element and heavy metal concentrations are higher in the blood plasma of *S. lewini* individuals than from muscle and liver tissues of *S. lewini* individuals reported in other studies (Table 1; Berges-Tiznado et al., 2015; Pancaldi et al., 2019; Ruelas-Inzunza et al., 2020). We suggest two potential explanations for this trend. Firstly, apparent high concentrations may result from the differences in biochemistry and turnover rate between blood plasma and muscle/liver tissues in this species, as observed in other taxa (Kim et al., 2012; Whitehead et al., 2020). Alternatively, the population sampled in this study may be exposed to higher levels of trace elements and heavy metals through their local environment. There is known to be significant variation in the chemical composition of sediments and water column subdivisions in this region, due to wastewater discharge from the city of La Paz, and nearby phosphate mining activity (González-Yajimovich et al., 2010; Páez-Osuna et al., 2017). If the latter is responsible, this raises urgent questions regarding the health and stability of *S. lewini* populations in this area. Whilst relatively high levels of heavy metals have been found to be of minimal physiological influence in other taxa (Merly et al., 2019) and we found evidence for detoxification mechanisms operating in *S. lewini*, no haematic information regarding condition of health of sampled individuals was available. Thus, high heavy metal concentrations could instead be symptomatic of population declines which have been occurring in the region for years (Gallagher and Klimley, 2018; Pérez-Jiménez, 2014). Further studies are urgently required to determine the extent to which *S. lewini* populations are affected by the elemental concentrations reported in this study, and any indirect consequences this might have for the wider ecosystem. Importantly, these hypotheses are not mutually exclusive and both anatomical and local biogeochemical differences may contribute to observed elemental concentrations.

A comparison of the concentration levels of element with maximum limits permitted for the human consumption of a food products provided by the Food and Drug Administration and the World Health Organization (FAO Legal Notice No 66/2003) produced some concerning results. A total of 18 sampled individuals recorded Se concentration, higher than the limit established by the FAO at 1.0 µg/g, as well as one individual with mercury concentration (0.52 µg/g) in excess of the limit established by the FAO for human consumption (0.50 µg/g). Additionally, Cd concentrations were also found to exceed the limit established in 2003 by the FAO and by the official Mexican regulation (NOM 242-SSA1, 2009) (0.05 ppm) in 40 out of 55 sampled individuals. Whilst this would be concerning regardless of the population from which individuals were sampled, shark consumption in this region is high at 17.34 g per capita per day, which represents over 20 times the national average (Ruelas-Inzuna et al., 2020). Whilst many species of sharks are consumed in the region, *S. lewini* represents one of those most frequently available. In the absence of proportionate and effective conservation measures to protect *S. lewini* from overfishing in Mexican waters, measures limiting shark consumption or enforcing existing limits for heavy metal and trace element consumption are urgently required in the interests of public health.

Differences between sex and size:

We did not recover evidence of any statistically significant relationship between elemental concentrations and sex (Figure 3). This result is not unexpected and matches findings in other taxa of similar trophic position (Adel et al., 2017; Escobar-Sánchez et al., 2011; Merly et al., 2019). This suggests that elemental uptake and processing in *S. lewini* does not differ significantly between the sexes. Whilst sex-based and size-based differences in habitat usage and trophic ecology are known in this species (Estupiñán-Montaño et al., 2021; Hoyos-Padilla et al. 2014; Klimley, 1987;), the inclusion of only of juveniles in this study likely nullified these potential confounding factors. On the basis of these results, we suggest that sex-based differences in elemental concentrations reported in other studies of *S. lewini* that include mature individuals result from differential exposure, rather than differential uptake and/or processing of said elements.

Whilst no relationship between sex and elemental concentrations was recovered, we did find evidence of significant relationships between size and the concentration of several elements. Iron and chromium concentrations were both higher in subadult individuals than immature individuals (Table 2), and iron, chromium, copper, and zinc concentrations all showed significant positive relationships with total length (Table 3). These results differ somewhat from existing literature concerning ontogenetic trends in elemental accumulation in elasmobranchs, where Zn and Cr typically demonstrate negative relationships with body length (Boldrocchi et al., 2019; Endo et al., 2008). Comparatively low concentrations of zinc in smaller individuals are concerning given that physiological demand for zinc is thought to be greatest during early ontogeny (Endo et al., 2008), however it is important to recognize that the relatively high turnover rate of blood plasma compared to muscle and hepatic tissues (Kim et al., 2012; Whitehead et al., 2020) means that observed elemental concentrations are likely less related to growth and more transient in nature (Merly et al., 2019). Neither sex nor total length was found to correlate significantly with Se:Hg ratio, in agreement with studies concerning other tissues and organs of *S. lewini* (Berges-Tiznado et al., 2015). This suggests that the protective effects of selenium against mercury toxicity remain consistent between the sexes and throughout ontogeny.

Conclusion:

This study is the first to report the concentration of heavy metals and trace elements in the blood plasma of the scalloped hammerhead shark (*S. lewini*), and amongst the first to analyze elemental concentrations in the blood plasma of any elasmobranch. Individuals of *S. lewini* appear to possess higher concentrations of several trace elements and heavy metals than populations from other regions, with concentrations of some elements such as Cadmium exceeding the limit determined safe for human consumption by national and international food standards agencies. High elemental concentrations may reflect biochemical differences between blood plasma and other tissues; however, they may also be symptomatic of high levels of exposure triggered by anthropogenic activities such as mining. Whilst *S. lewini* appears to possess detoxification mechanisms against some pollutants such as mercury, it remains to be seen whether marine pollution has significant physiological implications in this population. We also provide evidence of elemental accumulation through ontogeny, the nature of which differs from that reported in previous studies. There is much uncertainty underlying the relationship between different trace elements and heavy metals, and future studies are urgently required to assess the implications of these results not only for human health, but for the conservation prospective of *S. lewini* populations and the wider ecological community.

Acknowledgements:

Authors would like to thank volunteers and interns of Pelagios Kakunja A.C for assisting in fieldwork and data collection procedures as well as the local artisanal fishing communities for allowing the sampling protocols of their catch to support the research and conservation of sharks in this region. Also, to the laboratory technicians from the University of Milano-Bicocca and the Centro Interdisciplinario de Ciencias Marinas for support during the analysis process. FGM thanks to Instituto Politecncio Nacional for the fellowships granted (COFAA and EDI).

CRediT authorship contribution statement

Darren A. Whitehead: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – Original draft and editing, Visualization, **Joel H. Gayford:** Formal analysis, Investigation, Resources, Writing – Original draft and editing, **Jacopo Gobbato**: Formal analysis, Writing – editing, **Giulia Boldrin:** Methodology, Formal analysis, Investigation, Writing – original draft, **Maria Tringali:** Formal analysis, Methadology, Visualization, **James T. Ketchum:** Supervision, Project administration, Supervision, Funding acquisition, **Felipe Galvan Magaña:** Writing – editing, supervision, **Davide Seveso:** Writing – editing, supervision and **Simone Montano:** Conceptualization, Writing – editing, supervision.

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Figures & Tables

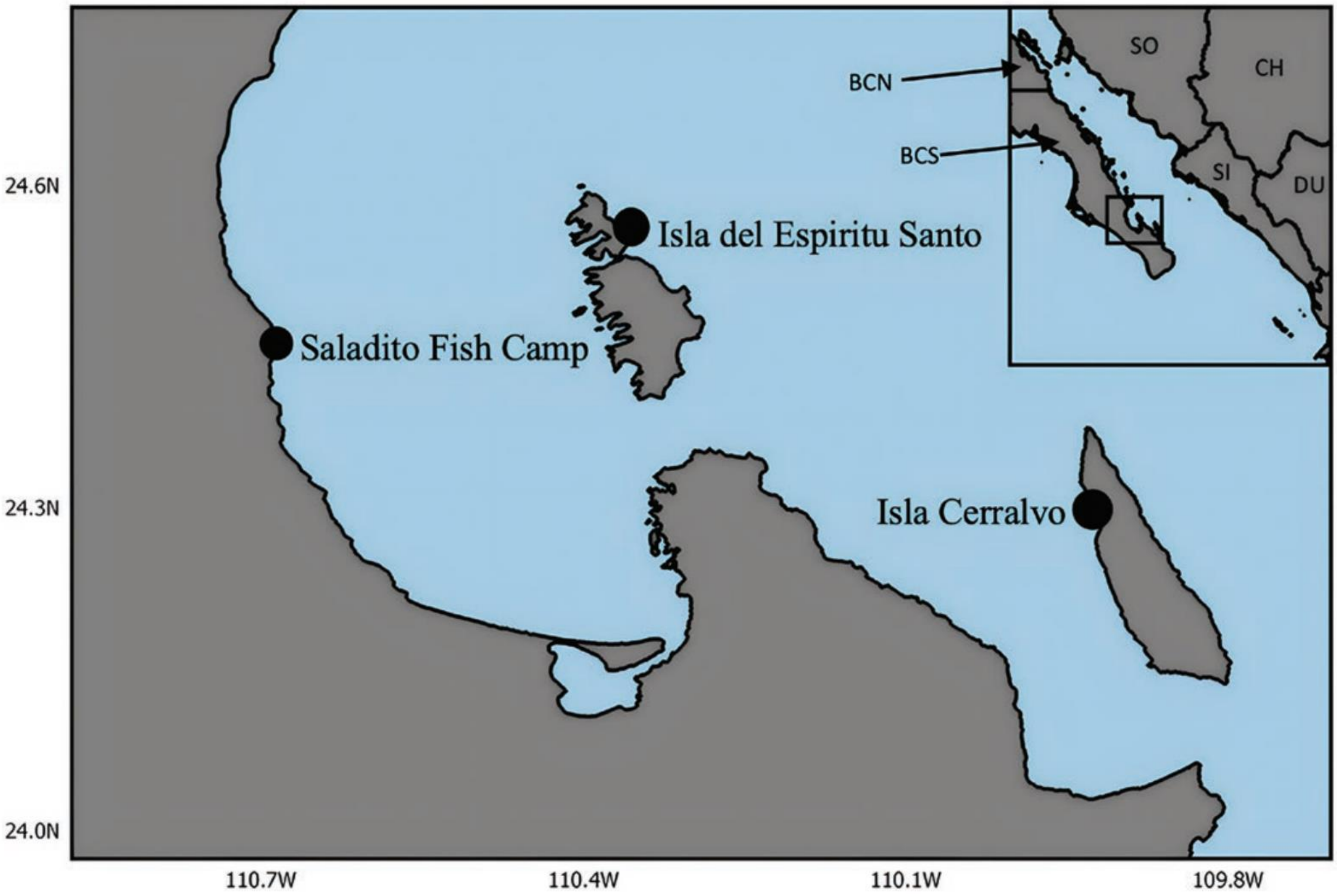

Figure 1. Map of the study site. Geographical labels on the inset refer to states of Mexico: Baja California Norte (BCN), Baja California Sur (BCS), Sonora (SO), Sinaloa (SI), Chihuahua (CH), Durango (D).

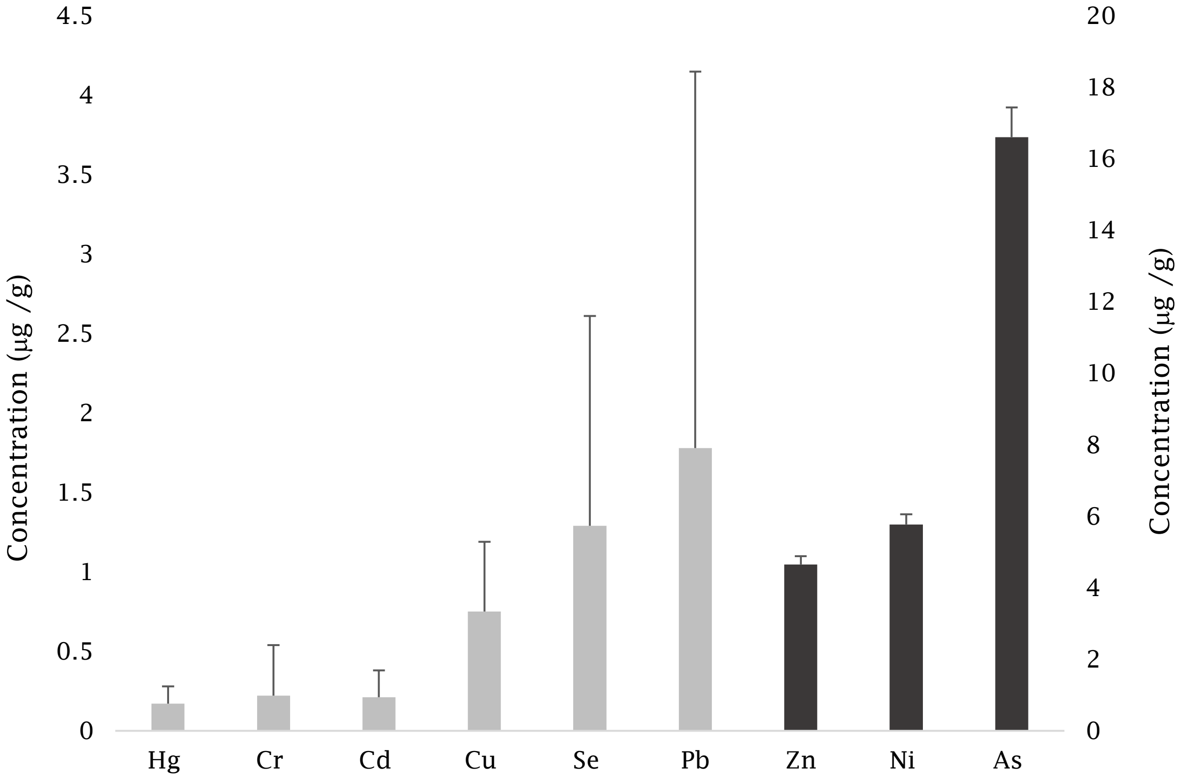

Figure 2. Concentration of all heavy metals and trace elements analyzed: mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu), selenium (Se) zinc (Zn), nickel (Ni) and arsenic (As). Iron (Fe) is excluded from the figure as it has significantly higher concentration as key component of hemoglobin (Wells, 1999). Elements in grey corresponds to the left concentration scale, whereas the elements in black to the right concentration scale.

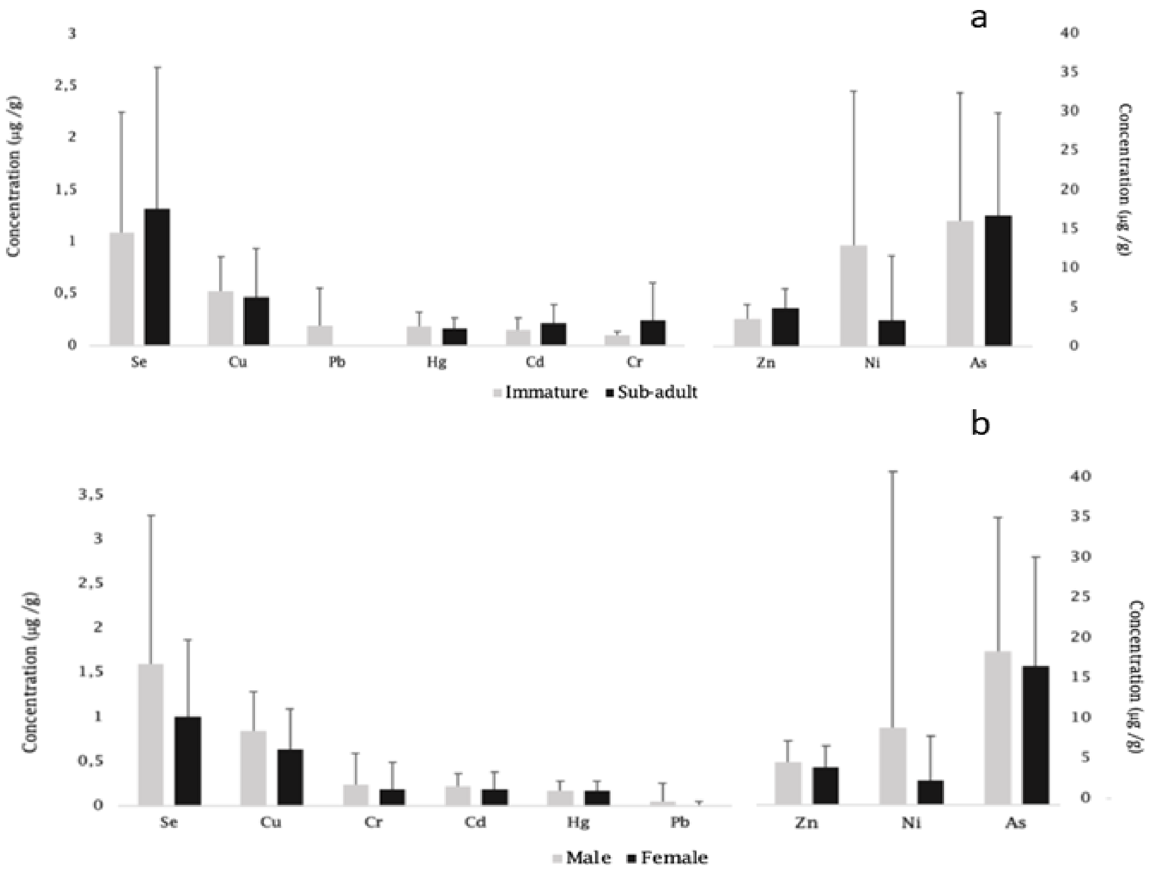

Figure 3. Mean elemental concentration: mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu), selenium (Se) zinc (Zn), nickel (Ni) and arsenic (As) for (a) immature and subadult and (b) male and female.

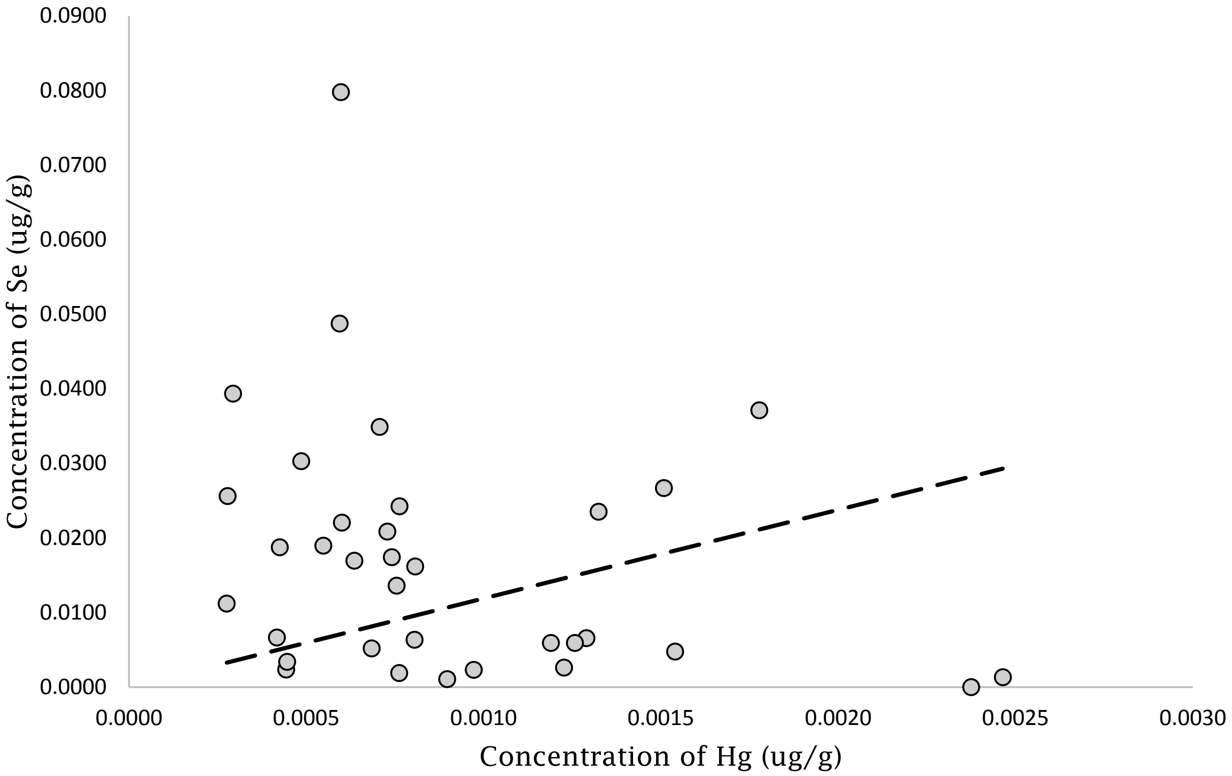

Figure 4. molar ratio between Se and Hg concentrations for sampled individuals.

Table 1. Overview of the concentration values of trace elements and heavy metals in the samples analysed. The limits for human consumption are displayed when assessed by FAO (Legal Notice No 66/2003).

Elements	**Minimum (µg/g)**	**Maximum (µg/g)**	**Mean (µg/g ± SD)**	**Limit for human consumption (FAO) (µg/g)**
Mercury (Hg)	0.06	0.52	0.17 ± 0.11	0.50
Selenium (Se)	0	6.30	1.29 ± 1.32	1.00
Zinc (Zn)	0	9.98	4.65 ± 2.38	30.00
Lead (Pb)	nd	5.26	1.78 ± 2.37	0.2
Arsenic (As)	0	16.61	16.61 ± 13.87	*
Iron (Fe)	0.81	918.60	459.56 ± 232.54	*
Chromium (Cr)	0	1.80	0.22 ± 0.32	*
Cadium (Cd)	0	0.85	0.21 ± 0.17	0.05
Nickel (Ni)	0	144.13	5.77 ± 20.60	*
Copper (Cu)	0	1.61	0.75 ± 0.44	*

* no limit established yet by FAO

Table 2. Statistically significance difference and correlation between concentration of Fe and Cr in Immature and Sub-adult individuals.

Elements	Spearman's Rho Test	*P* value	Mann-Whitney Test	*P* value
Iron (Fe)	0.330	0.016	2.377	0.017
Chromium (Cr)	0.327	0.015	2.400	0.016

Table 3. Statistically significance correlation between elements concentrations and TL of the analyzed individuals.

Elements	Spearman's Rho Test	*P* value
Iron (Fe)	0,339	0.013
Chromium (Cr)	0,564	<0.001
Zinc (Zn)	0,335	0.012
Copper (Cu)	0,322	0.020

Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: