



Essential (Cu, Zn) and nonessential (Pb, Cd) metals in the muscle of leopard groupers (*Mycteroperca rosacea*) from a mining port in the Gulf of California, Mexico: human health risk assessment

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Abstract

Mining activities are a current environmental issue due to heavy metal release and subsequent metal uptake by organisms. In this study, we quantified the concentrations of essential (Cu, Zn) and toxic (Cd, Pb) elements in the muscle of 248 leopard groupers, *Mycteroperca rosacea*, captured by spearfishing and free diving close to a mining district in the Gulf of California during 2014–2015. We analysed metals using high-resolution inductively coupled plasma source mass spectrometry (HR-ICP–MS). We analysed metal concentrations by fish size, sex, maturity, season, year and risk factor for human consumption. The results indicated common levels of essential elements (Cu: 11 ± 34.3 $\mu\text{g/g}$, Zn: 377 ± 1390 $\mu\text{g/g}$) in comparison with toxic elements (Cd: 0.06 ± 0.1 $\mu\text{g/g}$, Pb: 0.98 ± 1.5 $\mu\text{g/g}$). Cadmium was within the permissible limit of Mexican standards (0.5 $\mu\text{g/g}$), but lead content bordered its limit (1.0 $\mu\text{g/g}$). Heavy metal concentrations were comparable between males and females. Metal variations were not significantly correlated with sex, maturity, season or year ($p > 0.05$). The evaluation of benefits (daily mineral intake) and risks (target hazard quotients) to health indicated that these fish did not represent a risk of adverse effects to consumers within worldwide limits, while the nutritional benefits were high.

Keywords Heavy metals · Bioaccumulation · Epinephelidae · Groupers · Mining hotspot

Introduction

The presence of heavy metals in marine ecosystems is one of the main contamination issues that can lead to serious ecological, health and economic consequences (Ali and Khan 2019). Heavy metals are derived mainly from anthropogenic activities, such as the dumping of residual waters and mining wastes (Huerta-Díaz et al. 2014), which can contain persistent elements such as mercury (Hg), lead (Pb) and cadmium (Cd), which have unknown biological functions but are considered to be potentially toxic elements at even low concentrations (Ali and Khan 2019). Anthropogenic inputs related to mining waste can also contain copper (Cu) and zinc (Zn), which are essential micronutrients for physiological functions (Xu et al. 2017; Rehman et al. 2019). However, if metal content exceeds permissible limits (Zn 50 and Cu 5 $\mu\text{g/g}$ w.w., UK-EEA food standards), then even essential elements could also be harmful to human health, although occurrences of acute Zn and Cu poisoning have been reported (FAO, WHO, 2001). Thus, performing

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nutritional and toxicological analyses of organisms in human diet is beneficial in assessing the potential health risks that they may represent to the population, especially to children, pregnant women and lactating mothers.

Marine fish species are a key component in human diets worldwide because they contain essential amino acids, fatty acids (especially omega-3 and omega-6), protein, vitamins and minerals (Pal et al. 2018). For humans, the FAO (2017) recommends consuming fish at least 2 to 4 times per week to reduce the risk of cardiovascular diseases, diabetes and obesity. Global consumption for fish rose to above 20.5 kg *per capita* in 2016. In this sense, tunas, snappers and groupers represent 90% of the global catch (FAO 2017) and therefore, these fishes are the most widely eaten in the world.

Multiple grouper species are important in commercial fishery operations as well as in aquaculture and sport fishing activities. In Mexico, the leopard grouper, *Mycteroperca rosacea* (Streets 1877), is one of the most important species caught in the Gulf of California (Thomson et al. 2000). The annual production averaged 6360 tons/year in 2014 and 2015, with sardines, shrimp and sharks comprising the fourth component in terms of catch volume (CONAPESCA 2014). Leopard grouper meat is high-quality and carries a high price in local (\$7–10 USD per kilo) and national markets.

Ecologically, leopard groupers are among the main predators in coastal environments (Craig and Hastings 2007). They occupy a high trophic level (~4.5) (Froese and Pauly 2021) with slow growth (from 15 to 21 years) (Díaz-Uribe et al. 2001), which makes them susceptible to accumulating heavy metals in tissues and organs. Overall, higher levels of heavy metals are usually associated with carnivorous and long-lived fish species (Evers et al. 2009). Despite its restricted distribution, high economic value and the scarce information on its biology, *M. rosacea* is no longer designated as “vulnerable” by the International Union for the Conservation of Nature (IUCN) and has a current designation of “least concern” (Erisman and Craig 2018). Although there have been no reported changes in leopard grouper populations over the past 30 years, enforcement may be needed due to intense fishing pressure (Erisman and Craig 2018).

Fishing for leopard grouper occurs most intensely along the Gulf of California coastlines; however, consumption of their meat occurs both locally and nationally. The primary objective of this study was to evaluate metal concentrations in leopard grouper tissues as a function of fish biology (size, sex and maturity) and collection time (season and year). A second objective was to assess the potential human health benefits or risks related to concentrations of Cu, Pb, Cd and Zn in the muscle tissue of the leopard grouper *M. rosacea*.

Materials and methods

Study area and sample collection

We obtained specimens of *Mycteroperca rosacea* in the port of Santa Rosalía (27° 20.353' N; 112° 15.797' W), Baja California Sur, in the Gulf of California, Mexico (Fig. 1). Since 1885, this port has been involved in the copper extraction industry through the French mining company “Compagnie du Boleo, S.A.”. Currently, the company operates under the name “Minera y Metalúrgica del Boleo S.A. de C.V.” (Huerta-Díaz et al. 2014). An estimated three million tons of slag has accumulated at mine sites, and undetermined amounts have entered the gulf’s marine environments (Shumilin et al. 2013; Huerta-Díaz et al. 2014). In addition to the mining industry, Santa Rosalía is a primary port for fishing and tourism vessels, cargo and passenger ferries, and small boats (Huerta-Díaz et al. 2014). Therefore, the marine environment is potentially polluted and marine organisms that inhabit the area, including the leopard grouper, may contain heavy metals.

We captured organisms monthly (from March 2014 to May 2015) by spearfishing while free diving to obtain a good representation of varied sizes. We stored specimens on ice until processing at the Fish Ecology Laboratory at CICIMAR-IPN, where we recorded total length (TL, cm) and weight (g). Sex was identified by direct observation of the gonads and later corroborated with histological analyses (Nikolsky 1963; Pérez-Olivas et al. 2018). We followed the four stages of maturity as reported by Pérez-Olivas et al. (2018).

We caught a total of 345 *M. rosacea* individuals, ranging in size from 21 to 74 cm total length; 93 specimens were male, and 185 were female. Our specimens included a total of 15 bisexual immature organisms, as well as 52 individuals for which we were unable to determine sex even via histological analysis, all of which we excluded from the comparative analyses of sex and stage of maturity.

We grouped data by sex (males and females), maturity stage (stage 1, stage 2, stage 3 and stage 4) and size (small: < 36 cm, medium: > 36 cm, and ≤ 51 cm, large: > 51 cm). We defined seasonality according to temperature records for the study period obtained from MODIS-AQUA satellite images with 1.1 km resolution. We recorded anomalies based on the annual average of 23 °C (Moreno-Sánchez et al. 2019). We assigned months with positive temperature anomalies to the warm season and assigned negative anomalies to the cold season.

We dissected fish specimens in the laboratory. We removed the skin and collected 5.0 g of muscle tissue from the anterior dorsal part of each individual. We conducted the extraction of muscle samples with care to avoid contamination and exposure. We washed scalpels with Milli-Q water during the entire process. We tagged each sample, placed them in plastic bags and stored them frozen at –20 °C.

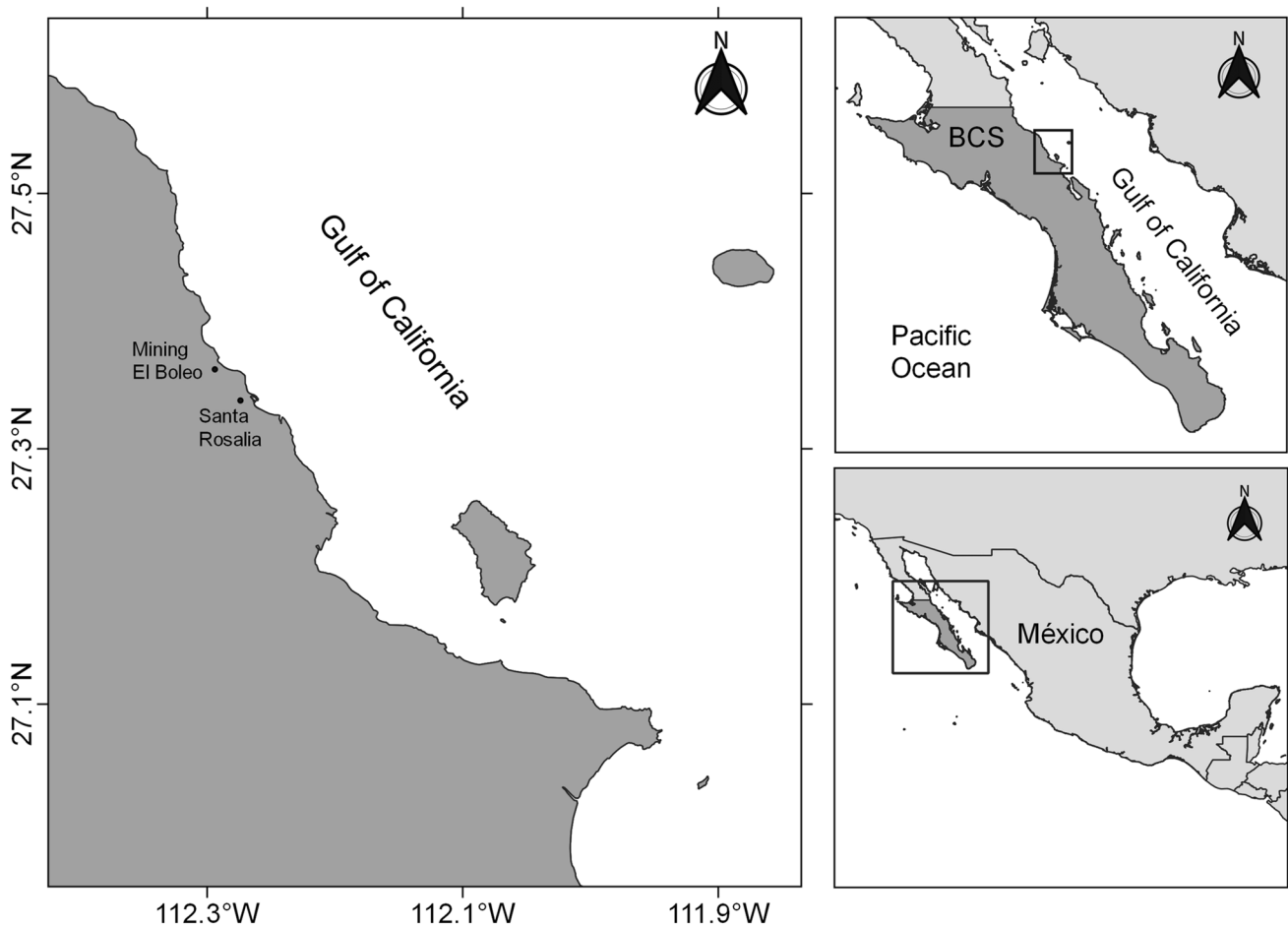


Fig. 1 Geographic location of the port of Santa Rosalía, Baja California Sur, Mexico

Heavy metal analysis

We lyophilized muscle tissue samples at 0.120 mBar pressure and $-40\text{ }^{\circ}\text{C}$ for 72 h (Labconco, FreeZone 2.5). We calculated the water content (%) by weighing the differences between fresh frozen and dried samples. The dried samples were ground using an agate mortar, homogenized and packed into trace metal-cleaned plastic vials.

Then, we sent 248 composite muscle samples to the Stable Isotope Laboratory at the ICMYL-UNAM at Mazatlán for elemental analysis. We processed samples and analysed in HEPA-filtered air (Class 1000) in a trace metal-free laboratory using high-purity reagents (trace metal grade) and water (18 MW cm^{-1} ; Milli-Q academic). We digested aliquots of fish muscle, blanks and certified reference material in Teflon vials (Savillex) with 10 mL concentrated nitric acid (HNO_3). We then placed the containers on a mod-block unit ($120\text{ }^{\circ}\text{C}$) for 4 h. After digestion, we transferred samples to polyethylene vials and diluted with Milli-Q water to a known volume (25 mL).

We conducted analyses for metals using a Thermo Scientific Element XR magnetic sector high-resolution inductively

coupled plasma source mass spectrometer (HR-ICP-MS) (Soto-Jiménez et al. 2008). We ascertained the accuracy of the analyses with concurrent processing of certified reference material (CRM) composed of Dog-Fish muscle (DORM-3) from the Institute for National Measurement Standards of the National Research Council Canada. The recovery values of CRM for Cd were 92%, Cu 94%, Pb 88% and Zn 92%. The detection limits were $<6\text{ ng g}^{-1}$ dry weight for Cd, $<10\text{ ng g}^{-1}$ for Pb and $<20\text{ ng g}^{-1}$ for Cu and Zn. We calculated concentrations of metals in fish in $\mu\text{g/g}$ dry weight but reported as averages \pm standard deviations in $\mu\text{g/g}$ wet weight.

Toxicological evaluation

To establish the amount of leopard grouper fillet that the human population could consume without a health risk, the MPCF (maximum possible consumption of fish meat (containing Cd or Pb) per week) was calculated using the following formula: $MPCF = PTWI/MT_j$, where PTWI is the provisional tolerable weekly intake of Cd and Pb, in $\mu\text{g week}^{-1}\text{ kg}^{-1}$ of body weight (Cd: $2.5\text{ }\mu\text{g kg}^{-1}\text{ w.w.}$; Pb:

25 $\mu\text{g kg}^{-1}$ w.w.; EFSA, 2012), and MT is the metal concentration ($\mu\text{g/g}$, w.w.) in muscle tissue of leopard grouper. We estimated the calculation assuming an average weight among the general population (70 kg), considering women (60 kg, pregnant and lactating) and children (16 kg, 4–6 years old). Additionally, we used average weights for the Santa Rosalía population (75 kg for men, 69 kg for women and 18 kg for 4-year to 6-year-old children) (INEGI 2015). We also used these average weights for additional evaluations.

The daily mineral intake of essential elements (Cu, Zn) was calculated based on the consumption recommended by the Institute of Medicine of the National Academies of the United States (www.iom.edu) using the following equation: $DMI = C * 100/RDI$, where DMI = daily mineral intake of essential elements, C = Cu or Zn concentration in 100 g of fresh fish weight and RDI = recommended daily intake, estimated as safe and adequate for each group in the population. CuRDI: men, women and children = 900 $\mu\text{g/day}$, pregnant and lactating women = 1000 $\mu\text{g/day}$; ZnRDI: men, women, pregnant and lactating women = 40 $\mu\text{g/day}$, children = 34 $\mu\text{g/day}$. We estimated these values for a healthy population.

Additionally, we calculated the potential health risk due to harmful effects from long-term leopard grouper consumption (e.g. months or years) as the target hazard quotient, where high THQ values (> 1) would represent a health risk. We calculated THQ as follows:

$$THQ = \frac{EF * ED * FIR * C}{RfD * BW * AT}$$

In this equation, C is the concentration of Cu, Pb, Cd or Zn ($\mu\text{g/g}$); EF is the exposure frequency (days/year); ED is the exposure duration (years/time); FIR is the fish intake rate (g/day); RfD is the daily fish intake rate (g/day); BW is the average body weight of the human population (kg); and AT is the average exposure time (days). We obtained all parameters except C , FIR and BW from actual data for the Santa Rosalía, BCS, Mexico population. We obtained the remaining data used in the formula, shown in Table 1, from the FAO (2017), US EPA (2015) and Yi et al. (2011).

Because heavy metal (Cu, Pb, Cd and Zn) interactions can cause multiple effects (Gu et al., 2017), we considered the added effect of these elements in the THQ, as recommended

by Chien et al. (2002) and Gu et al. (2017), as shown in the following equation:

$$\text{TotalTHQ(TTHQ)} = \sum THQ_{\text{Cu,Pb,Cd,Zn}}$$

Data analysis

We conducted simple linear regression (LR) analysis to assess the association between the concentration of each heavy metal and the size of leopard groupers. We separated the database into categories (sex, maturity stages and season), and fitted an LR of the heavy metal concentration as a function of size for each heavy metal and each category. We used this analysis to evaluate the hypothesis that the b coefficient of the LR model was zero (e.g. there was no association between heavy metal concentration and size). We performed all analyses using packages found in R (R Core Team 2017).

Results

Heavy metal concentrations in muscle tissue

Table 2 shows a summary of the heavy metal concentrations in the muscle of leopard grouper *M. rosacea* caught in Santa Rosalía (average \pm SD, maximum and minimum) and a comparative analysis of sex, maturity stage and total length. The sex groups, males, females and hermaphrodites showed comparable ranges for all metals (Table 2). Because of the comparable values and the high intravariability among sex groups, we observed no significant differences.

Heavy metal concentrations in maturity stages were stage 1 (Cu: 18.1 ± 58.4 $\mu\text{g/g}$; Zn: 588 ± 2394 $\mu\text{g/g}$; Cd: 0.1 ± 0.2 $\mu\text{g/g}$; Pb: 1.5 ± 2.4 $\mu\text{g/g}$), stage 2 (Cu: 16.8 ± 55.6 $\mu\text{g/g}$; Zn: 517.9 ± 2256 $\mu\text{g/g}$; Cd: 0.1 ± 0.2 $\mu\text{g/g}$; Pb: 1.5 ± 2.4 $\mu\text{g/g}$), stage 3 (Cu: 13.8 ± 56.7 $\mu\text{g/g}$; Zn: 439.2 ± 2323 $\mu\text{g/g}$; Cd: 0.1 ± 0.2 $\mu\text{g/g}$; Pb: 1.4 ± 2.3 $\mu\text{g/g}$) and stage 4 (Cu: 14.1 ± 58.1 $\mu\text{g/g}$; Zn: 447.3 ± 2362 $\mu\text{g/g}$; Cd: 0.1 ± 0.2 $\mu\text{g/g}$; Pb: 1.6 ± 2.5 $\mu\text{g/g}$). There were no significant differences between metal levels in maturity stages.

Table 1 Parameters and values used for the THQ formula for each heavy metal

Factor	Definition	Unit	Value	Reference
EF	Exposure frequency	Days/year	365	Yi et al. (2011)
ED	Duration	Years	78	Yi et al. (2011)
FIR	Fish intake rate	g/day	32.8	
RfD	Reference dose	$\mu\text{g/kg/day}$	1.0E^{-01} (Cd), 2.0E^{+01} (Pb), 4.0E^{+01} (Cu), 3.0E^{+02} (Zn)	US EPA (2015)
BW	Average weight	kg	70 ± 75	WHO; INEGI (2015)
AT	Average exposure time	Days	$365 * 78 = 28.470$	Yi et al. (2011)

Table 2 Heavy metal concentrations (mean \pm SD; $\mu\text{g/g}$ wet weight) in *Mycteroperca rosacea* muscle in a mining port in the Gulf of California, Mexico

Category	<i>n</i>	Cu	Zn	Cd	Pb
Overall	248	11.6 \pm 34.3 (0.01–361) ^c	377.3 \pm 1389.9 (0.02–0.06 \pm 0.13 (0.001–1.13) 13,165) ^c	0.08 \pm 0.2 (0.01–1.1)	0.98 \pm 1.56 (0.03–9.8) ^b
Sex					
Females	132	12.7 \pm 39.9 (0.01–361) ^c	433.8 \pm 1628.5 (0.02–0.05 \pm 0.12 (0.001–0.69) 13,165) ^c	0.08 \pm 0.2 (0.01–1.1)	1.06 \pm 1.71 (0.03–8.9) ^{a,b}
Males	70	13.6 \pm 33.6 (0.1–226) ^c	448.8 \pm 1348 (0.01–9442) ^c	0.08 \pm 0.2 (0.01–1.1)	0.84 \pm 1.0 (0.02–9.8) ^b
Hermaphrodites	15	7.7 \pm 7.7 (0.02–20) ^c	164.7 \pm 219.9 (0.1–807) ^c	0.07 \pm 0.1 (0.001–0.3)	0.79 \pm 1.3 (0.02–5.3) ^b
Size					
Small	129	12 \pm 34 (0.2–361) ^c	385 \pm 1644 (0.1–13,165) ^c	0.06 \pm 0.1 (0.01–1.1)	0.98 \pm 1.6 (0.1–9.8) ^b
Medium	99	11 \pm 32 (0.01–361) ^c	328 \pm 1269 (0.02–13,165) ^c	0.06 \pm 0.1 (0.01–0.7)	1.0 \pm 1.5 (0.02–9.8) ^{a,b}
Large	20	7 \pm 32 (0.2–90) ^c	246 \pm 1330 (0.8–6901) ^c	0.03 \pm 0.07 (0.01–0.1)	0.86 \pm 1.4 (0.05–2.7) ^b
Maturity stage					
Maturity stage 1	141	18.1 \pm 58.4 (0.3–283) ^c	588 \pm 2394 (2.3–12,006) ^c	0.1 \pm 0.2 (0.01–0.8) ^{a,b}	1.5 \pm 2.4 (0.07–15.4) ^{a,b}
Maturity stage 2	47	16.8 \pm 55.6 (0.3–123) ^c	517.9 \pm 2256 (6.0–5767) ^c	0.1 \pm 0.2 (0.01–1.3) ^{a,b}	1.5 \pm 2.4 (0.06–11.0) ^{a,b}
Maturity stage 3	76	13.8 \pm 56.7 (0.3–664) ^c	430.2 \pm 2323 (3.7–24,197) ^c	0.1 \pm 0.2 (0.01–0.9) ^{a,b}	1.4 \pm 2.3 (0.06–6.5) ^{a,b}
Maturity stage 4	8	14.1 \pm 58.1 (0.6–132) ^c	447.3 \pm 2362 (12.2–2231) ^c	0.1 \pm 0.2 (0.02–0.1) ^{a,b}	1.6 \pm 2.5 (0.05–1.7) ^{a,b}
Season					
Warm season	80	2.0 \pm 3.0 (0.2–23)	50 \pm 66 (0.8–411) ^c	0.01 \pm 0.03 (0.02–0.11)	1.0 \pm 2.0 (0.05–9.8) ^{a,b}
Cold season	168	16 \pm 35 (0.1–361) ^c	539 \pm 1412 (0.1–13,165) ^c	0.11 \pm 0.15 (0.01–1.1) ^{a,b}	1.0 \pm 1.6 (0.02–8.9) ^{a,b}
Year					
2014	180	8.8 \pm 31.5 (0.17–361) ^c	251 \pm 1239 (0.1–13,165) ^c	0.08 \pm 0.2 (0.02–1.1)	0.97 \pm 1.6 (0.03–9.9) ^b
2015	68	26 \pm 38.4 (0.2–205) ^c	867 \pm 1499.7 (5.1–8702) ^c	0.2 \pm 0.3 (0.1–1.1) ^b	1.5 \pm 1.9 (0.1–1.9) ^{a,b}

^aValues above recommended limits set by the official Mexican norm NOM-242-SSA1-2009 (Cd 0.5 and Pb 1.0 $\mu\text{g/g}$ w.w.)

^bUS-EPA, FDA criterion, surpassing established limits of 0.5 for Cd and 0.5 for Pb

^cUnited Kingdom norm (UK-EEA food standards) of Zn 50 and Cu 5 $\mu\text{g/g}$ w.w

Regarding size, the metal levels were as follows: small fish (Cu: 12 \pm 34 $\mu\text{g/g}$; Zn: 385 \pm 1644 $\mu\text{g/g}$; Cd: 0.06 \pm 0.1 $\mu\text{g/g}$; Pb: 0.98 \pm 1.6 $\mu\text{g/g}$), then medium-sized fish (Cu: 11 \pm 32 $\mu\text{g/g}$; Zn: 328 \pm 1269 $\mu\text{g/g}$; Cd: 0.06 \pm 0.1 $\mu\text{g/g}$; Pb: 1.0 \pm 1.5 $\mu\text{g/g}$) and large specimens presented lower concentrations (Cu: 7.0 \pm 32 $\mu\text{g/g}$; Zn: 246 \pm 1330 $\mu\text{g/g}$; Cd: 0.03 \pm 0.07 $\mu\text{g/g}$; Pb: 0.86 \pm 1.4 $\mu\text{g/g}$). We observed no significant differences among the size groups.

A comparative analysis related to the collection time showed that the heavy metal concentrations in organisms collected in 2015 (Cu: 26 \pm 38.4 $\mu\text{g/g}$; Zn: 867 \pm 1499 $\mu\text{g/g}$; Cd: 0.2 \pm 0.3; Pb: 1.5 \pm 1.9 $\mu\text{g/g}$) were significantly higher ($p < 0.05$) than those collected in 2014 (Cu: 8.8 \pm 31.5 $\mu\text{g/g}$; Zn: 251 \pm 1239 $\mu\text{g/g}$; Cd: 0.08 \pm 0.2 $\mu\text{g/g}$; Pb: 0.97 \pm 1.6 $\mu\text{g/g}$). In addition, significant differences ($p < 0.05$) were observed for Cu, Zn and Cd between the warm (Cu: 2.0 \pm 3.0 $\mu\text{g/g}$; Zn: 50 \pm 66 $\mu\text{g/g}$; Cd: 0.01 \pm 0.03 $\mu\text{g/g}$) and cold seasons (Cu: 16 \pm 35 $\mu\text{g/g}$; Zn: 539 \pm 1412 $\mu\text{g/g}$; Cd: 0.11 \pm 0.15 $\mu\text{g/g}$) but not for lead (warm: 1.0 \pm 2.0 $\mu\text{g/g}$; cold: 1.0 \pm 1.6 $\mu\text{g/g}$).

The results of the fitted simple linear regression (LR) suggested that the association between heavy metal concentration in muscle and the size of leopard groupers was weak

(low R^2 values) and not significant (p ($b=0$) > 0.05 in all cases) (Table 3).

A comparison of metal concentrations found in the grouper muscle samples showed that the essential elements (Cu = 11.61 \pm 34.36 $\mu\text{g/g}$ and Zn = 377.33 \pm 1389.98 $\mu\text{g/g}$) presented global values that exceeded the norms set by the UK (UK-EEA food standards Cu: 5.0 $\mu\text{g/g}$; Zn: 50 $\mu\text{g/g}$), whereas Cd, a nonessential element (Cd = 0.06 \pm 0.13 $\mu\text{g/g}$ vs. Cd = 0.05 $\mu\text{g/g}$), was within the ranges established by NOM-242 (Table 2). In the case of Pb, the global average borders the limit established by NOM-242. In fact, almost half of the samples ($n = 114$, 46%) were above the limit of 1 $\mu\text{g/g}$.

Ecotoxicological evaluation

According to the general calculations of the maximum possible consumption of fish meat per week (MPCF) for Cd values, we suggest that children under 6 years can consume fish fillets of up to 667 g per week and others can consume up to 2000 g of leopard grouper. However, Pb values indicated that the frequency of leopard grouper consumption should decrease (Table S1). The average values of the risk coefficient (THQ_{Cu}: 0.1 \pm 0.3, THQ_{Pb}: 0 \pm 0.1, THQ_{Cd}: 0.3 \pm 1.4, THQ_{Zn}: 0.2 \pm 0.6)

Table 3 Coefficient values of the linear regression of variations by sex, maturity stages and season in the muscle of leopard grouper *Mycteroperca rosacea* in Santa Rosalía, BCS, Mexico

Variables	Linear regression coefficients					
	<i>n</i>	<i>R</i> ²	<i>b</i>	<i>a</i>	<i>p</i> (<i>a</i> =0)	<i>p</i> (<i>b</i> =0)
Cu						
F	132	0.004	0.3	2,047,030	1	0.46
M	69	0.01	0.4	−2,975,734	0.87	0.36
Maturity stage 1	141	0.01	0.5	−1,018,392	0.97	0.45
Maturity stage 2	47	0.02	−0.48	26,628,406	0.02	0.14
Maturity stage 3	76	0.01	0.6	−11,830,348	0.74	0.43
Maturity stage 4	8	0.14	2688	−76,258,327	0.48	0.40
Cold	148	0.01	0.5	−2,897,376	0.85	0.19
Warm	47	0.05	−0.1	6,542,092	0.01	0.12
Pb						
F	132	0.0005	−0.005	13,403,743	0.07	0.79
M	69	0.04	0.02	−0.1501317	0.75	0.07
Maturity stage 1	141	0.007	−0.02	19,128.434	0.03	0.39
Maturity stage 2	47	0.105	0.07	−18,344,799	0.24	0.06
Maturity stage 3	76	0.04	0.02	−0.1766029	0.77	0.13
Maturity stage 4	8	0.11	0.03	−0.7844367	0.62	0.45
Cold	148	0.0004	−0.003	11,716,741	0.03	0.80
Warm	47	0.01	0.02	−0.1538668	0.90	0.42
Cd						
F	132	0.02	−0.002	0.138755835	0.002	0.06
M	69	0.005	0.001	0.010781516	0.92	0.54
Maturity stage 1	141	0.03	−0.002	0.158315518	0.002	0.05
Maturity stage 2	47	0.001	0.001	0.088152186	0.74	0.83
Maturity stage 3	76	0.003	0.0005	0.007955513	0.89	0.70
Maturity stage 4	8	0.14	0.002	−0.077131024	0.49	0.40
Cold	148	0.001	−0.0006	0.100902619	0.10	0.69
Warm	47	0.02	−0.0005	0.044550624	0.02	0.27
Zn						
F	132	0.01	252,802,895	−48,738,201	0.43	0.13
M	69	0.009	151,811,566	−15,627,183	0.83	0.42
Maturity stage 1	141	0.008	−127,235,772	74,047,734	0.12	0.35
Maturity stage 2	47	0.005	87,707,420	9,110,244	0.91	0.67
Maturity stage 3	76	0.01	302,079,514	−62,109,127	0.65	0.37
Maturity stage 4	8	0.13	453,892,443	−129,630,397	0.48	0.41
Cold	148	0.02	297,236,154	−53,587,711	0.38	0.07
Warm	47	0.02	0.69	1,298,858	0.62	0.31

showed there was no risk from leopard grouper ingestion; however, the maximum range of THQ_{Cd} values could surpass established limits (> 1). In contrast, the suggested amounts of essential elements (Cu and Zn) were higher for the three human population groups (children, women and men).

The benefits that consumption of this fish provide for vulnerable groups in the population (children, pregnant and lactating women) indicated that each 100 g of leopard grouper fillet represented over 1% Cu and 100% Zn. The percentages of Cu daily mineral intake oscillated between 1.2 and 1.3, while the percentages of Zn daily mineral intake ranged from 943 to 1110.

Discussion

The leopard grouper *Mycteroperca rosacea* is one of the main predatory species on rocky reefs (Thomson et al. 2000; Moreno-Sánchez et al. 2019). Due to its carnivorous habits and slow growth (Díaz-Urbe et al. 2001), it can be susceptible to bioaccumulating heavy metals, as occurs with other species that share these characteristics, such as *Scomberomorus sierra*, *Nematistius pectoralis*, *Caulolatilus princeps* and *Lutjanus colorado* (Frías-Espericueta et al. 2010; Ruelas-Inzunza et al. 2010, 2014). However, Cd concentrations (0.06 µg/g) were below the limit set by the Mexican norm

NOM-242, whereas Pb concentrations found in *M. rosacea* muscle tissue (0.98 µg/g) were on average close to the limit set by the Mexican norm (Pb: 1.0 µg/g; Cd: 0.5 µg/g). The average values of essential metals (Cu: 11.61 ± 34.36 µg/g; Zn: 377.33 ± 1389.98 µg/g) were well above the maximum criteria allowed by international regulations (UK-EEA food standards Cu: 5.0 µg/g; Zn: 50 µg/g).

Previously reported levels of Cd (0.2 µg/g) and Pb (2.5 µg/g) have been up to three times higher than those recorded here for *M. rosacea* in other commercially important species in Sinaloa, Mexico (*S. sierra*, *C. princeps* and *L. colorado*). Coastal ecosystems in Sinaloa have a significant anthropogenic influence with raw or partially processed effluents from aquaculture, agriculture, the food processing industry, urban wastewater and fisheries converge (Frías-Espéricueta et al. 2010; Ruelas-Inzunza et al. 2014). Concentrations of Cu, Zn, Pb and Cd above the limits established in the sediment quality criteria (Cu: 3860, Zn: 2600, Pb: 240, Cd: 240 mg/kg) have occurred in the Central Gulf of California (in Santa Rosalía). Cooper mining activities in the Santa Rosalía maritime port, from the beginning of the nineteenth century to the present, are the most important source of heavy metals to the adjacent coastal region (Jonathan et al. 2016). Although specific cases of inputs of contaminants are fundamental in explaining the presence of heavy metals in organisms (Zhang et al. 2017; Ali and Khan 2019), the concentrations of Pb and Cd found in *M. rosacea* do not reflect those inputs. However, the concentrations of essential elements such as Cu (11.6 µg/g) reported for *M. rosacea* in the Gulf of California were five times higher than those reported in Sinaloa for other fish such as *L. colorado* (Cu: 2.1 µg/g).

Few studies have investigated heavy metals in serranid species (Table 3), and these studies reported low metal concentrations in comparison with our study. However, two studies indicated elevated levels of Cd in *Mycteroperca fusca* (2.54 ± 10.3 µg/g; Franco-Fuentes et al. 2021) and *Mycteroperca olfax* (10.70 ± 7.93 µg/g; Lozano et al. 2009). The authors attributed these levels to differences in age, size, metabolic activity and feeding habits. In this sense, several studies reported that organisms that feed on invertebrates show lower heavy metal levels than those that include a greater proportion of fish in their diet (Escobar-Sánchez et al. 2016; Murillo-Cisneros et al. 2018; Sujitha et al. 2019). *Mycteroperca rosacea* is a predator that feeds primarily on the euphausiid *Nyctiphanes simplex*, which comprises 65% of the diet, in terms of relative importance (Moreno-Sánchez et al. 2019). Therefore, we would expect that due to the type of feeding shown by *M. rosacea* its average heavy metal concentrations would be at the limit (Pb) and/or below the limit (Cd) set by NOM-242-SSA-2009.

Other variables, such as size and sex, could affect the bioaccumulation of heavy metals (Xia et al., 2019).

For example, elements such as Hg and Cd have shown a positive relationship with fish size (Tremain and Adams 2012; Ruelas-Inzunza et al. 2014), where there were greater heavy metal concentrations at greater fish sizes, as reported for other grouper species (*Epinephelus*, *Mycteroperca* and *Cephalopholis*) (Tremain and Adams 2012). However, this correlation was not related to growth (in size, age or weight) for other heavy metals such as Pb, Zn and Cu (García-Hernández et al. 2007; Rodrigues et al. 2018; Xia et al. 2019). In our study, there was no evident relationship between total length and the analysed heavy metals. García-Hernández et al. (2007) showed that the lack of correlation between size and heavy metal concentrations (Hg, specifically) can be related to different fish species feeding on the same food components.

Nyctiphanes simplex is the main prey for *M. rosacea* in Santa Rosalía; however, this grouper can vary the proportion of food items in its diet according to size (Moreno-Sánchez et al. 2019). For example, small-sized grouper (< 36 cm; *n*: 129) fed exclusively on euphausiids (*N. simplex*), whereas medium-sized fish (> 36 cm < 51 cm; *n*: 99) had a mixed diet (invertebrates and fish), and large organisms (> 51 cm; *n*: 20) consumed a greater proportion of fish (*S. sagax*, *Microlepidotus inornatus*, *Chromis atrilobata*) (Moreno-Sánchez et al. 2019). As mentioned previously, this would imply that large organisms would show greater heavy metal concentrations (Murillo-Cisneros et al. 2018). However, although differences in the diet do reflect heavy metal concentrations by size (small, medium and large), the greatest heavy metal concentrations were recorded in the small and medium-sized organisms (see Table 2) rather than in the largest. Sujitha et al. (2019) reported that in different crustacean species (e.g. *Panulirus interruptus*, *Penaeus stylirostris*), high Zn (> 80 µg/g) and Cu (> 45 µg/g) levels occurred, which could explain the presence of greater quantities of those elements in smaller *M. rosacea*.

The occurrence of greater Cu and Zn concentrations in small-sized fish could be due to small individuals showing accelerated growth in their first years of life (1–3 years), as these elements are required for critical physiological processes. Moreover, the elimination rate of toxic metals is more efficient (Rajeshkumar and Li 2018). Estimates for size at first maturity of *M. rosacea* are 40.7 cm total length (Pérez-Olivas et al. 2018), which coincides with essential element concentrations being greater in small and medium-sized fish. That is, we would expect that groupers of this size would have higher heavy metal levels because Cu and Zn are important micronutrients for gonad maturation and are necessary to carry out reproduction successfully (Jeziarska et al. 2009). We suggest that the low Pb and Cd levels recorded could be due to more efficient elimination at those sizes (Yi and Zhang 2012), at least in *M. rosacea*.

Elevated levels of Pb and Cd at all maturity stages suggest increased metabolic activity and high lipid content of the gonads that stimulate rapid accumulation of lipophilic metallic species and are associated with metallothionein synthesis in the liver (El-Greisy and El-Gamal 2015). Metallothionein proteins bind to metals, and Cd forms a nontoxic complex, which tissues retain, resulting in bioaccumulation. This phenomenon shows that stage of sexual maturity plays a role in understanding the bioavailability of metals and detoxification of both essential and nonessential metals (Hemmadi 2016).

Compared with reports for the El Niño events of 1997 and 1998, the El Niño phenomenon of 2015 was the most intense event recorded, with temperatures over 0.76° higher than those of the previous record (Pérez-Olivas et al. 2018). Because of this, there was a difference of 2.2 °C between 2014 and 2015. This temperature difference coincided with greater metal concentrations in 2015 (Cu: 26 µg/g, Zn: 867 µg/g, Cd: 0.2 µg/g, Pb: 1.5 µg/g) than in 2014 (Cu: 8.8 µg/g, Zn: 251 µg/g, Cd: 0.08 µg/g, Pb: 0.97 µg/g). These increases in heavy metal concentrations coincide with a report by Huerta-Díaz et al. (2014) for the same study area regarding high heavy metal levels resulting from the mixing and removal of particulate materials in marine sediments caused by natural phenomena such as hurricanes and storms.

In 2014, hurricane “Odile” impacted Baja California Sur (CONAGUA 2014) in the months categorized as the warm season, coinciding with enrichment in heavy metals during the previously mentioned phenomenon. Therefore, the heavy metal enrichment observed in 2015 could reflect the impact of the hurricane entraining heavy metals through pluvial precipitation and temporary streams flowing into the port. Other environmental and industrial activities in Santa Rosalía could be involved in increased heavy metal concentrations. Such increases have occurred as reported in studies on heavy metals in north-western Mexico (Frías-Espéricueta et al. 2010; Ruelas-Inzunza et al. 2010; 2014).

Heavy metals are a potential problem in areas close to coasts and constitute a human health risk. However, there is currently a discrepancy between recommendations by public health sectors (FAO 2017) and studies that advise reducing the consumption of fish fillet. Additionally, fish consumption may be greater than we assumed because the frequency of fish consumption in coastal communities is often greater than reported.

In this study, a consumption of 700 g does not represent a risk to health. However, the frequency of leopard grouper consumption by the population of Santa Rosalía was greater than what we recommend (CONAPESCA 2014). Essential elements such as Cu and Zn are required for the correct absorption of vitamins B₆, B₁₂, C and A, among others, especially in vulnerable groups (children, pregnant women and lactating women). According to daily mineral intake

values, the *M. rosacea* fillet is generally recommended and beneficial to the population. This fish offers high nutritional value, and it meets worldwide levels (FAO 2017) and is even higher than what has been reported for other fish in north-western Mexico (Frías-Espéricueta et al. 2010).

The risk factor (THQ) showed no adverse long-term effects, whereas we advise caution for Cd due to the maximum values obtained. In the present study, we suggest continuous monitoring of Cd levels to more deeply evaluate the THQ and the consideration of later years because estimated values correspond to 2014 and 2015.

Conclusion

This is the first study focusing on heavy metals in *Mycteroperca rosacea*. The results on metal concentrations suggest that, although the samples of leopard groupers (*M. rosacea*) come from an area adjacent to a mining district, “El Boleo” in Santa Rosalía, Mexico, no essential metals (Cd and Pb) were below the limit set by the EPA, FDA and Mexican norms (NOM-242). We need further monitoring for Pb because its average concentration was close to the limit set by the Mexican norm. The leopard grouper did not represent adverse effects to the consumer; in contrast, the nutritional benefits were high. Enrichment of heavy metals during the warm season may occur as a result of natural phenomenon (e.g. hurricanes). *Mycteroperca rosacea* continues to be marketed; therefore, it is important to know the ingestion rate of this species in Mexico to understand metal impacts on human populations.

Supplementary information.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-022-18753-7>.

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Author contribution Marina S. Irigoyen-Arredondo: conceptualization, data analysis, writing—original draft preparation. Xchel G. Moreno-Sánchez: conceptualization, visualization, investigation. Ofelia Escobar-Sánchez: funding acquisition, project administration, data curation, writing—reviewing and Editing. Martín F. Soto-Jiménez: data curation, writing—reviewing and editing. Emigdio Marín-Enríquez: software. L. Andrés Abitia-Cárdenas: conceptualization, supervision, investigation, funding acquisition.

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