Chemosphere 242 (2020) 125180

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Cadmium, mercury, and selenium in muscle of the scalloped hammerhead *Sphyrna lewini* from the tropical Eastern Pacific: Variation with age, molar ratios and human health risk

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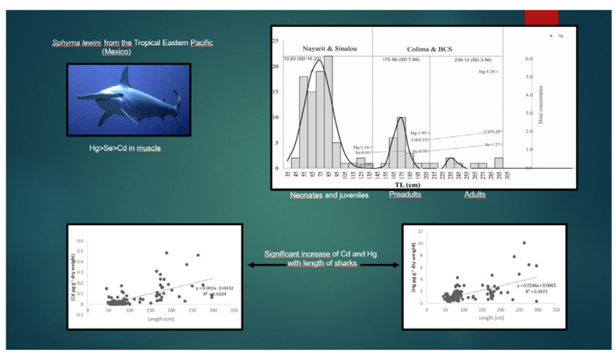
HIGHLIGHTS

- Mean concentrations of Cd and Hg increased according to mean length of sharks.
- Overall, HQ and HI values were below one so there is no health risk to consumers.
- Health benefit values were positive except in sharks from BCS so they might not be beneficial.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 June 2019
Received in revised form
11 October 2019
Accepted 20 October 2019
Available online 24 October 2019

Handling Editor: Martine Leermakers

Keywords:
Essential and non-essential elements
Edible portion
Sphyrnidae
Gulf of California
Mexican pacific

ABSTRACT

With the aim of assessing health risk to shark consumers, cadmium, mercury, and selenium were measured in muscle of *Sphyrna lewini* from four coastal states (Baja California Sur, Sinaloa, Nayarit, and Colima) in western Mexico. According to length of specimens, three age modes were found: juveniles and neonates (the majority of the individuals), preadults and adults. Average concentrations ($\mu g\ g^{-1}$ dry weight) in all the studied individuals followed the order cadmium (0.06), selenium (0.94), and mercury (1.56). The mean concentrations of cadmium and mercury increased significantly ($p < 0.001$) with mean length of specimens. Overall, hazard quotient and hazard index values were below one so there is no health risk to consumers. According to molar ratios of Hg and Se in the edible portion (muscle) of sharks, and depending on the areas of collection, individuals from Baja California Sur might not be beneficial to consumers.

1. Introduction

On the basis of the role that diverse elements play in the

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https://doi.org/10.1016/j.chemosphere.2019.125180
0045-6535/

metabolism, they can be classified as essential (E) and non-essential (NE). Essential elements are required for metabolism (Abtahi et al., 2017 and their levels are regulated by homeostatic mechanisms in organisms; *i.e.,* they are required at certain concentrations, depending on the individual and the element. Examples of E elements are iron (Fe), copper (Cu), zinc (Zn), and selenium (Se); although they are necessary for metabolic activities, at elevated concentrations they might exert damages to individuals (Vinceti et al., 2018). On the other hand, NE elements are not necessary for the metabolic activities in biota; it implies that their occurrence may cause toxic effects even at low concentrations. Deleterious effects range from lethal to sublethal effects, including behavioural alterations in biota (Wilk et al., 2017). Among the most dangerous non-essential elements are As, Cd, Hg, and Pb. Cd and Hg were selected because of their elevated toxicity to fish; these elements are among the ten most hazardous substances according to the Agency for Toxic Substances and Disease Registry (ATSDR) of the US Department of Health and Human Services (Wang, 2012). Cd may induce functional and structural damages in gills, kidney liver and intestine of fishes (Kumar and Singh, 2010). In the case of Hg, its occurrence in marine fish is necessary to understand given its complex biogeochemistry in the marine environment (Kwon et al., 2013) and the health risk associated to human consumption. Regarding Se, it has been shown that this element is effective to detoxify Hg in fish and mammals (Dang and Wang, 2011).

An important issue related to the occurrence of E and NE elements in the environment is that they are supplied by natural processes as well as anthropogenic activities (Callender, 2005; Páez-Osuna and Osuna-Martínez, 2015); therefore, these elements are present in aquatic ecosystems worldwide.

Global emissions of Cd to the atmosphere indicated that stationary fossil fuel combustion (23%) and non-ferrous metal production (73%) were the main contributors in the mid nineties (Pacyna and Pacyna, 2001). In the case of Hg, global anthropogenic emissions have been estimated in 2320 metric tons per year (Pirrone et al., 2010). The anthropogenic supply of Se to the environment is mainly as a by-product of irrigated agriculture, refining of crude oil, and mining of coal, phosphate, copper, and uranium (Young et al., 2010).

Coastal and marine contamination is increasing around the world (Mestre et al., 2012), but the environmental levels of many contaminants (included Cd and Hg) which can elicit adverse effects are largely unknown for marine megafauna. Bioaccumulation of these toxic substances has become a concern due to the possibility of their transfer to the food chain and its impact on diverse species of marine wildlife (Cortés-Gómez et al., 2017), including the humans.

Diet is considered as the main pathway of entrance of diverse pollutants to biota, including man. In the case of Cd, Hg and Se, marine fish are among the main contributors to humans (Okocha and Adedeji, 2011; Fréry et al., 2001; Navarro-Alarcon and Cabrera-Vique, 2008); in this context, it is necessary to monitor their occurrence in fish of elevated trophic levels since Hg and Cd may be biomagnified and their levels in the edible portion of fish might constitue a risk for consumers. On the other hand, the presence of Se in fish may protect against Hg toxicity (Kaneko and Ralston, 2007; Ralston, 2008). It has been studied that an excess of Se over Hg protects against Hg toxicity; the negative effects occur when Se is sequestered by Hg and the activity of selenoenzymes is inhibited (Falnoga et al., 2006; Huggins et al., 2009). Considering the antagonism between Se and Hg in the context of human health (Filippini et al., 2018; Vinceti et al., 2018), the Se health benefit value (HBV_{Se}) is an indicator of the amount of Se in excess or deficit in relation to Hg (as methyl-Hg, CH_3Hg) in food (Ralston et al., 2016) that turns into a benefit or risk, respectively. The objectives of the study were, a) to measure Cd, Hg and Se concentrations in muscle tissue of *S. lewini* from four coastal states in western Mexico (tropical Eastern Pacific, TEP), b) to determine elemental variations with length of specimens, c) to assess health risk to consumers according to elemental concentrations, Hg and Se interactions, and rate of shark consumption.

2. Materials and methods

Sharks were collected in the continental shelf in front of Baja California Sur (BCS), Sinaloa, Nayarit, and Colima (TEP) by local fishermen using gill nets (Fig. 1) between August 2013 and October 2015. After taxonomic identification (Fischer et al., 1995), total length was determined. A portion of muscle tissue was obtained from the dorsal anterior part of every individual using stainless steel scalpels and tweezers. Muscle samples were transported to the laboratory in ice boxes at low temperatures (~5 °C). Plastic utensils and glassware were washed with HCl 2 M and HNO_3 2 M and rinsed with milli-Q water according to Moody and Lindstrom (1997). Muscle samples were freeze-dried during 72 h (−50 °C and 120×10^{-3} mBar); dried samples were ground and homogenized manually in an agate mortar with pestle. Powdered samples were digested during 3 h with concentrated HNO_3 in capped Teflon vials on a hot plate at 120 °C (MESL, 1997). Cd and Se were measured by graphite furnace atomic absorption spectrophotometry (GF-AAS) and Zeeman effect in a PerkinElmer (AAnalyst 800) equipment. Hg was analyzed by cold vapor-atomic absorption spectrophotometry (CV-AAS) in a Buck Scientific equipment. Quality control of analytical runs was assessed by using duplicates, ultrapure water (milli-Q, 18.2 MΩ cm), blanks, trace metal grade acids and certified reference materials. Fish protein (DORM-3) was used as reference material for Cd and Hg; measured values of Cd (0.28 $\mu g\ g^{-1}$), and Hg (0.36 $\mu g\ g^{-1}$) were within certified values (Cd $0.29 \pm 0.02\ \mu g\ g^{-1}$, Hg $0.38 \pm 0.06\ \mu g\ g^{-1}$). For Se, fish liver (DOLT-3) was used as certified reference material, measured values of Se (6.61 $\mu g\ g^{-1}$) were within certified concentrations ($7.06 \pm 0.45\ \mu g\ g^{-1}$). Limits of detection (in $\mu g\ g^{-1}$) were estimated as two standard deviations of a blank (Cd 0.003, Hg 0.02, Se 0.08). Results are reported as $\mu g\ g^{-1}$ on a dry weight basis. Conversions of wet weight to dry weight concentrations were made by considering 65% of humidity content in muscle tissue.

Levels of Cd and Hg in shark meat were compared with maximum permissible limits in foodstuffs considered in the national and international legislations. Health risk was assessed through the hazard index (HI) proposed by Newman and Unger (2002): $HI = HQ_{Cd} + HQ_{Hg}$; where HQ_{Cd} and HQ_{Hg} are the hazard quotients of Cd and Hg concentrations in the edible portion of fish. The estimation of hazard quotients was calculated as $HQ = E/RfD$, where E is the exposure level or intake of Cd and Hg, and RfD is the reference dose for Cd (1.0 $\mu g\ kg^{-1}$ body weight day^{-1}) and Hg (0.5 $\mu g\ kg^{-1}$ body weight day^{-1}) (US EPA, 2000). The exposure level (E) was calculated as $E = C \times I/W$; C is the concentration of Cd and Hg (on a wet weight basis) in the muscle tissue of sharks, I is the ingestion rate of sharks. The rates of shark ingestion (in g per day^{-1}) per capita were estimated for the general population in Mexico (0.88) and in the states of interest (Sinaloa, 6.54; BCS, 17.34; Nayarit, 13.67; and Colima, 2.19) (CONAPESCA, 2017). W is the weight of an average adult (70 kg). Conversion of Cd and Hg levels from dry weight (dw) to fresh weight (fw) were made according to: Elementfw = Elementdw * (100 - % humidity)/100, using the humidity percentage (65%) in the muscle tissue of sharks.

Additionally, the Se health benefit value (HBV_{Se}) was estimated according to eq. (1) (Ralston et al., 2016).

$$HBV_{Se} = ([Se-Hg]/Se) \times (Se + Hg) \quad (1)$$

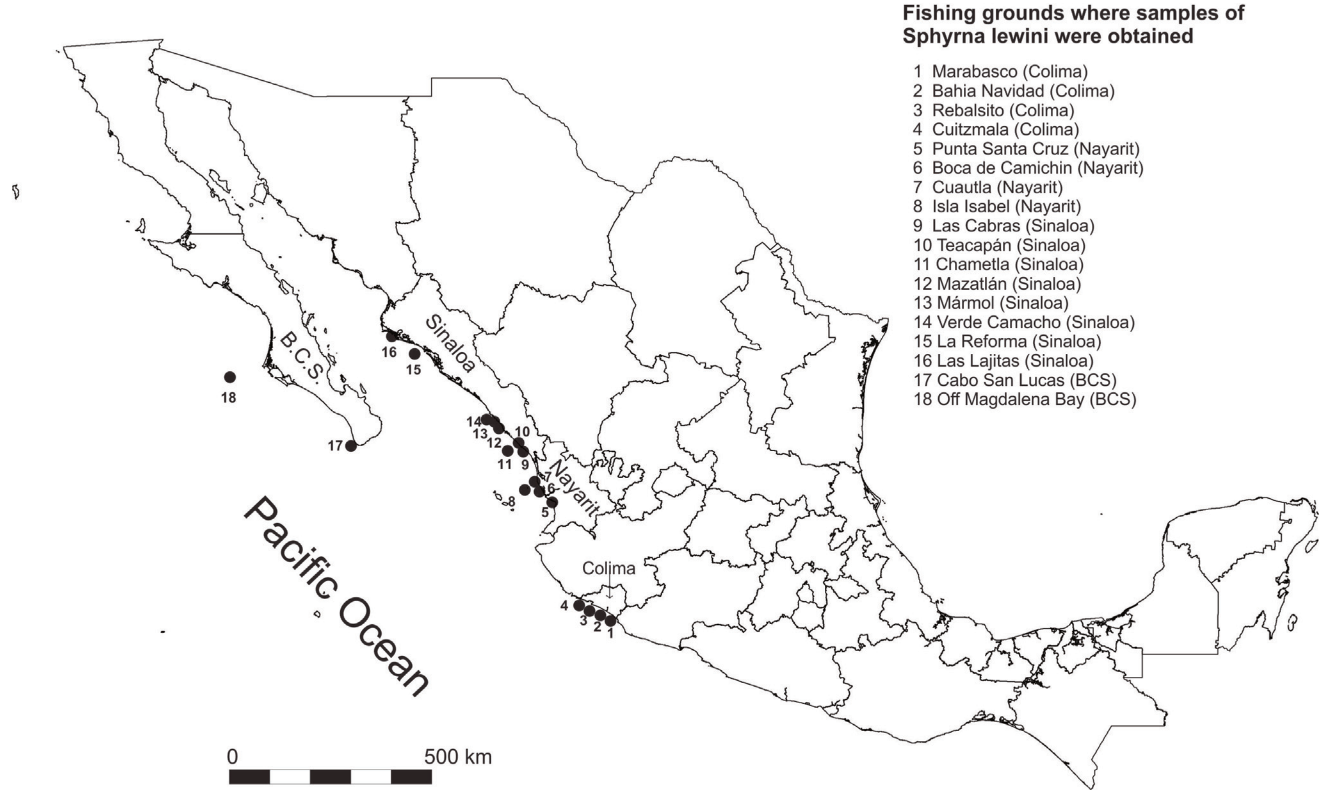

Fig. 1. Coastal states where sharks were collected in the continental shelf of the Tropical Eastern Pacific (TEP).

The concentrations of Se and Hg are given as μmol kg^{-1} on a wet weight basis. Positive results indicate that Se exceeds Hg and it is beneficial to consumers; negative values mean the contrary; the magnitude of the value indicates Se surplus or deficit related to the consumption of muscle of *S. lewini*.

For statistical tests, elemental concentrations that were not detectable were computed as half the limit of detection (López-Alonso et al., 2000). Average and standard deviation of total length of sharks and elemental concentrations in muscle were calculated. A length frequency histogram of the TL was elaborated and the cohorts were identified trough a Kernel Density Estimate (KDE) (Silverman, 1986). The univariate kernel density estimator used is given by the equation: Okocha and Adedeji, 2011; Pirrone et al., 2010 Where h is the bandwidth and $K(x)$ is the Gaussian kernel function. Comparisons of Cd, Hg and Se among sharks from the studies areas were made by a one-way ANOVA. Significant variations of elemental concentrations with length of specimens were defined by a Pearson correlation test.

3. Results and discussion

Total length of specimens and levels of Cd, Hg, and Se are presented in every studied area and for all the study (Table 1). Mean total length of collected specimens ranged from 73.7 to 216.0 cm. The age of the organisms was estimated according to Anislado-Tolentino et al. (2008) with the same species and study area using the corresponding equation for males [$TL = 364\ (1 - e^{-0.23\ (t+1.18)})$] and females [$TL = 376\ (1 - e^{-0.1\ (t+1.16)})$]. Based on the results of the KDE, three modes were estimated for the organisms analyzed in this study (Fig. 2):

First mode was $P(L) = \frac{1}{16.2\sqrt{2\pi}} e^{\frac{-(x-72.8)^2}{2*16.2^2}}$, which included the majority of individuals, belonging to the states of Nayarit and Sinaloa; these were juveniles and neonates (from ~0.7 to ~1.0 year old).

The second mode was $P(L) = \frac{1}{7.9\sqrt{2\pi}} e^{\frac{-(x-173.0)^2}{2*7.9^2}}$, which included preadults with a mean length of 173.0 (from 4.33 to 5.6 years old), they belonged to the states of Colima and BCS.

The third mode, which included the least number of individuals, was $P(L) = \frac{1}{3.6\sqrt{2\pi}} e^{\frac{-(x-239.1)^2}{2*3.6^2}}$. This mode included adult organisms (from 7.8 to 9.4 years old), from the states of Colima and BCS.

The mean concentration of the analyzed metals in the individuals from each mode increased according to the mean length (at higher mean length, higher mean metal concentration). The mean concentration of Hg increased significantly as the mean length increased from neonates to adults ($F_{(2,\ 113)} = 44.01$, $p < 0.001$), and these concentrations were all significantly different from each other according to the Tukey HSD test. The mean

Table 1
Length interval, mean total length, and mean elemental concentrations (μg g^{-1} dry weight) in muscle of scalloped hammerhead sharks *Sphyrna lewini* from the studied areas.

Area	N	Length Interval (cm)	Mean total length (cm)	Cd	Hg	Se
Sinaloa	64	45–126	73.7 ± 15.9	0.027 ± 0.033	1.10 ± 0.61	0.91 ± 0.56
BCS	10	174–297	216 ± 50.6	0.16 ± 0.10	3.06 ± 2.62	1.04 ± 0.9
Nayarit	22	53–132	77.2 ± 19.2	0.018 ± 0.019	1.32 ± 0.94	0.92 ± 0.77
Colima	20	151–276	187.9 ± 33.9	0.16 ± 0.133	2.52 ± 2.18	1.02 ± 0.44
All	116		106 ± 59	0.06 ± 0.09	1.56 ± 1.48	0.94 ± 0.62

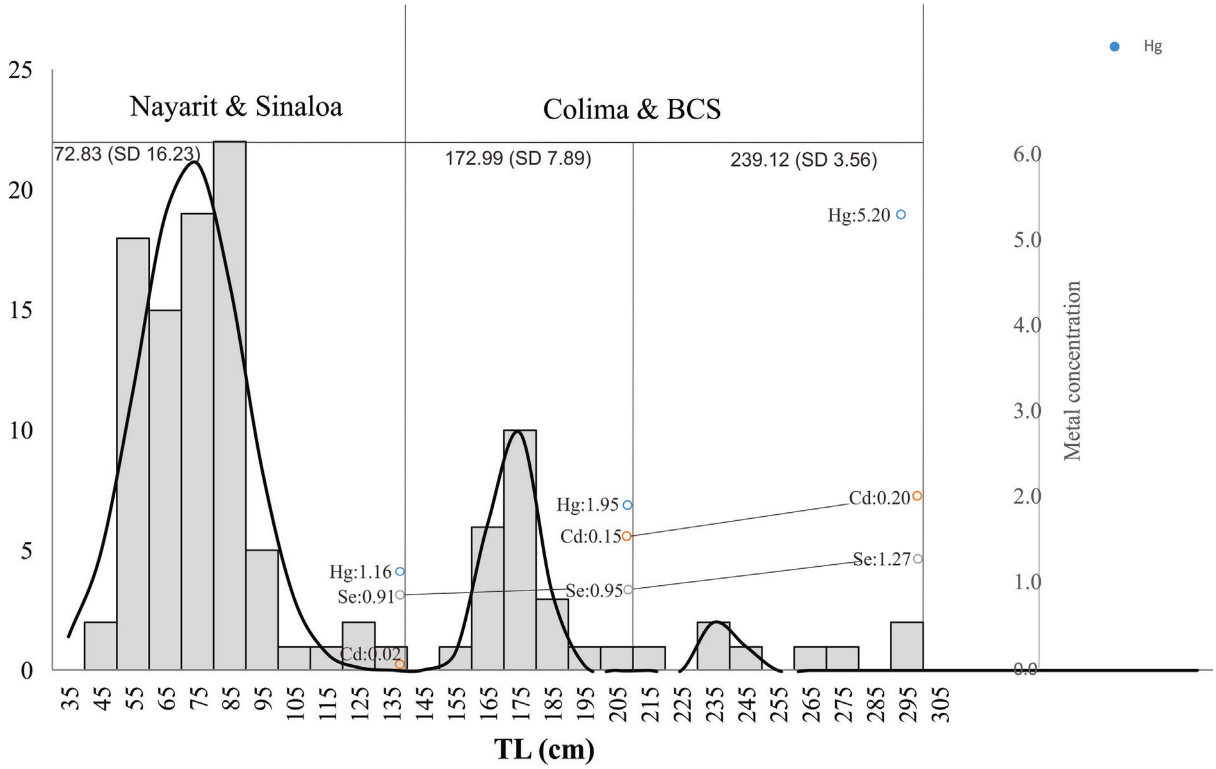

Fig. 2. Length frequency histogram and multimodal analysis of the organisms analyzed in the present study, showing the mean concentration (μg g^{-1} dry weight) of the analyzed elements in the muscle tissue for each mode. The names at the top indicate the states from where the individuals come. The numbers at the top indicate the mean length (cm) for every mode with its standard deviation (SD).

concentration of Cd also increased significantly as the mean length increased ($F_{(2,\ 113)} = 45.442$, $p < 0.001$), however, the Tukey HSD test indicated that these differences were only between the mean concentration of Cd found in the neonate and juvenile organisms, with preadults and adults, but the mean Cd concentration between preadults and adults differences were not found (elemental concentrations that were not significantly different are linked with a black line in Fig. 2).

Levels of Hg and Cd in muscle increased significantly ($p < 0.001$) with shark length (Fig. 3a and b); in the case of Se (Fig. 3c), muscle concentrations did not show a defined pattern. Essential elements like Se are necessary for diverse metabolic activities at certain levels so they are usually regulated by aquatic organisms; on the contrary, Cd and Hg are non-essential elements that are more difficult to regulate. In diverse shark species and other predators, positive correlations of Hg with length of individuals have been reported (Choy et al., 2009; Pethybridge et al., 2010; Bergés-Tiznado et al., 2019). Besides length of specimens, other factors may influence Hg concentrations; i.e. if diet and foraging habitat change with the size of specimens, metal levels may also increase (McKinney et al., 2016). With respect to Cd, in studies with sharks and other marine predators a significant increase of elemental concentrations in muscle (Kojadinovic et al., 2007; Damiano et al., 2011; Moreno-Sierra et al., 2016) and liver (Endo et al., 2017) with size of specimens have been found. In the case of Se, concentrations did not increase significantly ($p > 0.05$) with size of sharks; in a comparative study with marine biota, it was found that while Hg levels increases with the trophic levels, Se decreases (Karimi et al., 2013). Similar to our study, Se levels in smoothhound shark (*Mustelus mustelus*) from South Africa were weakly correlated with size of specimens (Bosch et al., 2016).

Concentrations of Cd, Hg, and Se in muscle of *S. lewini* from TEP were compared with values reported in sharks (Sphyrinidae) from TEP and other areas (Table 2). It is noticeable that Hg in muscle of *S. lewini* from this study exhibited similar concentrations to those previously reported (2011–2012) in the same species from the Gulf of California by Bergés-Tiznado et al. (2015). The highest concentration of Cd (range 0.34–2.86 μg g^{-1} wet weight) corresponded to *S. lewini* from the Gulf of Mexico and Caribbean. With respect to Hg and Se, the highest values (Hg 12.15 and Se 3.24 μg g^{-1} wet weight) were reported in muscle of the smooth hammerhead *Sphyrna zygaena* from the Mediterranean Sea. The compared elements varied in magnitude orders, Cd and Hg (two orders of magnitude), and Se (one order of magnitude). The above fluctuations are probably related to the capacity of fish to regulate elemental concentrations. Se is required for metabolic activities in sharks; *i.e.,* Se is an essential element that must occur within certain levels in fish. Contrastingly, Cd and Hg are not essential elements so they are not required for metabolism. In a global review dealing with the occurrence of organic and inorganic pollutants in sharks, Hg and Cd were considered to be in high levels (Kibria and Haron, 2015).

Legal concentrations of Cd in Mexico and internationally (Nauen, 1983) are 0.5 μg g^{-1} (wet weight), no specimens had values above the limit (Fig. 4a). With respect to Hg, mean concentration (0.55 μg g^{-1} ww) considering all the sharks was above the limits considered in the Mexican (0.5 μg g^{-1} ww; NOM-242, 1997) and Belgian (0.3 μg g^{-1} ww; Nauen, 1983) legislations (Fig. 4b) but below the value (1.0 μg g^{-1} ww) set in the European Union (CEC , 2006). Considering individual Hg concentrations, the Mexican and Belgian limits were exceeded in 38 and 66% of the samples, respectively. In the case of Se (Fig. 4c), all the values were below the threshold (2.0 μg g^{-1} ww) in fish and fish products established in New Zealand, but 45% of the samples were above the Chilean limit (0.30 μg g^{-1} ww) for any type of foodstuff (Nauen, 1983).

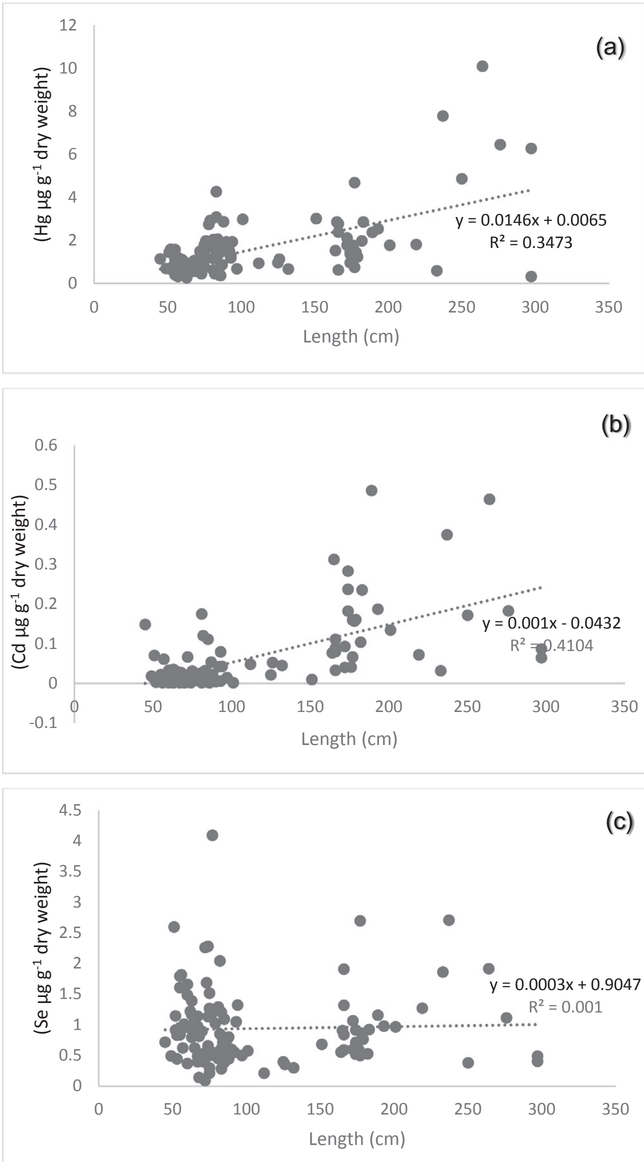

Fig. 3. Correlations of Hg (a), Cd (b) and Se (c) concentrations ($\mu g\ g^{-1}$ dry weight) in muscle with total length of *Sphyrna lewini* considering all the individuals.

The hazard quotients (HQ) for Cd and Hg, and the hazard indexes (HI) are presented in Table 3. In general, HQ values were higher for Hg than for Cd. The highest HQ figures for Hg (0.0135) and Cd (0.5298) were found in BCS; as a consequence, the most elevated HIs were also registered in BCS. Considering the rate of fish consumption in the studied states and in the whole country, HQ and HI were more elevated in the studied states than in the whole country. The above pattern is related to the higher rate of fishery products consumption in the coastal states in comparison to the average rate for the Mexican population (Zamora-Arellano et al., 2017); e.g. shark consumption in BCS (17.34 g per day per capita) is higher than the national average (0.88 g per day per capita) by a factor of 20. There are diverse factors that influence the patterns of fish consumption, among the most relevant are social, economic, and cultural issues (Meyer and Schwartz, 2000). In a comparison of diverse sectors of the population in the Great Lakes region, fishermen and anglers were the groups with more risk of pollutant exposure due to fish consumption (Lauber et al., 2017). In a study with the whitecheek shark *Carcharhinus dussumieri* from the Persian Gulf, related to the presence of Cd, Cu, Hg, Pb and Zn, no risk for human health associated to shark consumption was found (Adel et al., 2018). In another study with the bamboo shark *Chiloscyllium arabicum* from the Persian Gulf, the authors concluded that the risk for consumers was low for Cd, Cu, Ni, Pb and Zn but Hg was close to the risk threshold (Adel et al., 2018). For the Mexican coasts, more studies related to patterns of fish consumption are necessary to assess health risk more precisely.

Mass and molar concentrations of Hg and Se in muscle tissue of sharks and corresponding HBV_{Se} were variable depending on the study area (Table 4). Considering all the sharks, HBV_{Se} value (1.48) was positive; *i.e.*, their consumption provides benefits to consumers. Depending on the areas of shark collection, all values except those for fish from BCS (HBV_{Se} −1.68) might not be beneficial to consumers; *i.e.*, Hg toxicity may occurr and synthesis or activity of Se dependent enzymes be altered (Watanabe et al., 1999; Ralston et al., 2008). Sharks from BCS where the largest; accordingly, Hg levels were the most elevated and Se concentrations were comparable to those in sharks from the other areas. The above factors may have influenced the negative value of the HBV_{Se}. Nevertheless, it is relevant to consider the occurrence of additional Se from other diet sources (Watanabe et al., 1999) and the intraspecific variation of Se:Hg ratios (Burger et al., 2012). Variation of Se:Hg molar ratios versus total length of specimens is shown in Fig. 5, the relation was negative and significant ($p < 0.05$). Since Se:Hg ratios are variable within a species (Burger and Gochfeld, 2012), some individuals might be potentially toxic. In our study, the average Se:Hg molar ratio considering all the specimens was 1.53. It is considered that a Se:Hg ratio of one means that there is enough Se to protect from Hg toxicity (Kaneko and Ralston, 2007; Ralston, 2008). Taking into account the protective Se:Hg ratio, many sharks had ratios above one; however, it is noticeable that most of the biggest individuals (>100 cm) had Se:Hg molar ratios below the unit.

4. Conclusions

Total length of sharks was variable but the majority of the individuals were juveniles and neonates from Sinaloa and Nayarit states, followed by preadults and adults from Colima and BCS, respectively. Muscle concentrations of Hg and Cd increased

Table 2
Comparison of Cd, Hg, and Se concentrations ($\mu g\ g^{-1}$ wet weight) in muscle tissue of sharks (family Sphyrnidae) from diverse areas.

Species	Common name	Cd	Hg	Se	Site	Reference
Sphyrna lewini	Scalloped hammerhead	0.34–2.86	0.21–1.9	–	Gulf of Mexico and Caribbean	Mohammed and Mohammed (2017)
Sphyrna lewini	Scalloped hammerhead	0.01	0.39–0.44	–	New Guinea and Australia	Powell et al. (1981)
Sphyrna lewini	Scalloped hammerhead	–	0.63	1.2	SE Gulf of California	Bergés-Tiznado et al. (2015)
Sphyrna zygaena	Smooth hammerhead	0.03	12.15	3.24	Mediterranean	Storelli et al. (2003)
Sphyrna zygaena	Smooth hammerhead	–	0.16	0.34	TEP	Escobar-Sánchez et al. (2010)
Sphyrna lewini	Scalloped hammerhead	0.02	0.55	0.33	TEP	This study

TEP, Tropical eastern Pacific.

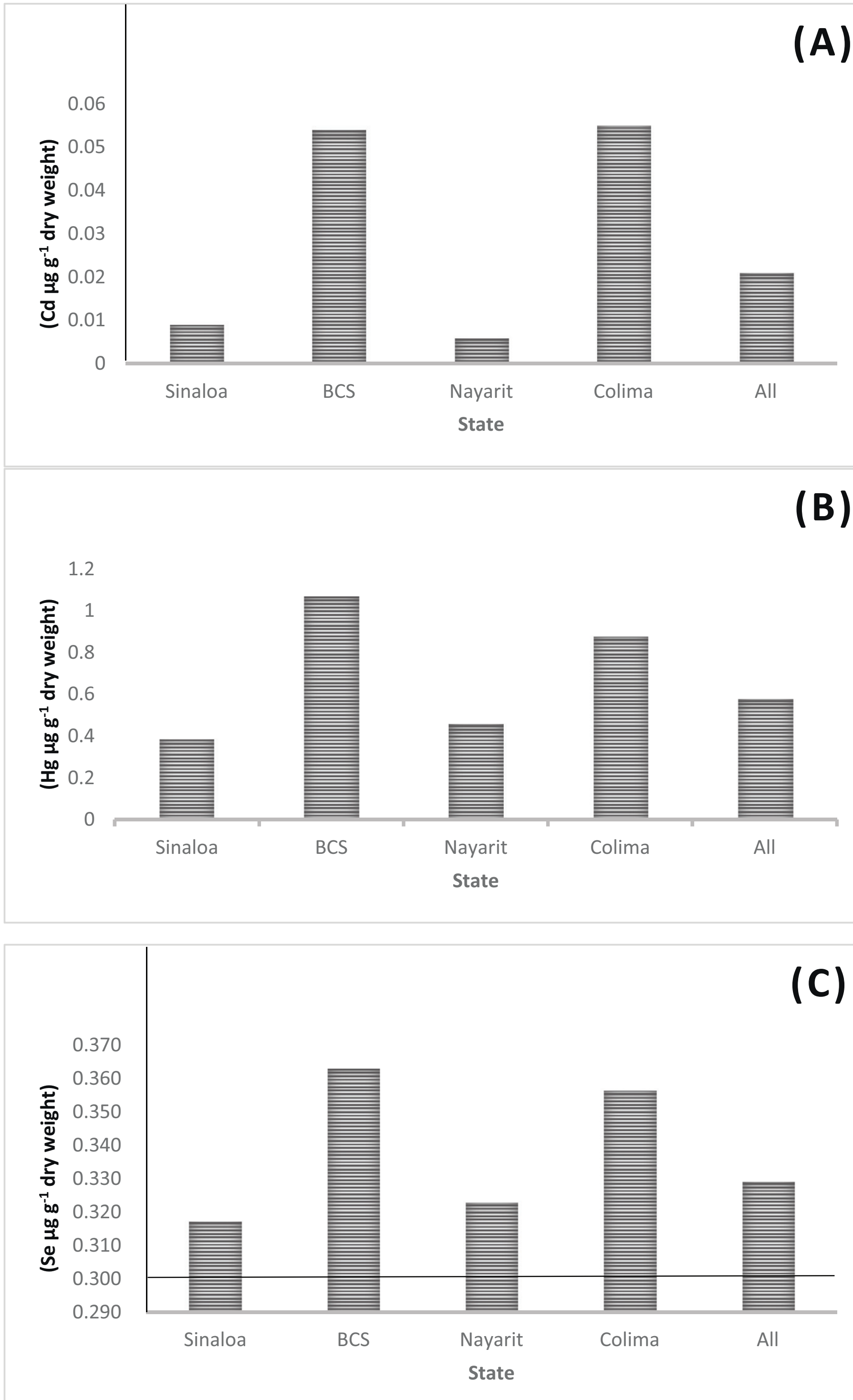

Fig. 4. Comparison of measured concentrations (µg g^{-1} wet weight) of Cd, Hg, and Se in the edible portion of sharks with maximum permissible limits.

Table 3
Average concentrations (µg g^{-1} ww) of Cd and Hg, and corresponding hazard quotients and hazard indexes in muscle of *S. lewini* from the mexican Pacific.

State	N	Cd	Hg	HQ_{CdMex}	$HQ_{CdState}$	HQ_{HgMex}	$HQ_{HgState}$	HI_{Mex}	HI_{State}
Sinaloa	64	0.009	0.39	0.00012	0.0009	0.0097	0.0721	0.0098	0.0730
BCS	10	0.054	1.07	0.00068	0.0135	0.0269	0.5298	0.0276	0.5433
Nayarit	22	0.006	0.46	0.00008	0.0012	0.0117	0.1811	0.0118	0.1823
Colima	20	0.055	0.88	0.00696	0.0017	0.0223	0.0554	0.0293	0.0571
All	116	0.021	0.55	0.00026	0.0022	0.0138	0.1293	0.0141	0.1315

Table 4
Mass (μg g^{-1} wet weight) and molar (μmol kg^{-1} wet weight) concentrations of Hg and Se, and HBV_{Se} in muscle of *S. lewini* from the mexican Pacific.

State	N	Mass concentration		Molar concentration		HBV_{Se}
		Hg	Se	Hg	Se	
Sinaloa	64	0.39	0.32	1.94	4.05	3.12
BCS	10	1.07	0.36	5.33	4.55	−1.68
Nayarit	22	0.46	0.32	2.29	4.05	2.76
Colima	20	0.88	0.36	4.38	4.56	0.34
All	116	0.70	0.34	3.48	4.30	1.48

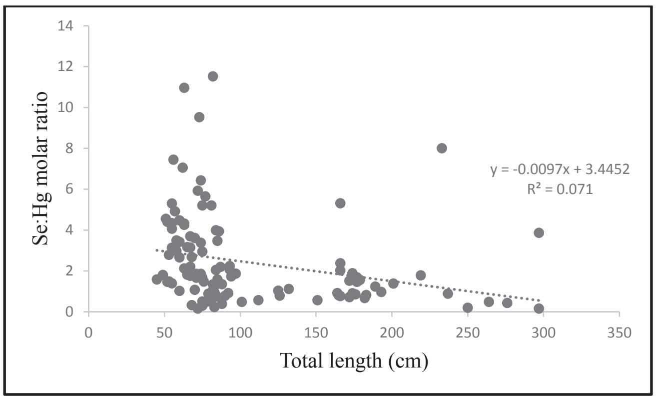

Fig. 5. Relation of Se:Hg molar ratios in muscle of *Sphyrna lewini* with total length (cm) of specimens.

significantly ($p < 0.001$) with shark length; with respect to Se, there was not a defined pattern. Concentrations of Hg in a previous report and the current study with *S. lewini* from the Gulf of California region were similar. Regarding the maximum permissible limits of Cd, Hg and Se in fish for human consumption, only Hg concentrations were above the legal limits set in the Mexican (38%) and Belgian (66%) legislations. Overall, HQ values were higher for Hg than for Cd but none of the figures were above the unit; similarly, HI were below one so there is no health risk. The estimated HBV_{Se} value considering all the individuals was positive, but taking into account the different areas of collection, sharks from BCS had a HBV_{Se} (−1.68) that might not be beneficial to consumers.

Author contributions

Ruelas-Inzunza: conceptualization, methodology, original draft preparation. Amezcua: funding acquisition, formal analysis, visualization. Coiraton: investigation, methodology. Páez-Osuna: resources, writing-review & editing.

Funding

This work was supported by the Ministry of Public Education (grant number 5164.19-P, 2019) of Mexico. We had additional support from UNAM-PAPIIT-IG201215, Alianza WWF FundaciónCarlos Slim and the Western Division of the American Fisheries Society. Pelagios Kakunjá, A.C. administered the funding.

Acknowledgements

To C. Suárez-Gutiérrez and G. Ramírez Rezendiz for the preparation of figures; to H. Bojórquez-Leyva, T. Sarabia-Frías and Z. Flores-Rodríguez for their help in the laboratory. We also acknowledge G. Alonso-Sosa from Pesca Siglo XXI and artisanalfishers for providing samples.

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