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Bioaccumulation of cd and hg in Muscle of Juvenile Pacific Sharpnose Shark *Rhizoprionodon longurio* from the SE Gulf of California

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Abstract
In this study, the concentrations of Cd and Hg were measured in muscle of juvenile individuals at an importan fishing ground in southeastern Gulf of California to assess the health risk to human consumers considering elemental levels and rate of shark consumption in NW Mexico. Twenty-eight individuals were sampled in September 2019. Quantification of Hg was made by cold vapor-atomic absorption spectrophotometry, analyses of Cd were made by graphite furnace atomic absorption spectrophotometry. In general, average Hg (1.27 μg g^{-1} dry weight) concentrations were higher than Cd (0.059). In comparison to results of Cd and Hg in muscle of several species of genus *Rhizoprionodon* sp., our reported concentrations were comparable. Maximum permissible limits (Cd 0.5 and Hg 0.5 μg g^{-1} wet weight) in fish products for human consumption were not exceeded. Health risk assessment to shark consumers indicated that Hg is of more concern than Cd but no hazards exist.

Keywords Non-essential elements · Sharks · Mexico · Health risk assessment

Essential elements (EE) are required within certain limits for metabolism of aquatic biota (Perelló et al. 2008). On the other hand, non-essential (NE) elements are not necessary for organisms and may be deleterious at low concentrations. Mercury (Hg) and cadmium (Cd) are NE elements that are supplied to the environment by natural processes and human activities. Among anthropogenic activities, production of non-ferrous metals and combustion of fossil fuels are the main contributors of Cd to the atmosphere (Pacyna and Pacyna 2001). With respect to Hg, it is emitted to the atmosphere through smelting, coal combustion, incineration, production of batteries and thermometers, and disposal of Hg-laden wastes from gold mining operations (Millward and Turner 2010). Fish of high position in food webs may accumulate elevated concentrations of pollutants (Ali and Khan 2018); sharks are top predators that may accumulate high levels of NE elements and they constitute relevant sources of Cd and Hg to human consumers (Okocha and Adedeji 2011; Fréry et al. 2001).

The Pacific sharpnose shark (*Rhizoprionodon longurio*) is a migratory species of important contributions to small-scale landings in the SE Gulf of California from November to May, and it is one of the main components of shark landings in the region (Furlong-Estrada et al. 2015). However, and despite its abundance and economic importance, information related to environmental parameters is missing. In coastal communities, shark muscle constitutes an important source of proteins but also a potential source of biomagnified Hg and Cd (Hurtado-Banda et al. 2012). There are a few studies in relation to the differences of Cd and Hg concentrations between females and males of sharks. Considering that studied sharpnose sharks were of similar size and weight, our hypothesis is that there are not differences of Cd and Hg in muscle between sexes. In this context, it is necessary to determine the levels of Cd and Hg in *R. longurio* from NW Mexico. In the present study, Cd and Hg were measured in muscle of *R. longurio* to determine the degree of bioaccumulation and to assess the health risk to human consumers according to levels of Cd and Hg and the rate of shark consumption in NW Mexico.

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Materials and Methods

Sharpnose sharks were collected off the estuarine system of Santa Maria La Reforma (Fig. 1) on September 2019 by the small-scale shark fishery operating in this system using surface and bottom gillnets and surface longlines. All sample collection was opportunistic and carried out in accordance with relevant national guidelines and regulations.

Twenty eight sharks (20 males, 8 females) were taxonomically identified (Fischer et al. 1995) and weight and total length (TL) were determined (Table 1). Samples of muscle were obtained from every specimen with stainless steel scalpels and transported to the laboratory in ice boxes. To avoid contamination of samples during manipulation and laboratory processing, glassware and plastic utensils were acid washed (Moody and Lindstrom 1997). Muscle samples were freeze-dried (− 49°C; 150×10^{-3} mBar; 72 h) and manually ground in an agate mortar. Homogenized powdered samples were digested with concentrated nitric acid in capped vials for 3 h at 120°C (MESL 1997). Analyses of Cd were made by graphite furnace atomic absorption spectrophotometry (GF-AAS) and Zeeman effect in a PerkinElmer (AAnalyst 800) equipment; Hg was measured by cold vapor-atomic absorption spectrophotometry (CV-AAS) in a Buck Scientific equipment.

Table 1 Sex, mean total weight (g), mean total length (cm) and standard deviation (minimum-maximum) of collected specimens of Pacific sharpnose shark from the SE Gulf of California

Sex	N	Weight	Total length
Females	8	641 ± 94.3 (486–760)	54.21 ± 1.76 (51.7–55.9)
Males	20	688.5 ± 119.37 (545–1008)	54.24 ± 2.60 (51-60.4)
All	28	674.96 ± 113.17 (486–1008)	54.23 ± 2.36 (51-60.4)

n, number of individuals

Quality control of metal analyses included blanks, duplicates, ultrapure water (milli-Q, 18.2 MΩ cm), trace metal grade acids and reference materials. Measured concentrations of Cd (0.27 μg g^{-1}) and Hg (0.34 μg g^{-1}) in reference material (dogfish muscle DORM-3) were in agreement with certified mean values of Cd (0.29 μg g^{-1}) and Hg (0.38 μg g^{-1}). The limits of detection (two times the standard deviation of a blank) were 0.003 μg g^{-1} for Cd and 0.02 μg g^{-1} for Hg. Concentration units of Cd and Hg are given as μg g^{-1} dry weight. Conversions of concentration units from dry weight to wet weight and viceversa were made considering humidity percentage in muscle (75%) as reported elsewhere (Gil-Manrique et al. 2017). Concentrations of Cd and Hg in muscle of sharks were compared with maximum permissible limits in fishery products set in national and international legislations. Health risk of shark consumers was estimated

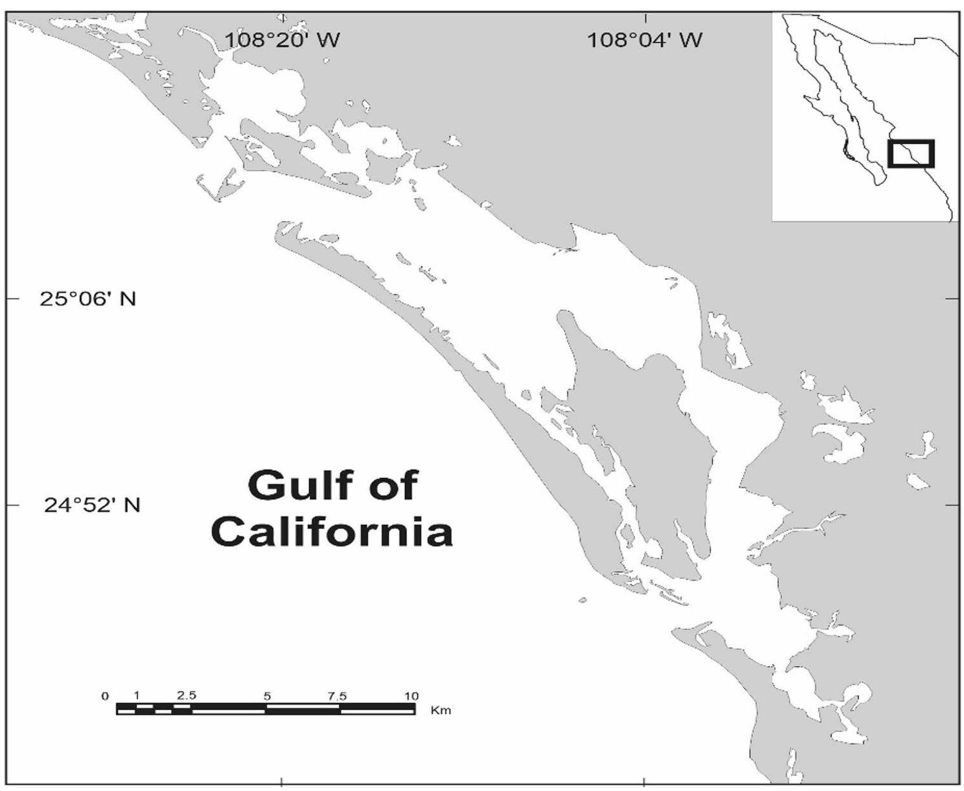

Fig. 1 Location of La Reforma lagoon (SE Gulf of California) where Pacific sharpnose sharks were collected

by the hazard index (HI) according to Newman and Unger (2002); the HI is the addtion of hazard quotients ($HI = HQ_{Cd} + HQ_{Hg}$) for the studied elements. For assessing the HQ we used the concentrations of Cd and Hg in the muscle of sharks, HQ = E/RfD, where E is the exposure to Cd and Hg through muscle shark consumption, and RfD is the reference dose (US EPA 2000) of the elements of interest (Cd, 0.5 µg kg^{-1} body weight day^{-1}; Hg, 0.3 µg kg^{-1} body weight day^{-1}). The exposure level (E) is calculated as E = C x I/W; where C is the concentration (µg g^{-1} wet weight basis) of Cd and Hg in the edible portion of shark, I is the average ingestion rate per capita of shark (6.54 g day^{-1}) in the region where sharks were captured (CONAPESCA 2017) and W is the weight of an average adult (70 kg). Levels of Cd and Hg were tested for homoscedasticity (Shapiro-Wilks test) and normality (Kolmogorov-Smirnov test); since data were not normally distributed, non-parametric tests were used to define significant differences. Comparison of elemental concentrations in muscle of males and females of *R. longurio* were made by U Mann-Whitney test. Similarly, comparisons between Cd and Hg concentrations in muscle of all individuals were made by a U Mann-Whitney test. Satistical analysis were performed in a specialized software (IBM SPSS Statistics 25) with a confidence level of 95%

Results and Discussion

Mean TL and weight of sharks indicate that they were juveniles (Table 1). The reported TL at birth of *R. longurio* from the Mexican Pacific ranges from 30 to 37 cm (Márquez-Farías et al. 2005) and this species reaches maturity below 100 cm (females 83 cm, males 93 cm) of TL (Alatorre-Ramírez et al. 2013). Overall, Hg concentrations were significantly ($p < 0.05$) higher than Cd (Table 2); this is in agreement with a global review (Amezcua et al. 2022) of Cd and Hg in sharks where Hg in muscle (1.507 µg g^{-1} wet weight) was higher than Cd (0.153 µg g^{-1} wet weight). Considering the sex of specimens, no significant ($p > 0.05$) differences of Hg and Cd concentrations were found between males and females. The elevated variability of Hg and Cd concentrations may be related to differences in the feeding items (Fisk et al. 2002) but also to physiological factors that turn into varying accumulation rates and the degree of impact of the zones where sharks inhabit (Frías-Espericueta et al. 2019).

Table 2 Mean concentrations and standard deviations (minimum-maximum) of Hg and Cd (µg g^{-1} dry weight) in muscle of the Pacific sharpnose shark from the SE Gulf of California

Sex	n	Hg	Cd
Females	8	1.24 ± 0.44 (0.21–1.67)	0.057 ± 0.040 (0.03–0.12)
Males	20	1.28 ± 0.82 (0.21–2.17)	0.060 ± 0.058 (0.02–0.21)
All	28	1.27 ± 0.72 (0.21–2.17)*	0.059 ± 0.053 (0.02–0.21)*

* Significantly different

Published information related to the occurrence of Cd and Hg in muscle of Pacific sharpnose shark and related species (genus *Rhizoprionodon*) is scarce. On a global basis, twenty studies have reported Cd and Hg concentrations in six *Rhizoprionodon* species (Table 3).

Most of the studies correspond to *R. acutus*. Overall, Cd concentrations were lower than Hg; for both elements, concentrations varied by two magnitude orders. The highest concentration of Cd (0.35 µg g^{-1}) was reported by Núñez-Nogueira (2005) in *R. terraenovae* from the Gulf of Mexico; in the case of Hg, the highest value (5.0 µg g^{-1}) corresponded to *R. lalandii* from a site in Rio de Janeiro (Amorim-Lopes et al. 2020) that is polluted by domestic and industrial untreated sewage and other anthropogenic sources (Fistarol et al. 2015).

In comparison with maximum permissible limits (Cd 0.5 µg g^{-1} wet weight; Hg, as methyl mercury, 0.5 µg g^{-1} wet weight) in fish for human consumption (NOM 2009), measured concentration of both elements (Cd 0.015 µg g^{-1} wet weight; Hg 0.353 µg g^{-1} wet weight) were within legal thresholds. Health risk assessment for shark consumers was made considering the individual (HQ) and combined (HI) occurrence of Cd and Hg in muscle (Table 4).

Values of HQ_{Hg} were more elevated than HQ_{Cd}, as indicative that the presence of Hg is of more concern to consumers than Cd in muscle of *R. longurio*. None of the HI values were above the unit; i.e. the consumption of muscle of *R. longurio* from La Reforma lagoon does not pose any hazard different to cancer to consumers during their life expectancy in terms of the presence of Hg and Cd. Though no HI value was above the unit, it is important to highlight that other EE and NE elements may contribute significantly to increase the HI. For example, HQ values of Hg related to consumption of *Sphyrna lewini* and HQ values of Cd associated to consumption of *Carcharhinus porosus* from Trinidad and Tobago were above one (Mohammed and Mohammed 2017). As concluding remarks we may say that considering all shark specimens, Hg concentrations were higher than Cd. Although both elemental concentrations were higher in males than in females, differences were not significant. Levels of Cd and Hg in muscle of *R. longurio* of this study were within the intervals reported for both elements in sharks of the same genus from all over the world. Health risk assessment to consumers indicate that at the rate of shark consumption and levels of Cd and Hg in muscle of *R. longurio* no health problems may occur.

Table 3 Concentrations (µg g^{-1} wet weight) of Cd and Hg in muscle of Pacific sharpnose shark from the SE Gulf of California and comparison with other sharks of the same genus (*Rhizoprionodon*: Carcharhinidae)

Species	Cd	Hg	Site	Reference
R. acutus	0.055	0.073	Gulf of Aden	Boldrocchi et al. (2019)
R. acutus	0.01	0.09	New Guinea	Powell et al. (1981)
R. acutus	-	1.51[a]	East Southafrica	McKinney et al. (2016)
R. acutus	0.002	0.184	Malaysia	Ong and Gan (2017)
R. acutus	-	0.397[a]	Gulf of Oman	Al-Reasi et al. (2007)
R. acutus	-	1.01	Northern Australia	Lyle (1986)
R. acutus	0.056	1.15	Persian Gulf	Adel et al. (2017)
R. lalandii	-	0.199	Rio de Janeiro	Viana et al. (2005)
R. lalandii	-	5.0	Rio de Janeiro	Amorim-Lopes et al. (2020)
R. longurio	-	0.35	SE Gulf of California	Frías-Espericueta et al. (2019)
R. longurio	-	0.92	SE Gulf of California	Hurtado-Banda et al. (2012)
R. longurio	-	4.3	SE Gulf of California	García-Hernández et al. (2007)
R. longurio	0.008	-	SE Gulf of California	Frías-Espericueta et al. (2014)
R. longurio	0.015	0.32	SE Gulf of California	This study
R. porosus	-	0.0106	Rio de Janeiro	Lacerda et al. (2000)
R. porosus	-	0.18	Rio de Janeiro	Amorim-Lopes et al. (2020)
R. taylori	-	0.56	Northern Australia	Lyle (1986)
R. taylori	-	0.666	Queensland Australia	Denton and Breck (1991)
R. terraenovae	0.35	0.76	Gulf of Mexico	Nuñez-Nogueira (2005)
R. terraenovae	0.01	-	SE United States	Sommerville et al. (2020)
R. terraenovae	-	1.42	Atlantic ocean	Hammerschlag et al. (2016)
R. terraenovae	-	1.99	SW Florida	Rumbold et al. (2014)
R. terraenovae	-	1.06	East-central Florida	Adams and McMichael (1994)

[a]median

Table 4 Hazard quotients (HQ) of Cd and Hg and correspondent hazard index (HI) associated to consumption of mucle of *R. longurio* from La Reforma lagoon (SE Gulf of California)

Sex	HQ_{Cd}	HQ_{Hg}	HI
Females	0.0026	0.0961	0.0987
Males	0.0028	0.0992	0.1020
All	0.0027	0.0984	0.1011

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