

# Mercury levels in myliobatid stingrays (Batoidea) from the Gulf of California: tissue distribution and health risk assessment

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**Abstract** With the aim of knowing Hg distribution in selected tissues of myliobatid stingrays and assessing health risk to Mexican population, Hg concentration was determined in the muscle and liver of four ray species. Total Hg levels were determined by cold vapor atomic absorption spectrophotometry. With respect to the muscle, devil rays (*Mobula* spp.) showed lower Hg levels ( $<0.22 \mu\text{g g}^{-1}$ ) than *Rhinoptera steindachneri* ( $0.37 \pm 0.25 \mu\text{g g}^{-1}$  wet weight). In the case of the liver, the highest Hg concentration was found in *Mobula japonica* ( $0.22 \pm 0.01 \mu\text{g g}^{-1}$ ). Hg levels in the muscle and liver varied according to the species; in some case, the liver accumulated more Hg than the muscle and the opposite pattern in other cases. *R. steindachneri* showed a significant difference between both tissues. No significant differences of Hg levels between males and females and between juveniles and adult specimens of *R. steindachneri* were found. Positive correlation between Hg concentrations and disc width and total weight was not significant for *R. steindachneri* ( $R_s < 0.36$ ,  $p > 0.05$ ). Batoids showed Hg values below the Mexican (NOM-027-SSA1-1993) limits ( $1.0 \mu\text{g g}^{-1}$ ) in fishes for human consumption. The species with the highest potential of Hg transfer to human

population is *R. steindachneri*; however, an adult (70 kg) could consume approximately 943 g per week without representing a health risk. Nevertheless, further and continuous monitoring is needed since batoids support an important fishery in Mexican waters, being a food resource and income to coastal communities.

**Keywords** Bioaccumulation · Heavy metal · Batoids · Pacific Ocean · Mexico

## Introduction

Batoids (rays, skates, stingrays) occur in all the oceans; they are commonly found in shallow waters of estuarine, coastal, and shelf regions and in depths up to 3,000 m (McEachran and Aschliman 2004; Frisk 2010). Among the wide range of habitats of batoids, coastal areas are accessible for fishing communities.

In Mexico, ray fisheries support around 30 % of the total national production, being the families Rhinobatidae, Dasyatidae, Gymnuridae, and Myliobatidae (DOF 2010), the batoids most frequently captured for commercial purposes (Bizarro et al. 2007). In Sonora State (NW Mexico), rays represent 63.4 % of the total artisanal catch of chondrichthyans (Bizarro et al. 2009).

Myliobatid stingrays are among the most frequently captured fish; their meat is marketed and consumed fresh, frozen, dried, and salted. It is commercialized in the domestic market and sometimes they are exported. Similar to sharks, batoids are currently protected by Official Mexican Norm 029 (NOM-029-2006) from May to July; however, they are captured by artisanal

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fishermen (intentionally or as bycatch) during this protected season. Besides their vulnerability to over-exploitation, batoids are susceptible to bioaccumulate heavy metals (because of their slow growth and long life spans), so batoid fish can accumulate some toxic elements like Hg for longer periods than short-lived species, such as bony fishes (Cai et al. 2007).

Human health effects may appear depending on the rate of fish consumption and the corresponding Hg concentrations. Similarly, fish that eat other species with certain levels of Hg might suffer some damage. The detailed information of Hg in batoid species is essential for assessing human health risk and product quality. Toxicological information on commercially exploited ray species in Mexican waters is limited (García-Hernández et al. 2007; Gutiérrez-Mejía et al. 2009). The studies have focused on sharks, but if we consider the commercial importance of batoids and the economic resources that they represent, this group needs to be monitored continuously. This study aims to provide information of Hg distribution in the muscle and liver of batoids frequently caught in selected areas of the Gulf of California and to assess human health risk considering Hg concentration in the edible portion (muscle) and rate of stingray consumption in the Mexican population.

## Material and methods

### Sample collection

Specimens were captured by local fisherman using gill nets. Samples were collected from May to November 2012 in selected sites located in the Eastern Gulf of California: Dautillos, Sinaloa (24°43'N, 107°58'W) and Santa Bárbara, Sonora (26°70'N, 109°65'W). During the field work, each fish was taxonomically identified; sex was determined and disc width (DW) and total weight (TW) were measured. Specimens were dissected to extract the liver and a portion of the muscle from the anterior–dorsal region. Samples were transported (with ice) to the laboratory and stored in a freezer at a low temperature (−20 °C).

### Laboratory analysis

All the glassware and plastic utensils were washed according to Moody and Lindstrom (1977). Samples were freeze-dried for 72 h (−49 °C and  $133 \times 10^{-3}$  mbar)

in a Labconco Freeze Dry System-FreeZone 6 and then ground in an agate mortar with pestle (Fischer Scientific). All samples were weighed before and after lyophilization process using an analytical balance OHAUS ( $\pm 0.001$  g) in order to estimate the humidity percentage. Total Hg (THg) concentrations (in  $\mu\text{g g}^{-1}$ ) were reported on a wet weight basis.

Grounded and homogenized aliquots (0.2–0.3 g) were digested in 5 mL of concentrated nitric acid ( $\text{HNO}_3$ , JT Baker for trace metal analysis) using capped teflon vials (Savillex™) on a hot plate (Barnstead Thermolyne) during 3 h (120 °C). Later, digested samples were made up to 23 mL with Milli-Q water and stored in polyethylene containers.

Readings of THg were done by cold vapor atomic absorption spectrophotometry (CV-AAS) in a Hg analyzer (Buck Scientific, model 410 A). Quality control was assessed by analyzing duplicate samples, blanks, and certified reference materials (DORM-3, fish protein; DOLT-4, dogfish liver) with every batch of samples. The limit of detection (LD) was  $0.011 \mu\text{g g}^{-1}$ ; the recovery percentage was 115.4 % ( $0.440 \pm 0.003 \mu\text{g g}^{-1}$ ) for DORM-3 (certified value  $0.382 \pm 0.06 \mu\text{g g}^{-1}$ ) and 104 % ( $2.685 \pm 0.002 \mu\text{g g}^{-1}$ ) for DOLT-4 (certified value  $2.58 \pm 0.22 \mu\text{g g}^{-1}$ ). THg values did not adjust to a normal distribution (Kolmogorov–Smirnov test); differences in THg levels by sex or size were determined by a Kruskal–Wallis (K–W) test. The relationship between Hg concentration and DW or TW was defined by a Spearman correlation (Rs) analysis.

### Toxicological assessment

Health risk assessment from batoid consumption was estimated by calculating the maximum possible consumption of fish per week (MPCF; gram of edible portion of rays per kilogram of body weight) using the following formula:

$$\text{MPCF} = \text{PTWI}/\text{THg}_j,$$

where PTWI is the provisional tolerable weekly intake established by World Health Organization (WHO),  $\text{THg}_j$  is the average of THg concentration in batoid “j”. For THg, the PTWI value is  $5.0 \mu\text{g kg}^{-1}$  body weight per week. For pregnant or lactating women, this value is restricted to  $2.45 \mu\text{g}^{-1} \text{kg}^{-1}$  body weight per week (Ordiano-Flores 2009). For adults, women, and

children, the average weights of 70, 60, and 16 kg, respectively, were considered in this analysis.

**Results**

Myliobatids obtained from the eastern coast of the Gulf of California were *Rhinoptera steindachneri* (golden cow-nose ray), *Mobula japanica* (spinetail mobula), *Mobula thurstoni* (smoothtail mobula), and *Mobula munkiana* (Munk's devil ray). *Mobula* species are commonly known as devil rays or “cubanas” in Mexican waters. Hereon, they will be mentioned as devil rays. Sampling sites as well as biological information of collected specimens are given in Table 1. Muscle and liver samples of *M. japanica* were represented by minimal number of individuals ( $n=1$  for Dautillos, Sinaloa and  $n=2$  for Santa Barbara, Sonora).

THg distribution in the muscle and liver of analyzed species is presented in Table 2. THg levels of *R. steindachneri* ranged from 0.04 to 0.79  $\mu\text{g g}^{-1}$  ( $0.37 \pm 0.25 \mu\text{g g}^{-1}$ ) for the muscle, while liver values were between 0.03 and 0.40  $\mu\text{g g}^{-1}$  ( $0.17 \pm 0.10 \mu\text{g g}^{-1}$ ). Devil rays (*Mobula* spp.) showed lower Hg levels than *R. steindachneri* (Table 2).

In the golden cow-nose ray, THg concentrations in the muscle and liver were significantly different (K–W:  $H=7.36, p=0.01$ ). Different patterns of Hg distribution were observed in the analyzed tissues. In Sinaloa, *R. steindachneri* and *M. thurstoni* showed higher THg concentrations in the muscle than in the liver, while in Sonora, *M. japanica* and *M. munkiana* had higher THg levels in the hepatic tissue (Table 2).

With respect to Hg variation by sex of specimens, no significant differences between the tissues of males and

females of *R. steindachneri* were found. Females of *R. steindachneri* had a muscle THg concentration of 0.43  $\mu\text{g g}^{-1}$ , while males accumulated 0.30  $\mu\text{g g}^{-1}$  of THg (K–W:  $H=5.493, p=0.14$ ). In the case of liver, similar values were observed for both sexes (females= 0.17  $\mu\text{g g}^{-1}$ , males=0.15  $\mu\text{g g}^{-1}$ , K–W:  $H=3.846, p=0.15$ ) (Fig. 1). The same THg value was found in the muscle from juveniles (0.39  $\mu\text{g g}^{-1}, n=12$ ) and adults (0.39  $\mu\text{g g}^{-1}, n=11$ ) of *R. steindachneri*, but the Hg accumulation in pre-adult specimen was comparatively lower (0.15  $\mu\text{g g}^{-1}, n=1$ ). No sufficient samples were obtained for the devil rays to categorize them by sex and size; however, some categories are shown in Fig. 2. The lowest THg values were observed in *M. thurstoni* from Sonora State. Adult specimens were only recorded in *M. munkiana*, and similar THg values were observed in juveniles (Fig. 2).

The correlation of THg in the muscle and liver with DW was positive but not significant ( $R_{\text{muscle}}=0.20, p=0.24$ ;  $R_{\text{liver}}=0.06, p=0.76$ ). With respect to TW, correlations were not significant ( $R_{\text{muscle}}=0.36, p=0.98$ ;  $R_{\text{liver}}=0.10, p=0.83$ ).

**Toxicological assessment**

All rays showed THg values below the international (Food and Drug Administration) and Mexican (NOM-027-SSA1-1993) limits in fishes (1.0  $\mu\text{g g}^{-1}$ ) for human consumption (Table 2). The average level of THg in the muscle of *R. steindachneri* (0.371  $\mu\text{g g}^{-1}$ ) was above the limit (0.3  $\mu\text{g g}^{-1}$ ) established by the Environmental Protection Agency (EPA).

According to the highest THg level in the edible portion, the species with a major potential of Hg transfer to human population is *R. steindachneri*; however, an

**Table 1** Sites of collection (Eastern Gulf of California) and biological information of myliobatids of interest (disc width is expressed in centimeters; total weight in kilograms)

Species	Common name	Locality	Sample size	Disc width (min–max)	Total weight (min–max)	Habit	Main food
<i>M. japanica</i>	Spinetail mobula	Dautillos, Sinaloa	1	134.8	22	Pelagic	Plankton
		Santa Bárbara, Sonora	2	135.7–157	18.7–32	Pelagic	Plankton
<i>M. munkiana</i>	Munk's devil ray	Santa Bárbara, Sonora	5	48.3–99	1.43–14	Pelagic	Plankton
<i>M. thurstoni</i>	Smoothtail mobula	Dautillos, Sinaloa	5	104–144.3	7–29	Pelagic	Plankton
		Santa Bárbara, Sonora	10	97.3–107.3	9.11–13.8	Pelagic	Plankton
<i>R. steindachneri</i>	Golden cow-nose ray	Dautillos, Sinaloa	25	41.9–84.6	1.3–9.86	Benthopelagic	Mollusks, Crustaceans

**Table 2** Details of THg levels ( $\mu\text{g g}^{-1}$  wet weight) in hepatic and muscle tissues of myliobatid rays from the Eastern Gulf of California, Mexico

Area	Species	Tissue/organ	Sample size	THg range	Mean $\pm$ S.D.	
Sinaloa	<i>R. steindachneri</i>	Muscle	25	0.04–0.79	0.37 $\pm$ 0.25 <sup>a</sup>	
		Liver	23	0.03–0.40	0.17 $\pm$ 0.10 <sup>a</sup>	
	<i>M. japonica</i>	Muscle	1	<LD		
		Liver	1	<LD		
	<i>M. thurstoni</i>	Muscle	4	0.03–0.18	0.09 $\pm$ 0.07	
		Liver	2	0.07	0.07 $\pm$ 0.01	
	Sonora	<i>M. japonica</i>	Muscle	2	0.13–0.15	0.14 $\pm$ 0.01
			Liver	2	0.21–0.22	0.22 $\pm$ 0.01
<i>M. thurstoni</i>		Muscle	4	0.16–0.23	0.20 $\pm$ 0.04	
		Liver	7	<LD–0.21	0.11 $\pm$ 0.09	
<i>M. munkiana</i>		Muscle	4	0.16–0.22	0.19 $\pm$ 0.03	
		Liver	5	0.08–0.30	0.20 $\pm$ 0.10	

LD limit of detection  
(0.011  $\mu\text{g g}^{-1}$ )

<sup>a</sup>Significantly different

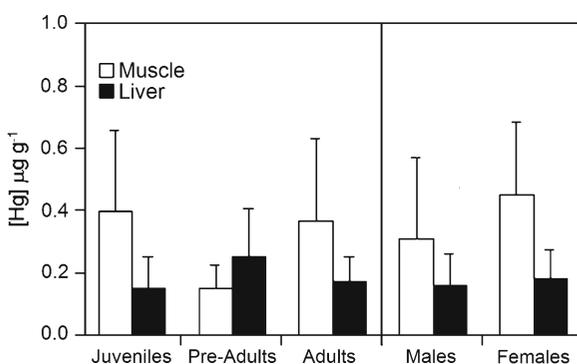
adult (70 kg) could consume approximately 1 kg (943 g week<sup>-1</sup>) without representing a human risk (Table 3). A higher and continuous consumption could bring negative effects.

## Discussion

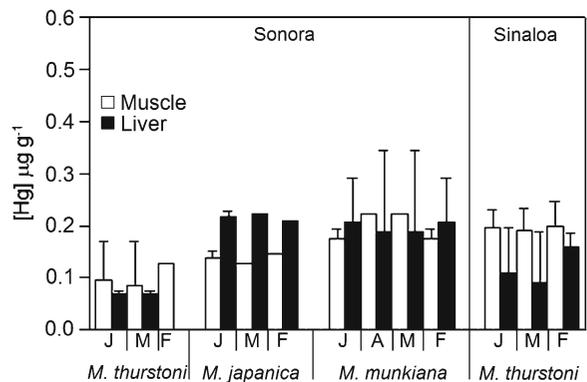
High trophic level species typically have elevated THg levels due to biomagnification in food webs. This has been observed mainly in sharks (Adams and McMichael 1999; Cai et al. 2007). However, in relative species like batoids, THg values are less than 0.79  $\mu\text{g g}^{-1}$  w.w (Joiris et al. 1997; Adams et al. 2003; García-Hernández et al. 2007; Gutiérrez-Mejía et al. 2009).

The golden cow-nose ray had the highest concentration (0.37  $\mu\text{g g}^{-1}$ ) of THg, while in devil rays, the value

was below 0.22  $\mu\text{g g}^{-1}$ . Slight variations in THg levels could be enhanced by differences in feeding habits (Monteiro et al. 1996). Devil rays (*Mobula* spp.) are filter feeders that feed on euphausiid crustacean such as *Nyctiphanes simplex* (Notarbartolo-di-Sciara 1988), while *R. steindachneri* feeds on mollusks, crustaceans, crabs, and others small invertebrates crushing their shells with their flattened teeth. Certain invertebrates may contain lower Hg levels than those found in many fish species (Adams et al. 2003); therefore, invertebrates could not transfer significant concentrations of THg to predators. Mollusk and crabs can have higher THg levels than planktonic organisms, due to sediment influence (Adams et al. 2003). Therefore, the THg levels in devil rays (planktonic feeders) are lower than in the golden cow-nose ray.



**Fig. 1** Total Hg levels ( $\mu\text{g g}^{-1}$  wet weight) by categories (sex and maturity state) in the muscle and liver of golden cow-nose ray, *R. steindachneri*, from Sinaloa State, Mexico. Error bars represent the standard deviation



**Fig. 2** Levels of Hg ( $\mu\text{g g}^{-1}$  wet weight) in the muscle and liver of devil rays caught in Sonora and Sinaloa, Mexico. J juveniles, A adults, M males, F females. Error bars represent the standard deviation

**Table 3** Maximum possible consumption of batoid meat per week (MPCF; g) in the northwestern Mexico

State	Locality	Species	Average (Hg)	MPCF (g)		
				Man (70 kg)	Woman (60 kg)	Child (16 kg)
Sinaloa	Dautillos	<i>M. thurstoni</i>	0.09	3,889	1,633	436
		<i>M. japonica</i>	<LD	–	–	–
		<i>R. steindachneri</i>	0.371	943	396	106
Sonora	Santa Bárbara	<i>M. japonica</i>	0.137	2,555	1,073	286
		<i>M. thurstoni</i>	0.195	1,795	754	201
		<i>M. munkiana</i>	0.187	1,872	786	210

LD limit of detection

In general, coastal areas are considered as contaminated by anthropogenic influences; therefore, coastal species are susceptible to this pollution (Moreno et al. 1984). In spite of *R. steindachneri* feeding close to sediments near the coast, which makes it vulnerable to effluents with high concentrations of trace elements, it was not reflected in high Hg concentrations in the muscle and liver. Likewise, the devil rays are influenced by coastal areas, due to their migration from oceanic to coastal waters. Although, Sinaloa and Sonora areas have intensive agricultural activity, aquaculture, fisheries, and residential and industrial discharges that affect the marine ecosystem (Gutiérrez-Galindo et al. 1988; Gutiérrez-Mejía et al. 2009) with contaminants like THg, the analyzed species did not reflect this influence. However, high THg levels have been observed in other fish from these areas (Ruelas-Inzunza and Páez-Osuna 2005; García-Hernández et al. 2007).

Distribution of THg in the muscle and liver

It is known that concentrations of THg in different tissues can provide insights on the ways of assimilation and detoxification of this element in the organisms. However, contrasting information has been found with respect to the organs or tissues where THg is mostly accumulated. Although it is generally accepted that the liver is the main organ for THg accumulation (Gutiérrez-Mejía et al. 2009), after exposure to this metal, levels can increase in the muscle, and later, it is stored in the liver or eliminated by the kidney (Harrison and Klaverkamp 1990; Goldstein et al. 1996).

Similar to our study, Núñez-Nogueira et al. (1998) and Gutiérrez-Mejía et al. (2009) found the highest THg level in the muscle than in the liver of sharks

(*Rhizoprionodon terranovae*, *Carcharhinus limbatus*) and rays (*R. steindachneri*), but other workers reported a more elevated concentration of THg in the liver of fish species (Nam et al. 2011). Moreover, aside from the fact that Hg concentrations are not the same in all the tissues and organs, there are differences within the same species. In the tiger shark (*Galeocerdo cuvier*), muscle Hg levels were higher than liver concentrations in immature sharks, but the inverse trend was observed in mature individuals (Endo et al. 2008).

Correlation of THg with disc width and sex of individuals

In many studies, THg concentration has been positively correlated with size (TL, DW) or weight of specimens (Hueter et al. 1995; Cai et al. 2007; Endo et al. 2008). This trend was not found in this study, i.e., correlations were positive but not significant. A major sample size would provide a more consistent and stronger trend for a relationship of THg concentration with batoid sizes.

Some hypotheses have been proposed for explaining the relationship of size with THg levels in elasmobranchs. Gutiérrez-Mejía et al. (2009) suggested that adults have lower metabolic rates than juveniles, and it may take longer to “metabolize” metals, resulting in higher concentration in older individuals. Others authors (Núñez-Nogueira et al. 1998) mention that older elasmobranchs have more efficient mechanisms for eliminating THg (detoxification) than juvenile individuals. In our study, no differences of THg levels were observed between adults and juveniles in *R. steindachneri* and *M. munkiana*. Considering that females transfer certain quantity (8–60 %) of THg to embryos during the gestation (Adams and McMichael 1999), males did not

bioaccumulate higher levels of THg. Thus, juveniles could be accumulating THg from early stages (since gestation). Moreover, the small differences between age categories can suggest that the organisms (categorized on the basis of size and sex) are in the same area feeding on the same prey items.

### Human health risk

Following the Minamata disaster which occurred in Japan during the 1950s, there was a greater concern of the risk of Hg poisoning from eating contaminated marine products. Thus, the monitoring and assessment of commercial species has become more important for the public health. Myliobatids are caught by small-scale regional fisheries along the Mexican Pacific Ocean, and their meat is commercialized fresh, dried, and salted. However, devil rays (genera *Mobula*) cannot be sold for commercial uses, because of legal restrictions and their status of endangered species in the IUCN Red List. Nevertheless, the meat of devil rays is still offered for consumption.

The potential risk to humans from consumption of edible muscle of myliobatids is low. The THg levels in devil rays would allow a consumption of over 1.0 kg per week of meat by a man of 70 kg of weight, while for women and children, this quantity is reduced (<500 g). However, more details are necessary on the consumption patterns of batoid fishes by human population (frequency, quantity, etc.), mainly in fishing communities where the consumption of this food resource is more frequent.

Fish is the main source of dietary exposure to Hg. A potential health risk for consumers has been found mainly in elasmobranchs from the northwestern coast of Mexico. The scalloped hammerhead shark, *Sphyrna lewini* showed the highest THg levels and therefore the highest hazard quotient (1.04) (Ruelas-Inzunza et al. 2011) among the compared fish species. High quotients have been related to the trophic level of fish. Thus, carnivorous fishes (mainly ichthyophagous feeders) transfer THg to humans in a higher extent than omnivorous and herbivorous species (De Pinho et al. 2002; Ruelas-Inzunza et al. 2011). The differences in feeding habits of analyzed rays constitute an indication of the risk for consumption of certain species. The majority of batoids (including myliobatids) feed on invertebrates, although other batoid species prey on bony fishes and could have higher levels of Hg than invertebrate consumers.

Moreover, if the general population has a low consumption of fish, the health risk would be minimum. In this context, it has been estimated that fish is consumed at a rate of 25 g person<sup>-1</sup> day<sup>-1</sup> in the Mexican territory (Ruelas-Inzunza et al. 2011). Therefore, the risk of exposure to high concentrations of Hg through batoid consumption is not high. Nevertheless, susceptible sectors of the population (children, pregnant women) must be monitored, since organic Hg is able to cross the placenta and may consequently concentrate in the fat tissue and the brain of the embryo and fetus (Maycock and Benford 2007).

### Conclusion

Batoids have played an important role in coastal and oceanic ecosystems; they also support an important fishery in Mexican waters, being a food resource and income to coastal communities. However, a poor monitoring of heavy metals in batoids has been done. In spite of the small sample size for some ray species, this study is a useful overview on the Hg levels in these myliobatid species. THg levels in the edible portion of analyzed specimens did not represent a high risk to consumers. Further studies on consumption rates of myliobatid fish, physiological aspects related to Hg toxicokinetics, and the simultaneous occurrence of other trace elements are necessary.

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