

Short Communication

Trace elements in the whale shark *Rhincodon typus* liver: an indicator of the health status of the ecosystem base (plankton)

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ABSTRACT. Trace elements were determined in three areas of the right (RL) and left (LL) lobe of the liver obtained from a whale shark (*Rhincodon typus*) stranded in Mexico. Mean \pm standard error concentrations in $\mu\text{g g}^{-1}$ wet weight were for zinc (Zn) RL: 22.5 ± 2.1 ; LL: 26.5 ± 7.1 , arsenic (As) RL: 33.0 ± 1.6 ; LL: 20.0 ± 9.9 and cadmium (Cd) RL: 15.5 ± 0.9 ; LL: 11.3 ± 3.7 ; copper (Cu) RL: 3.2 ± 0.3 ; LL: 2.2 ± 0.8 , selenium (Se) RL: 0.5 ± 0.1 ; LL: 1.2 ± 0.6 , mercury (Hg) RL: 0.06 ± 0.02 ; LL: 0.05 ± 0.004 and lead (Pb) RL: 0.05 ± 0.02 ; LL: 0.05 ± 0.01 . Concentrations showed significant ($P < 0.05$) differences within the same lobe but not between lobes. The trace element levels found in this whale shark represent the baseline levels of the trophic base's health status in the study area.

Keywords: *Rhincodon typus*; arsenic; mercury; selenium; cadmium molar ratio; Gulf of California

The liver is a highly metabolic organ where up taking, storing, and excretion of nutrients and other molecules occur (Hinton et al. 2001). Several studies describe this organ as a target for toxic substances (Pethybridge et al. 2010), as bioaccumulation of contaminants often occurs due to the large blood supply and its interaction with anthropogenic compounds as heavy metals and methylmercury (Bosch et al. 2016). One of the liver's primary functions is detoxification through the transformation of poorly excretable chemicals into more excretable ones, and the synthesis of metallothionein proteins that bind to different metals (Hinton et al. 2001). Therefore, the liver plays a key role in an organism's health. Top predators with large lipid-rich livers, such as most sharks, are particularly susceptible to the uptake and bioaccumulation of contaminants (Barrera-García et al. 2013), which can increase throughout their lifetime. Bioaccumulation occurs differentially between organs and tissues in a process

known as organotropism (Correa et al. 2014), where specific organs are the main storage and detoxification sites of trace elements (Mieiro et al. 2011). The interaction between different elements is an additional tool for body detoxification. For example, selenium Se is an essential element that maintains cellular oxidative homeostasis and mitigates the toxicity of heavy metals such as mercury Hg (Endo et al. 2005) and cadmium Cd (Jamwal et al. 2018) through the action of selenoproteins (Belzile et al. 2006).

The whale shark *Rhincodon typus* is a circumglobal species found in several Gulf of California areas (Ketchum et al. 2013). It is a long-lived animal with a life span of more than 100 years; additionally, it is an opportunistic filter-feeding shark (Colman 1997). Feeding aggregations in the Gulf of California show that whale sharks prey mostly on zooplankton patches that are rich in copepods (Nelson & Eckert 2007), euphausiids (Ketchum et al. 2013), and chaetognaths

(Pancaldi et al. 2019b). Studies on trace elements bioaccumulation in this species are scarce and show that whale sharks accumulate arsenic As, Hg, Cd, copper Cu, lead Pb, selenium, and zinc Zn in the liver (Wang et al. 2015, Pancaldi et al. 2019a). This study complements a report on the element's concentrations in this animal's tissues (Pancaldi et al. 2019a, Whitehead et al. 2019). The main objectives of this study were to examine the concentrations of essential (Cu, Se, Zn) and non-essential (As, Cd, Hg and Pb) elements as well as to determine the potential antagonism between Se, Zn, Hg, Cd and As throughout the molar ratio in three areas (proximal, median and distal) of the right and left lobes of the liver in a stranded whale shark from La Paz, Gulf of California, Mexico. Our results are also compared with heavy metal limits established for edible fish by the Food and Agriculture Organization (FAO) (CODEX 2009).

On February 16, 2018, a whale shark was found stranded inside La Paz Bay Lagoon (24°4.8'N, 110°10.8'W; Fig. 1), Baja California Sur, Mexico. The dead shark was immediately transported to a laboratory where the liver was extracted. Duplicate samples from the proximal, median, and distal part of both lobes of the liver were taken with a sterilized knife and preserved at -20°C in plastic containers previously cleaned and rinsed with HCl (10%) and HNO₃ (20%) for three days; subsequently, they were freeze-dried (72 h, -49°C and 133×10⁻³ mbar), pulverized and homogenized. Lyophilized aliquots (0.25 g), blanks, and reference material were digested in Teflon vials with caps (Savillex) using concentrated HNO₃ (70%, trace metal analysis, JT Baker) and H₂O₂ to 120°C for 4 h. Digested samples were gauged with a solution of In 115 (Pancaldi et al. 2019a,b). The humid percentages of tissues are shown in Table 1. Mexican wildlife authorities approved ethics requirements and protocols for samples' obtention (SGPA/DGVS 05605/17).

Trace element analyses (Cu, Se, Zn, Hg, As, Cd, and Pb) were performed using a high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) Thermo Scientific Element 2XR and cold vapor atomic absorption spectrometry (Pancaldi et al. 2019a,b). Quality control of the analysis included blanks and certified reference material (DOLT-5 dogfish liver). Blanks were performed with no anomalies detected. Recovery percentages were 112% Cu, 105% Se, 101% Zn, 92% As, 106% Hg and 100% Cd and Pb. Trace element concentrations are expressed on wet weight (ww). Non-parametric tests were used as data did not follow a normal distribution. Mann-Whitney test was applied to the mean concentrations between lobes, Kruskal Wallis and Dunn test was applied between mean concentrations of an element within the same lobe;

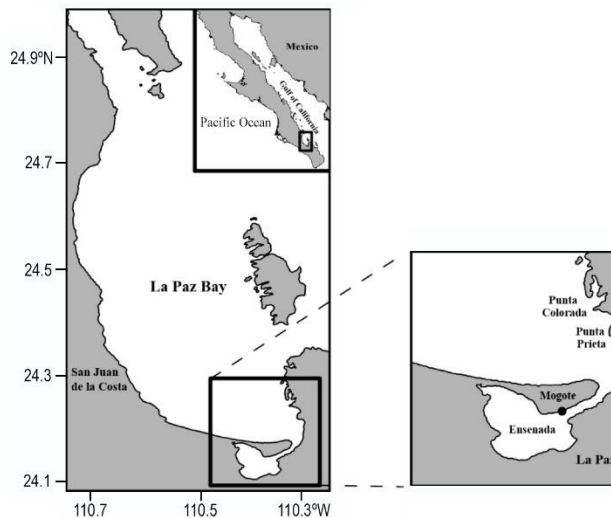


Figure 1. Map of La Paz Bay with a close up to La Paz Lagoon and the whale shark's (*Rhincodon typus*) feeding area (Mogote) (dark dot indicates the site where the stranded whale shark was found).

t-test was applied to the molar ratios to assess differences greater than 1.

The stranded whale shark measured 548 cm in total length (TL), and it was identified as an immature male (Whitehead et al. 2019). The liver's right lobe (Fig. 2) measured 152 cm TL and 38 cm in its wider area. The gallbladder was attached to the right lobe near the proximal area. The left lobe of the liver measured 151 cm TL and 39 cm in its wider area. In general, As, Zn and Cd showed the highest concentrations in both lobes, while Cu, Hg, and Pb were the less concentrated elements (Table 1). In the right lobe, the concentration (mean ± standard error, SE μg g⁻¹) of As, Zn, and Cd were 33.0 ± 1.6, 22.5 ± 2.1, and 15.5 ± 0.9, respectively; while for Cu, Se, Hg, and Pb, it was 3.2 ± 0.3, 0.5 ± 0.1, 0.07 ± 0.02, and 0.05 ± 0.01. In the left lobe, As and Cd's concentration was lower compared to the right lobe, while Zn was higher (Table 1). These results suggest a heterogeneous distribution of As, Zn, and Cd in the whale shark liver. Despite these differences, all trace elements' mean concentrations were not different (Mann-Whitney test *P* > 0.05) between lobes.

It is important to highlight that in the southwest Gulf of California, around the Mogote area (Fig. 1) where feeding aggregations of *R. typus* occur, sediments are naturally enriched in As, and Cd, and they have been related to phosphorites present in the watershed (Pérez-Triboullier et al. 2015). Phosphorite mining activity in San Juan de la Costa during the past 80 years could have affected As and Cd's levels in whale shark feeding grounds, leading finally to bioaccumulation in the liver.

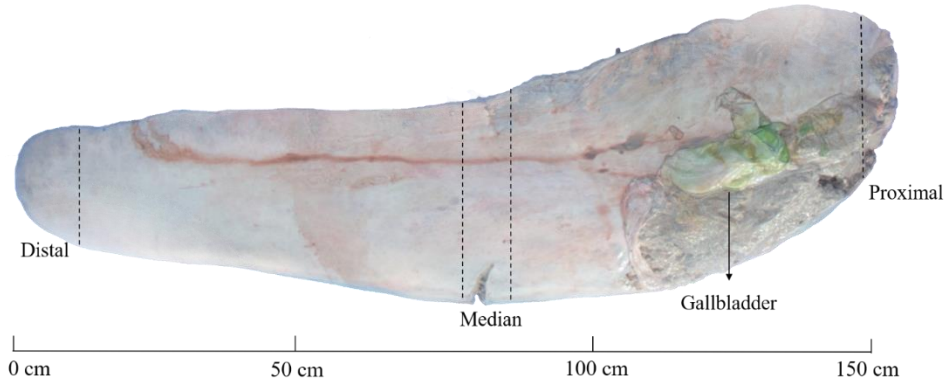


Figure 2. *Rhincodon typus* right lobe of the liver with the proximal, median, and distal area along with the gallbladder.

Table 1. Trace element concentrations, mean and standard error (SE), and molar ratio Se:Hg, Se:Cd, Se:As, Zn:Cd, Zn:As in the right and left lobe of the whale shark *Rhincodon typus* liver. Concentrations are expressed in $\mu\text{g g}^{-1}$ ww. Mann-Whitney test applied to elements mean concentrations showed no significant differences between lobes ($P > 0.05$). Asterisks indicate molar ratio that are significantly different than 1 ($*P < 0.05$; $**P < 0.001$). A *t*-test performed this statistical analysis. Limits of Cd, Hg (as methyl-Hg), and Pb established for edible fish products showed from the FAO (CODEX 2009), and for As from the Mexican norm NOM-242-SSA1-2009 (DOF 2011).

Lobe	Sample	Humidity (%)	Cu	Se	Zn	As	Cd	Hg	Pb	Se:Hg	Se:Cd	Se:As	Zn:Cd	Zn:As
Right	Proximal	32.8	3.1	0.6	26.3	30.0	16.5	0.05	0.08	30.0	0.05	0.02	2.7	1.0
	Median	15.2	3.8	0.5	19.1	33.5	13.7	0.10	0.04	13.8	0.06	0.02	2.4	0.7
	Distal	42.3	2.7	0.4	22.1	35.3	16.4	0.04	0.02	20.8	0.03	0.01	2.3	0.7
	Mean		3.2	0.5	22.5	33.0	15.5	0.06	0.05	21.4	0.05**	0.01**	2.5*	0.8
	SE		0.3	0.1	2.1	1.6	0.9	0.02	0.02	4.6	0.01	0.003	0.1	0.1
Left	Proximal	21.4	0.7	2.3	13.5	0.9	4.6	0.05	0.03	108.4	0.70	2.50	5.0	17.6
	Median	19.3	2.8	0.8	27.7	25.8	11.8	0.04	0.05	44.6	0.09	0.03	4.0	1.2
	Distal	44.8	3.1	0.4	38.1	33.7	17.5	0.06	0.06	17.6	0.03	0.01	3.7	1.3
	Mean		2.2	1.2	26.5	20.0	11.3	0.05	0.05	57.0	0.30	0.84	4.3*	6.7
	SE		0.8	0.6	7.1	9.9	3.7	0.004	0.01	26.9	0.20	0.82	0.4	5.4
Maximum limit														
Edible fish products														

High levels of Cd and Zn in the liver of sharks have been explained by the action of metallothioneins, which are concentrated in the hepatic tissue retaining these elements (Marcovecchio et al. 1991). Conversely, Cu, Se, Hg, and Pb were found in lower concentrations (Table 1). Significant differences in the right lobe were found between As with Cu, As with Se, and As with Hg concentrations, which showed a $P < 0.05$ (Kruskal-Wallis and posterior Dunn-test applied). Other significant differences ($P < 0.05$) were found between Zn with Se and with Hg in the same lobe. Finally, Cd and Pb concentrations and between Cd and Hg showed a $P < 0.05$ (Kruskal-Wallis and posterior Dunn-test applied).

In the left lobe, Zn showed the highest concentrations (38.1 and 27.7 $\mu\text{g g}^{-1}$ in distal and median, respectively), which were also higher than the same element's levels in the right lobe. Arsenic concentrations were 33.7 $\mu\text{g g}^{-1}$ in the distal and 25.8 $\mu\text{g g}^{-1}$ in the median area. Cd concentrations were 17.5 $\mu\text{g g}^{-1}$ in the distal and 11.8 $\mu\text{g g}^{-1}$ in the median area. Dunn-test applied to the mean concentrations of Zn, As, and Cd showed significant differences ($P < 0.05$) with Hg and Pb. Unexpectedly, Zn, As, and Cd was found in lower concentrations in the left lobe's proximal area (13.5 $\mu\text{g g}^{-1}$ Zn, 0.9 $\mu\text{g g}^{-1}$ As, and 4.6 $\mu\text{g g}^{-1}$ Cd) compared to the proximal area of the right one.

The proximal area of the right lobe of the whale shark liver is located near the gallbladder, which contains the bile. The vicinity between the proximal area and the gallbladder in the right lobe could be a factor that increases concentrations of As and Cd. Hg was found in similar concentrations in the proximal area of the right (0.050 $\mu\text{g g}^{-1}$) and left lobe (0.054 $\mu\text{g g}^{-1}$). The Hg levels in both lobes were higher (0.040-0.100 $\mu\text{g g}^{-1}$ in the right lobe, and 0.040-0.058 $\mu\text{g g}^{-1}$ in left lobe) compared to the levels reported in the liver of a dead whale shark from China ($0.00330 \pm 0.00001 \mu\text{g}$

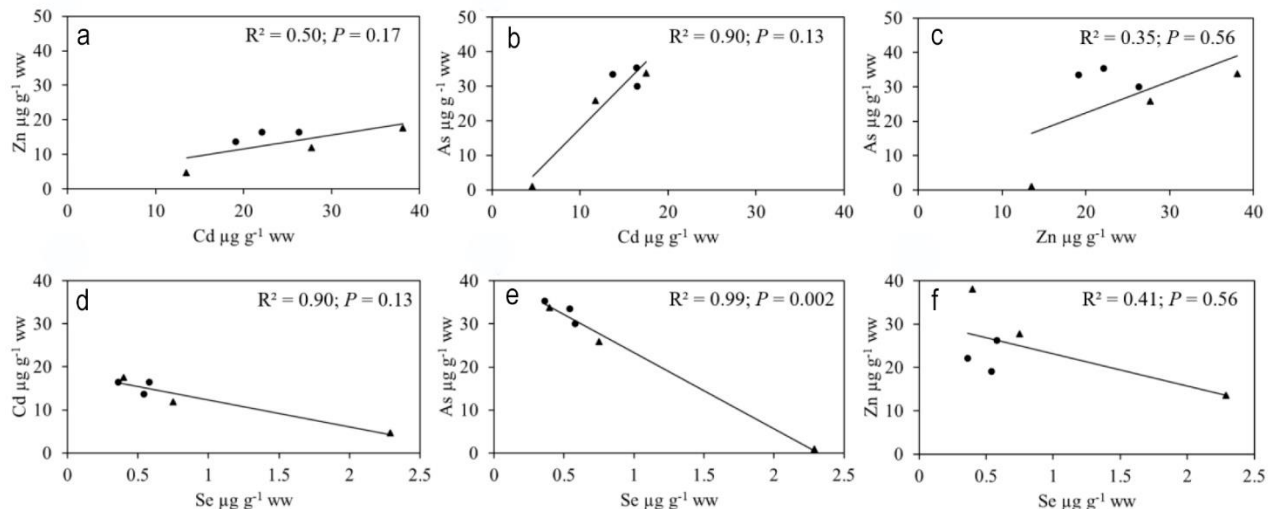


Figure 3. Trace element variation (Spearman R^2 and P are included) between a) Zn and Cd, b) As and Cd, c) As and Zn, d) Cd and Se, e) As and Se, f) Zn and Se. Circles represent concentrations from the right lobe and triangles from the left lobe in $\mu\text{g g}^{-1}$ w/w.

g^{-1} ; Wang et al. 2015). Nevertheless, Hg levels found in the whale shark from La Paz were lower compared to the levels reported in the liver of the carnivorous blue shark *Prionace glauca* from the Mexican Pacific ($0.22 \pm 0.35 \mu\text{g g}^{-1}$; Barrera-García et al. 2013) or the predatory shark *Sphyrna lewini* from the Gulf of California (Bergés-Tiznado et al. 2015). The uptake of Hg principally occurs through the diet; therefore, Hg concentrations are expected to be higher in top predator sharks rather than filter-feeding species. Considering that the sampled shark feeds most of the time in La Paz Bay, low Hg concentrations in its liver indicate that the trophic base, from which the shark depends, also present low Hg concentration in this area.

Selenium with Hg and Cd's antagonist effect has been observed in several organisms, including elasmobranchs (Siscar et al. 2014). Even though these interactions' mechanism is not well understood, the molar ratio between the elements is proposed to be 1:1 (or close) for Se to exhibit efficient behavior against metal toxicity (Ralston et al. 2016). Se showed the highest ($2.3 \mu\text{g g}^{-1}$) level in the left lobe's proximal area; consequently, molar ratio Se:Hg in this area presented the highest value (108:1), which indicates a more evident detoxification process in this area. Molar Se:Hg ratio was 44.6:1 in the median area and 17.6:1 in the distal area (Table 1), indicating that these areas of the left lobe contain sufficient Se to detoxify the action of Hg. In the right lobe, Se:Hg molar ratio was 29.6:1, 20.8:1, and 13.8:1, in the proximal, distal, and the median area, respectively, which also indicates sufficient Se to detoxify Hg action in the three regions.

Molar Se:Cd ratio was <1 (Table 1) in all sampled areas of the liver, suggesting that Se concentrations are not enough to protect the organ from Cd damage. Cd is a non-essential metal, and it is known to affect the uptake of calcium through fish gills (Niyogi & Wood 2004). Also, exposure to this element may lead to the accumulation of reactive oxygen species and oxidative damage (Waisberg et al. 2003, Bertin & Averbek 2006). In contrast, results show that the molar Zn:Cd ratio was >1 (Table 1), which shows that in the whale shark, Zn might exert a more effective detoxification action against Cd concerning Se. These same results have been found in the liver of deep fish species (Siscar et al. 2014) and mammals (Jihen et al. 2009), where Zn is more efficient than Se in protecting the liver from Cd damages. The molar ratio Se:As was <1 in all sampled areas of the liver except in the left lobe's proximal area, while the molar ratio Zn:As was close to or greater than 1 (Table 1). According to these results, Zn could also act more efficiently against As concerning Se. An antagonistic effect of Zn over As has been observed in the cladoceran *Daphnia obtusa* (Gaete & Chavez 2008); however, information about the antagonistic effect of Se and Zn on As is scarce. Spearman correlation applied to trace elements shows strong relations (Figs. 3b,d,e). The antagonism between Se and As in whale shark liver (right and left lobe) is shown in Figure 3e, where Spearman correlation shows a negative and significant ($R^2 = 0.989$; $P = 0.002$; $n = 6$) correlation between these elements. A negative ($R^2 = 0.903$) and not significant ($P = 0.136$; $n = 6$) Spearman correlation was also found between Se and Cd (Fig. 3d). The highest concentration of Se ($2.30 \mu\text{g g}^{-1}$

g^{-1} ; Table 1) found in the whale shark liver corresponds to the lowest concentrations of Cd ($4.63 \mu\text{g g}^{-1}$) and As ($0.88 \mu\text{g g}^{-1}$), which indicate antagonism.

In conclusion, trace elements analyzed were not uniformly distributed in the whale shark liver; Cd, As, and Zn were found in high concentrations in both right and left lobes, probably due to natural enrichment of the area associated with remains of phosphorites. In contrast, Hg and Pb were found in lower concentrations. The left lobe proximal area presented the lowest concentrations of Zn, As, and Cd, and the highest Se concentrations, which could be due to the gallbladder's reduced distance, which is connected to the right lobe or a detoxification action from Se throughout the action of selenoproteins. Molar ratio Se:Hg indicates sufficient Se levels to detoxify Hg in the lobes. On the other hand, the molar ratio Zn:Cd was >1 , which shows that Zn acts more efficiently in Cd detoxification in this species. Whale shark means concentrations of Cd found in both lobes of the liver (Table 1) exceeded the maximum levels established by the FAO in edible fish products ($2.0 \mu\text{g g}^{-1}$ ww; CODEX 2009). Conversely, Hg and Pb concentrations found in the liver were lower than the maximum levels established in the CODEX (2009) (Hg (as methyl-Hg $0.5 \mu\text{g g}^{-1}$); Pb: $0.3 \mu\text{g g}^{-1}$ ww); similarly, as levels were below the maximum established by the Mexican norm NOM-242-SSA1-2009 ($80 \mu\text{g g}^{-1}$, DOF 2011). Although the whale shark is not an edible fish in Mexico and most of the world, its relatively high Cd level is remarkable, which would have to be considered if the liver of this animal were considered for human consumption.

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