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Research Article

Total mercury in female Pacific sharpnose sharks *Rhizoprionodon longurio* and their embryos

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ABSTRACT. We determined the Hg content of blood, placenta and umbilical cord of 20 pregnant females of the viviparous Pacific sharpnose shark, *Rhizoprionodon longurio* and of the livers of the embryos contained in their right and left uterus, aiming to provide information on the amount of this metal offloaded during pregnancy by the mother to the embryos. Hg content varied by close or higher than one order of magnitude in all tissues and showed the decreasing trend: maternal blood > umbilical cord > placenta > embryonic livers, with placenta and embryonic livers significantly lower than maternal blood. There were highly significant correlations ($P < 0.001$) between the Hg content of maternal blood, cord, and placenta. Those between embryonic livers and maternal blood, cord and placenta were not significant ($P > 0.05$). The results suggest transplacental Hg transfer and that the liver is not the main site of Hg accumulation.

Keywords: mercury, maternal offloading, shark embryos, blood, placenta, umbilical cord.

Mercurio total en hembras del tiburón bironche, *Rhizoprionodon longurio* y en sus embriones

RESUMEN. En el presente estudio se determinó el contenido de mercurio en la sangre, placenta y cordón umbilical de 20 hembras del tiburón bironche *Rhizoprionodon longurio* así como en el hígado de los embriones de los úteros derecho e izquierdo, con el objetivo de proveer información sobre la cantidad de este metal transferida por la madre a sus embriones durante su desarrollo. Los contenidos de Hg variaron hasta un orden de magnitud en todos los tejidos y presentaron el siguiente orden decreciente: sangre materna > cordón umbilical > placenta > hígado de los embriones. Los contenidos de la placenta y el hígado de los embriones fueron significativamente menores al determinado en la sangre materna. Se encontraron correlaciones altamente significativas ($P < 0,001$) entre el contenido de Hg de la sangre materna, cordón y placenta, mientras que las calculadas entre hígado de los embriones y la sangre materna, cordón y placenta no fueron significativas ($P > 0,05$). Los resultados sugieren que existe una libre transferencia de Hg y que el hígado no es el sitio principal de acumulación de Hg.

Palabras clave: mercurio, transferencia materna, embriones de tiburones, sangre, placenta, cordón umbilical.

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INTRODUCTION

Atmospheric transport and deposition are considered the main sources of Hg pollution, although industrial and urban wastes, mining, and agriculture are other important sources of Hg contamination of the aquatic environment (Harris *et al.*, 2012). This has a high environmental cost, because it entails lower availability of recreational areas, loss of biodiversity and of access to natural food products for human consumption, and adverse health effects (Bellanger *et al.*, 2013).

Along the food web, Hg excretion is generally lower than its absorption, and because of its progressive accumulation, this metal may reach high concentrations in the tissues of top predators (McMeans *et al.*, 2015). This may explain the levels of Hg close or above the precautionary limits for human consumption detected in Mexican Pacific sharks (Escobar-Sánchez *et al.*, 2011; Hurtado-Banda *et al.*, 2012). This is a source of concern for human health and for the conservation of these species, because the pregnant mothers of viviparous sharks transfer to their embryos the toxic substances accumulated in their organs and tissues (Lyons & Lowe, 2013; Mull *et al.*, 2013; Olin *et al.*, 2014).

In mammals, maternal blood supplies oxygen and nutrients to developing embryos, but it is also the source of their exposure to contaminants (Leino *et al.*, 2013) since, although the placenta may act as at least a partial barrier against Cd (Gundacker & Hengstschläger, 2012), Pb and Hg can readily cross this barrier (Gupta, 2012). Some results seem to show that embryos concentrate maternal blood-borne Hg, suggesting that they act a route of discharge of the excessive load of maternal mercury (Rudge *et al.*, 2009).

In placental sharks, yolk sac and stalk become progressively modified into placenta and umbilical cord, with gas exchange and hematrophic functions similar to those of mammals. These shared basic maternal-fetal relationships seem to indicate convergent evolution of the two groups (Haines *et al.*, 2006), with possible shared aspects of other functions of this organ such as acting as transport site or as partial barrier to some metals.

An important component of the winter landings of the artisanal fishing fleets of the Mexican Pacific NW is the sharpnose shark *Rhizoprionodon longurio*, which is a highly migratory placental viviparous species (Corro-Espinosa *et al.*, 2011). In this work, we evaluated the Hg content of blood and placenta of pregnant *R. longurio* females and of the liver of their respective embryos, to provide information on the mother to embryo transfer of Hg in this species, which might be an important mechanism of impaired reproductive success.

MATERIALS AND METHODS

Pregnant sharpnose sharks (20), obtained between January and March 2012 from local fishermen of Mazatlán (SE Gulf of California), were measured (total length, TL), and dissected in the laboratory with a stainless steel knife to obtain placenta and umbilical cord from the females and the liver of the embryos of both uteri. Blood (30 mL) was drawn from the ventral portion of females (Cizdziel *et al.*, 2003) using sterile plastic syringes, and immediately placed in polyethylene tubes. All tissues were lyophilized for 72 h and homogenized in a Teflon mortar. Three samples of each tissue were digested at 130°C in a mod-block unit, using sealed Teflon vessels with 5 mL of concentrated HNO_3 (trace metal grade). After digestion, samples were transferred to vials and diluted to 15 mL with Milli-Q water (Frías-Espericueta *et al.*, 2014).

All materials used during sampling and metal analysis were acid washed. Total Hg was determined by cold vapor atomic absorption spectrophotometry (CV-AAS) after reduction with $SnCl_2$ in a mercury analyzer (Buck Scientific). Certified reference material (DORM 3, National Research Council Canada) was used to assess the accuracy of the method, with a recovery of 105%, and blanks were included using the same procedure of the samples to check possible contamination. The limit of detection was 0.01 µg g^{-1} and the coefficient of variation was <10%.

The non-compliance with parametric assumptions led to employment of Mann-Whitney's tests to compare the mean Hg content of the livers of the embryos of the two uteri of each female. Since no significant differences were detected between uteri, the mean Hg content of the livers of all embryos obtained from each female was used for statistical comparisons between tissues, using non-parametric block ANOVA (Friedman´s) and Dunn's multiple comparison tests. Possible relations between the Hg values found in the tissues of mothers and embryos were determined with Spearman´s correlations tests. (ρ). All tests were with $\alpha = 0.05$ (Zar, 1999).

RESULTS

Each female, with TLs ranging from 99.8 to 118.1 cm (mean 107.5 ± 4.9 cm), carried 4 to 11 embryos. The total number of embryos obtained from the 20 females was 168. Their TLs varied between 26.38 and 34.25 cm, and there was no significant difference in mean size between the mean values of right and left uterus-borne embryos (29.46 ± 2.53 and 29.75 ± 2.52 cm, respectively).

The Hg concentrations ranged between 0.16 and 1.97 µg g^{-1} dry weight in maternal blood and from 0.10 to 0.72 and 0.06 to 0.71 µg g^{-1} in cord and placenta, respectively. The mean values were 0.54 ± 0.52, 0.32 ± 0.17 and 0.20 ± 0.15 µg g^{-1}, with a significant difference ($P < 0.05$) between blood and placenta, but not between blood and cord (Table 1). In embryonic livers total Hg ranged from 0.02 ± 0.01 to 0.16 ± 0.08 (right uterus) and between 0.04 ± 0.03 and 0.14 ± 0.02 µg g^{-1} (left uterus), and their global mean value was significantly lower than those of blood and cord, but not of the mean content of the placenta (Table 1).

There were significant correlations between the Hg content of blood and placenta ($\rho = 0.943$), blood and cord ($\rho = 0.847$), and between cord and placenta ($\rho = 0.778$), but the mercury contents of embryonic livers were not significantly related ($P > 0.05$) to the respective female tissues (Table 2). Additionally, there was no significant correlation between the Hg content of the embryonic livers with size and weight of the embryos ($\rho = 0.005$ and -0.1308, respectively, $P > 0.05$ in both cases) nor between total length of females with the respective blood Hg content ($\rho = 0.231$, $P > 0.05$).

DISCUSSION

The greatest maternal offloading of contaminant occurs during the first reproductive event, when the loads of contaminants accumulated by the mother are at their highest values (Lyons & Lowe, 2013). The mean TL of the pregnant mothers indicates ages close to 8-10 years, and the smallest was 99.8 cm, which is reached at 4 to 5 years of age (Castillo *et al.*, 1996). This indicates that none was primiparous, since the mean size at first maturity of this species is close to 93 cm (Corro-Espinosa *et al.*, 2011).

Several authors found a significant relation between the Hg content of fish species and their TL, age or weight (Adams *et al.*, 1999; Farkas *et al.*, 2001). This does not correspond to our results, possibly because of the low variability in size of our specimens, as well as the fairly wide range of blood Hg concentrations. On the other hand, the good correlations between Hg contents of blood, cord and placenta coincide with the general agreement that blood Hg concentration is strongly related to the Hg content of the remaining tissues (Cizdziel *et al.*, 2003; Schmitt & Brumbaugh,

Table 1. Mean Hg concentrations in µg/g, dw (± standard error) in blood, cord and placenta of 20 pregnant *Rhizoprionodon longurio* and of the livers of the respective embryos of the right (RU) and of the left (LU) uterus.

Female	Blood	Cord	Placenta	RU	LU
1	0.26 ± 0.01	0.10 ± 0.04	0.15 ± 0.00	0.16 ± 0.08	0.11 ± 0.02
2	0.16 ± 0.01	0.17 ± 0.04	0.11 ± 0.02	0.09 ± 0.03	0.05 ± 0.01
3	0.21 ± 0.01	0.19 ± 0.06	0.11 ± 0.02	0.06 ± 0.01	0.08 ± .03
4	0.47 ± 0.01	0.37 ± 0.07	0.26 ± 0.03	0.11 ± 0.02	0.09 ± 0.01
5	0.37 ± 0.05	0.20 ± 0.06	0.11 ± 0.03	0.07 ± 0.01	0.06 ± 0.01
6	1.65 ± 0.21	0.72 ± 0.15	0.45 ± 0.03	0.09 ± 0.05	0.09 ± 0.01
7	0.17 ± 0.01	0.14 ± 0.09	0.06 ± 0.02	0.04 ± 0.02	0.06 ± 0.01
8	1.27 ± 0.01	0.71 ± 0.15	0.33 ± 0.03	0.07 ± 0.01	0.07 ± 0.02
9	0.22 ± 0.00	0.21 ± 0.14	0.07 ± 0.03	0.04 ± 0.02	0.05 ± 0.01
10	0.36 ± 0.02	0.31 ± 0.11	0.11±0.01	0.05 ± 0.01	0.05 ± 0.01
11	0.36 ± 0.04	0.39 ± 0.07	0.20 ± 0.02	0.06 ± 0.01	0.06 ± 0.01
12	0.50 ± 0.00	0.25 ± 0.09	0.13 ± 0.02	0.04 ± 0.01	0.05
13	0.34 ± 0.02	0.37 ± 0.31	0.15 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
14	1.97±0.41	0.57 ± 0.13	0.71 ± 0.13	0.15 ± 0.03	0.14 ± 0.02
15	1.04 ± 0.16	0.34 ± 0.10	0.26 ± 0.01	0.07 ± 0.01	0.10 ± 0.05
16	0.33 ± 0.01	0.29 ± 0.16	0.15 ± 0.01	0.05 ± 0.04	0.05 ± 0.01
17	0.26 ± 0.00	0.20 ± 0.08	0.16 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
18	0.21 ± 0.03	0.20 ± 0.03	0.12 ± 0.01	0.03 ± 0.01	0.04 ± 0.03
19	0.32 ± 0.01	0.27 ± 0.05	0.13 ± 0.01	0.02 ± 0.01	0.06 ± 0.07
20	0.23 ± 0.00	0.32 ± 0.10	0.13 ± 0.01	0.04 ± 0.01	0.04 ± 0.06
Mean*	0.54 ± 0.52a	0.32 ± 0.17ab	0.20 ± 0.15bc	0.08 ± 0.04c	

*Total mean values of blood, cord, placenta and embryonic livers ± standard deviations. Equal or common letters indicate lack of significant differences (Friedman's and Dunn's tests, $\alpha = 0.05$; cd≤c≤bc≤ab and d<a).

Table 2. Spearman's correlation coefficients (ρ) between the Hg contents of tissues of females and embryos.

Relation	ρ	*P*
Placenta/blood	0.943	<0.001
Cord/blood	0.847	<0.001
Placenta/cord	0.778	<0.001
Embryonic liver/blood	0.107	>0.05
Embryonic liver/cord	0.117	>0.05
Embryonic liver/placenta	0.126	>0.05

2007). Among its several functions, blood distributes the products of digestion to all body tissues and carries metabolic wastes to the organs in charge of their excretion. This relation has been described also in humans (Ask *et al.*, 2002; Rudge *et al.*, 2009), although in these cases the Hg contents of cord or placental blood were higher than that of maternal blood.

The similar Hg contents of the embryonic livers obtained in right and left uterus confirm the lack of inter-uterine difference determined in other sharks by Adams *et al.* (1999), which was postulated by Frías-Espericueta *et al.* (2014) for other metals in the case of this species.

The sequence of progressively decreasing mean Hg concentrations of blood, cord, placenta and the lack of a significant difference between placenta and embryonic livers might indicate that the placenta acts as a partial barrier to transfer of toxic substances from mother to embryos. It also shows the existence of transplacental Hg transfer, possibly due to molecular mimicry with methionine of MeHg bound to thiol-groups (Ballatori, 2002). The content of the cord, intermediate between maternal blood and placenta, might therefore be due to the Hg fraction carried by maternal blood but not retained by the embryos, and possibly enriched by the embryos unloading their excess Hg to the mother.

There are many examples of higher Hg concentrations in fish liver tissues (Romeo *et al.*, 1999; Mieiro *et al.*, 2009; Guilherme *et al.*, 2010; Azevedo *et al.*, 2012), but a lower Hg liver content was observed in several fish species including sharks (Cizdziel *et al.*, 2003; Kenšová *et al.*, 2010; Lyons & Lowe, 2013). This difference coincides with the results obtained by Pethybridge *et al.* (2010) and Le Bourg *et al.* (2014) in embryos of placental and aplacental sharks, and is probably due to a lower content of inorganic than organic Hg, which has a high degree of affinity for the thiol groups of the muscle tissues (Soares *et al.*, 2011).

In placental viviparous sharks, mercury transfer from mother to embryos is considered a detoxification mechanism of the mother (Adams *et al.*, 1999), similar to the maternal offloading of organic pollutants described by Mull *et al.* (2013) and Olin *et al.* (2014) in the white shark *Carcharodon carcharias* and the bull shark, *Carcharhinus leucas*, respectively. Our results confirm that the placenta of the Pacific sharpnose shark *Rhizoprionodon longurio* is not an effective barrier to Hg transfer, which might be of concern because Hg might affect the normal embryonic development. This should be the focus of future studies concerning this, as well as other viviparous shark species.

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