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 HNO₃ (trace metal grade). After digestion, samples were transferred to vials and diluted to 15 mL with Milli-Q water (Frias-Espiercueta 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 SnCl₂ 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. (p). All tests were with α = 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 \( \mu g \, g^{-1} \) dry weight in maternal blood and from 0.10 to 0.72 and 0.06 to 0.71 \( \mu g \, g^{-1} \) in cord and placenta, respectively. The mean values were 0.54 \( \pm 0.52 \), 0.32 \( \pm 0.17 \) and 0.20 \( \pm 0.15 \) \( \mu g \, g^{-1} \), 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 \( \pm 0.01 \) to 0.16 \( \pm 0.08 \) (right uterus) and between 0.04 \( \pm 0.03 \) and 0.14 \( \pm 0.02 \) \( \mu g \, g^{-1} \) (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, \( P > 0.05 \)) between blood and cord (Table 1). In embryonic livers the mean size at first reproduction 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 fairily 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, 2003). 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).

### Table 1. Mean Hg concentrations in \( \mu g/g, \, dw \) (\( \pm \) 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.

<table>
<thead>
<tr>
<th>Female</th>
<th>Blood</th>
<th>Cord</th>
<th>Placenta</th>
<th>RU</th>
<th>LU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.26 ( \pm 0.01 )</td>
<td>0.10 ( \pm 0.04 )</td>
<td>0.15 ( \pm 0.00 )</td>
<td>0.16 ( \pm 0.08 )</td>
<td>0.11 ( \pm 0.02 )</td>
</tr>
<tr>
<td>2</td>
<td>0.16 ( \pm 0.01 )</td>
<td>0.17 ( \pm 0.04 )</td>
<td>0.11 ( \pm 0.02 )</td>
<td>0.09 ( \pm 0.03 )</td>
<td>0.05 ( \pm 0.01 )</td>
</tr>
<tr>
<td>3</td>
<td>0.21 ( \pm 0.01 )</td>
<td>0.19 ( \pm 0.06 )</td>
<td>0.11 ( \pm 0.02 )</td>
<td>0.06 ( \pm 0.01 )</td>
<td>0.08 ( \pm 0.03 )</td>
</tr>
<tr>
<td>4</td>
<td>0.47 ( \pm 0.01 )</td>
<td>0.37 ( \pm 0.07 )</td>
<td>0.26 ( \pm 0.03 )</td>
<td>0.11 ( \pm 0.02 )</td>
<td>0.09 ( \pm 0.01 )</td>
</tr>
<tr>
<td>5</td>
<td>0.37 ( \pm 0.05 )</td>
<td>0.20 ( \pm 0.06 )</td>
<td>0.11 ( \pm 0.03 )</td>
<td>0.07 ( \pm 0.01 )</td>
<td>0.06 ( \pm 0.01 )</td>
</tr>
<tr>
<td>6</td>
<td>1.65 ( \pm 0.21 )</td>
<td>0.72 ( \pm 0.15 )</td>
<td>0.45 ( \pm 0.03 )</td>
<td>0.09 ( \pm 0.05 )</td>
<td>0.09 ( \pm 0.01 )</td>
</tr>
<tr>
<td>7</td>
<td>0.17 ( \pm 0.01 )</td>
<td>0.14 ( \pm 0.09 )</td>
<td>0.06 ( \pm 0.02 )</td>
<td>0.04 ( \pm 0.02 )</td>
<td>0.06 ( \pm 0.01 )</td>
</tr>
<tr>
<td>8</td>
<td>1.27 ( \pm 0.01 )</td>
<td>0.71 ( \pm 0.15 )</td>
<td>0.33 ( \pm 0.03 )</td>
<td>0.07 ( \pm 0.01 )</td>
<td>0.07 ( \pm 0.02 )</td>
</tr>
<tr>
<td>9</td>
<td>0.22 ( \pm 0.00 )</td>
<td>0.21 ( \pm 0.14 )</td>
<td>0.07 ( \pm 0.03 )</td>
<td>0.04 ( \pm 0.02 )</td>
<td>0.05 ( \pm 0.01 )</td>
</tr>
<tr>
<td>10</td>
<td>0.36 ( \pm 0.02 )</td>
<td>0.31 ( \pm 0.11 )</td>
<td>0.11 ( \pm 0.01 )</td>
<td>0.05 ( \pm 0.01 )</td>
<td>0.05 ( \pm 0.01 )</td>
</tr>
<tr>
<td>11</td>
<td>0.36 ( \pm 0.04 )</td>
<td>0.39 ( \pm 0.07 )</td>
<td>0.20 ( \pm 0.02 )</td>
<td>0.06 ( \pm 0.01 )</td>
<td>0.06 ( \pm 0.01 )</td>
</tr>
<tr>
<td>12</td>
<td>0.50 ( \pm 0.00 )</td>
<td>0.25 ( \pm 0.09 )</td>
<td>0.13 ( \pm 0.02 )</td>
<td>0.04 ( \pm 0.01 )</td>
<td>0.05</td>
</tr>
<tr>
<td>13</td>
<td>0.34 ( \pm 0.02 )</td>
<td>0.37 ( \pm 0.31 )</td>
<td>0.15 ( \pm 0.01 )</td>
<td>0.05 ( \pm 0.01 )</td>
<td>0.05 ( \pm 0.01 )</td>
</tr>
<tr>
<td>14</td>
<td>1.97( \pm 0.41 )</td>
<td>0.57 ( \pm 0.13 )</td>
<td>0.71 ( \pm 0.13 )</td>
<td>0.15 ( \pm 0.03 )</td>
<td>0.14 ( \pm 0.02 )</td>
</tr>
<tr>
<td>15</td>
<td>1.04 ( \pm 0.16 )</td>
<td>0.34 ( \pm 0.10 )</td>
<td>0.26 ( \pm 0.01 )</td>
<td>0.07 ( \pm 0.01 )</td>
<td>0.10 ( \pm 0.05 )</td>
</tr>
<tr>
<td>16</td>
<td>0.33 ( \pm 0.01 )</td>
<td>0.29 ( \pm 0.16 )</td>
<td>0.15 ( \pm 0.01 )</td>
<td>0.05 ( \pm 0.04 )</td>
<td>0.05 ( \pm 0.01 )</td>
</tr>
<tr>
<td>17</td>
<td>0.26 ( \pm 0.00 )</td>
<td>0.20 ( \pm 0.08 )</td>
<td>0.16 ( \pm 0.01 )</td>
<td>0.05 ( \pm 0.01 )</td>
<td>0.06 ( \pm 0.01 )</td>
</tr>
<tr>
<td>18</td>
<td>0.21 ( \pm 0.03 )</td>
<td>0.20 ( \pm 0.03 )</td>
<td>0.12 ( \pm 0.01 )</td>
<td>0.03 ( \pm 0.01 )</td>
<td>0.04 ( \pm 0.03 )</td>
</tr>
<tr>
<td>19</td>
<td>0.32 ( \pm 0.01 )</td>
<td>0.27 ( \pm 0.05 )</td>
<td>0.13 ( \pm 0.01 )</td>
<td>0.02 ( \pm 0.01 )</td>
<td>0.06 ( \pm 0.07 )</td>
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<td>20</td>
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<td>0.13 ( \pm 0.01 )</td>
<td>0.04 ( \pm 0.01 )</td>
<td>0.04 ( \pm 0.06 )</td>
</tr>
</tbody>
</table>

\*Total mean values of blood, cord, placenta and embryonic livers \( \pm \) 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>ab).
0.126 >0.05

<table>
<thead>
<tr>
<th>Relation</th>
<th>ρ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta/blood</td>
<td>0.943</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cord/blood</td>
<td>0.847</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placenta/cord</td>
<td>0.778</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Embryonic liver/blood</td>
<td>0.107</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Embryonic liver/cord</td>
<td>0.117</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Embryonic liver/placenta</td>
<td>0.126</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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