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Maternal and embryonic trace element concentrations and stable isotope fractionation in the smalleye smooth-hound (Mustelus higmani)

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Maternal and embryonic trace element concentrations and stable isotope fractionation in the smalleye smooth-hound (*Mustelus higmani*)

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HIGHLIGHTS

- Mothers offload essential and nonessential elements to their young.
- Element concentrations were typically higher in liver then in muscle, with the exception of Hg.
- Element and $\delta^{13}C$ values declined with increasing embryo size.
- Embryo tissue isotope values reflect mother's diet and foraging location.
- Se may play a protective role against Hg toxicity.

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ABSTRACT

Here, we evaluate maternal offloading of 16 trace elements (Essential: Co, Cr, Cu, Fe, Mn, Ni, Se and Zn; Nonessential: Al, As, Ba, Cd, Hg, Pb, Tl and U) and determine mother-offspring isotopic fractionation of $\delta^{13}C$ and $\delta^{15}N$ in muscle and liver tissue of four pregnant *Mustelus higmani* and 18 associated embryos sampled from the Amazon Coast of Brazil. Embryo muscle tissue had significantly higher concentrations of most trace elements when compared to mothers, with the exception of Hg. Embryo liver accumulated more nonessential elements than muscle (n = 7 *vs*. 0, respectively), while the Se:Hg molar ratio was >1 in liver and muscle of both mothers and embryos. Livers of embryos were moderately enriched in $\delta^{13}C$ and $\delta^{15}N$ when compared to that of their mother. Negative correlations were observed between embryo body length and $\delta^{13}C$ and trace elements concentrations. We conclude that mothers offload a large portion of all essential elements and Al, As and Pb to their young and that the isotopic fractionation of embryos reflects maternal diet and habitat occupied, with $\delta^{13}C$ diluted with embryonic growth. We also show that muscle and liver accumulate trace elements at different rates relative to the body length of embryos. The Se:Hg molar ratio suggests that Se could play a protective role against Hg toxicity during early stages of *M. higmani* embryonic development.

1. Introduction

Maternal offloading is the process whereby pregnant females transfer a portion of their body burden of contaminants to their offspring during gestation (Addison and Brodie, 1987; Anas and Wilson, 1970). While maternal offloading of essential trace elements such as Cu, Fe, Se and Zn are critical for embryonic growth and development, the transfer of nonessential trace elements (Hg, As, Cd and Pb) has no known biological function and are considered to have deleterious health and developmental effects even at low concentrations (Bosch et al., 2016). During reproduction of

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vertebrates, large amounts of lipids are required by females for the formation of eggs or lactation. In elasmobranchs, these lipids are derived from the lipid-dense liver of the mother (Davidson and Cliff, 2010; Hussey et al., 2010; Pethybridge et al., 2011, 2014; Rossouw, 1987), that stores energy for egg production and acts as a detoxification organ through accumulating trace elements at high concentrations (Ardeshir et al., 2017). As a result, trace elements, particularly nonessential trace elements, can be transferred to developing eggs and therefore embryos (Ardeshir et al., 2017; Hall, 2001).

Compared to marine organisms such as turtles (Guirlet et al., 2008, 2010; Páez-Osuna et al., 2011) and whales (Borrell et al., 1995; Desforges et al., 2012), little is known with regard to the dynamics of maternal offloading of trace elements in sharks, with most research to date focused on a few toxic elements such as Hg (Frías-Espericueta et al., 2015; Hauser-Davis et al., 2020; Lyons and Lowe, 2013; van Hees and Ebert, 2017) and persistent organic pollutants (POPs) including PCBs, DDTs and pesticides (Lyons et al., 2013; Lyons and Adams, 2014). For example, quantifying the extent of maternal transfer of Hg and POPs has been examined for eleven shark species including the common thresher shark (*Alopias vulpinus*; Lyons and Lowe, 2013; Lyons et al., 2013), white shark (*Carcharodon carcharias*; Lyons et al., 2013; Mull et al., 2013) and bull shark (*Carcharhinus leucas*; Rumbold et al., 2014; Weijs et al., 2015), while the maternal offloading of a suite of trace elements has only been investigated in the Pacific sharpnose (Cd, Cu, Pb, and Zn; Frías-Espericueta et al., 2014), common thresher shark (essential [Co, Cr, Cu, Fe, Mn, Ni, Se, Zn] and nonessential [Ag, As, Cd, Hg, Pb]; Dutton and Venuti, 2019) and in the maternal plasma and uterine fluids of the ragged-tooth sharks (*Carcharhinus taurus*; Al, As, Cd, Pb and Se; Naidoo et al., 2017).

The application of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively) to investigate the diet and movement of sharks is a well-established technique (Estrada et al., 2003, 2006; Fisk et al., 2002; Kerr et al., 2006), which has proven useful for studying nutrient transfer between mothers and young (Le Bourg et al., 2014; McMeans et al., 2009; Vaudo et al., 2010). Given neonatal sharks use energy provided through maternal investment for weeks to months while they develop foraging skills (Hussey et al., 2010), understanding stable isotope fractionation between mother-embryo tissues is fundamental to provide insight into the dynamics of maternal provisioning (de Sousa Rangel et al., 2019, 2020; McMeans et al., 2009; Olin et al., 2018; Vaudo et al., 2010).

The smalleye smooth-hound, (*Mustelus higmani*) is a small (70 cm max length) placentotrophic shark, whereby the mother nourishes individual embryos (range: 20–29 cm total length: L_T) via a vascular placenta-like structure (Tagliafico et al., 2015). The gestation period of *M. higmani* is 10 months producing on average 3–4 offspring per litter (range: 1 to 7) (Heemstra, 1997; Tagliafico et al., 2015). It is an endemic species to South America, occurring in coastal waters ranging from the Gulf of Venezuela, via Curaçao and Trinidad, to southeastern Brazil (Froese and Pauly, 2020; Piorski et al., 2010). The species forages on benthic species in neritic waters, feeding primarily on lobsters and crabs (Cortés, 1999; Heemstra, 1997; Tagliafico et al., 2015). *Mustelus higmani* is often caught as bycatch, with 40% of the species catch a result of drift and bottom gillnet artisanal fisheries in Venezuela (Tavares et al., 2009), while in Brazilian waters the species is caught in bottom trawl shrimp fisheries (Feitosa et al., 2018).

The current study aimed to examine maternal and embryonic trace element concentrations (essential: Co, Cr, Cu, Fe, Mn, Ni, Se and Zn; nonessential: Al, As, Ba, Cd, Hg, Pb, Tl and U) and determine fractionation of carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in muscle and liver tissue of *M. higmani* obtained from fisheries bycatch off the Brazilian coast. Specifically, we compared (i) the occurrence and concentrations of trace elements and trends in stable isotope values in mothers relative to their embryos and (ii) variation in trace element concentrations and stable isotope values between two tissue types with different turnover rates (fast *vs.* slow: liver *vs.* muscle) and with increasing embryo length.

2. Material and methods

2.1. Study area

Specimens of *Mustelus higmani* used in this study were captured off the Amazon Coast of Brazil, which extends over 1059 km from the Cape of Oiapoque to São Marcos Bay (Fig. 1). The region is influenced by run-off from the Amazon River to the north of Marajó Island, and that of the Tocantins River to the south, that mixes approximately 6300 km^3/year of continental waters and 9.3×10^8 t/year of sediments with ocean waters (Meade, 1994). Together with the high sediment deposition caused by the action of erosion, the development of islands and flooded plains, contributes to the maintenance of estuarine and mangrove ecosystems.

2.2. Sampling

Samples of four *M. higmani*, assessed to be mid and late-stage gestation, were obtained from bycatch mortalities captured in shrimp trawl fisheries in 2016 (Table 1). Pregnant females and all associated embryos were first measured (L_T) and weighed. Muscle (from the base of the first dorsal fin) and liver samples were taken for trace element and stable isotope (SIA; $\delta^{15}N$ and $\delta^{13}C$) analyses. All tissue samples were kept frozen at −20 °C in polyethylene bags until analysis.

2.3. Trace elements analysis

The concentrations of 16 trace elements (Essential: Co, Cr, Cu, Fe, Mn, Ni, Se and Zn; Nonessential: Al, As, Ba, Cd, Hg, Pb, Tl and U) were determined using Induced Plasma Coupled Mass Spectrometry (ICP-MS). For the analysis, wet samples were first homogenized and a 0.1 g aliquot of each tissue was transferred to a PTFE bottle with 1.5 ml of HNO_3. Following a 30 min period, 0.5 ml of H_2O_2 was added. In the case of one litter with two small embryos, liver samples were pooled to obtain sufficient mass (minimum of 0.05 g) for analysis. Samples were then heated in a microwave oven (MarsXpress, CEM Corporation) over stages of temperature (1° stage: 800 W, 180 °C, 10 min; 2° stage: 1200 W, 200 °C, 5 min; 3° stage: 1000 W, 100 °C, 10 min), and then cooled for 20 min in a cold bath. The digested solutions were transferred to polyethylene bottles, topped up to 15 ml with 1% HNO_3, and stored at 4 °C until analysis by ICP-MS. For quality control, certified reference materials [DORM-3 fish protein (n = 3) and DOLT- 4 dogfish liver (n = 2); National Research Council Canada] were used, with the percentage recovery of all elements ranging from 75.7% to 109.9% for DORM-3 and from 75.3 to 90.5% for DOLT-4 (Table 1). In addition to the analysis of five automatic replicates of each sample, all samples were weighed, digested and analysed in duplicate. Six blanks were also analysed simultaneously with mother/embryo samples and all were below the detection limit of the respective elements.

2.4. Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analysis

Muscle tissue samples (~1 g) were dried in a standard laboratory oven at 60 °C for 24 h, and then homogenized to a fine powder using a porcelain mortar and pestle. Lipids were extracted by the addition of 1.9 ml of chloroform-methanol solution (1:2) to powdered muscle tissue into cryovials, and vortexed for 1 min.

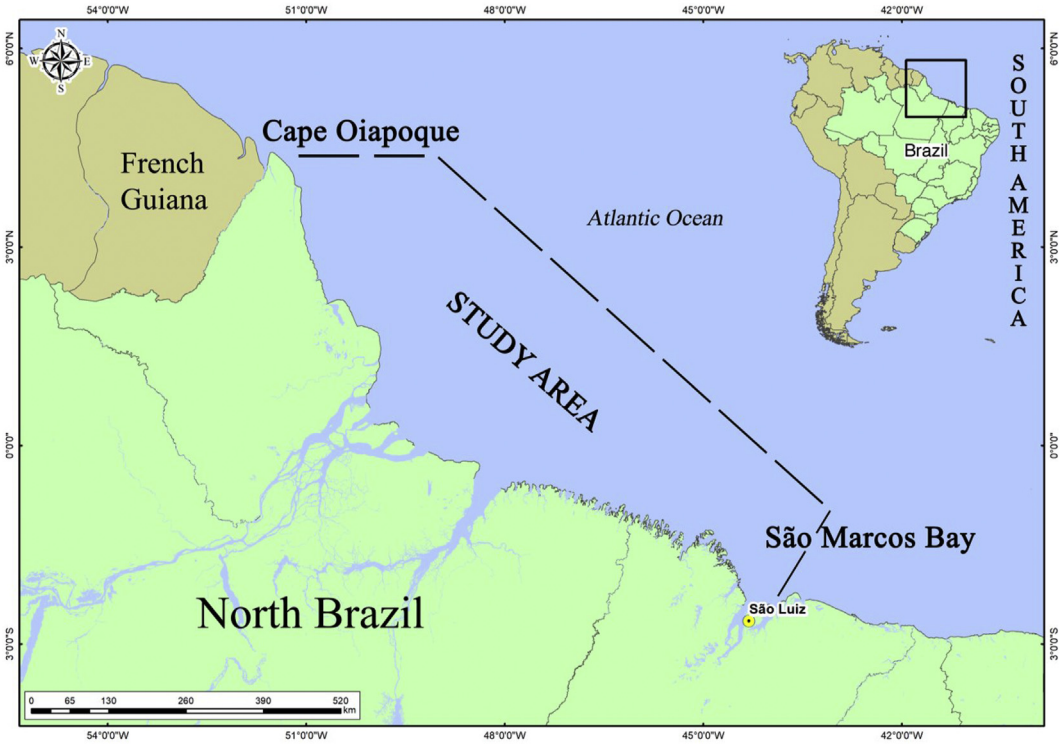

Fig. 1. Map of the study area located on the Amazon Coast of Brazil.

Table 1
Analytical recovery of the certified reference material (DORM-3 and DOLT-4) for the quality control of the muscle and liver tissue samples.

Element	DORM-3		DOLT-4	
	Recovery %	Mean ± SD	Recovery %	Mean
Al	93.6	1409.02 ± 704.62	–	–
Cr	92.1	1.72 ± 0.18	–	–
Mn	86.3	2.73 ± 0.38	–	–
Fe	91.7	314 ± 43.5	77.6	1422.81
Co	76.3	0.19 ± 0.01	–	–
Ni	76.2	1.02 ± 0.16	79.2	0.76
Cu	84	13.1 ± 1.16	90.5	28.24
Zn	75.7	39 ± 1.84	80.7	93.66
As	76.2	5.23 ± 0.71	75.3	7.27
Se	87.7	3.02 ± 0.48	78.2	6.49
Cd	76.79	0.22 ± 0.07	84.2	20.47
Ba		4.29 ± 0.34	–	–
Hg	109.9	0.45 ± 0.14	76.3	1.97
Tl		0.005 ± 0.003	–	–
Pb	86	0.34 ± 0.08	76.8	0.12
U	87.3	0.04 ± 0.004	–	–

Cryovials were then placed in a water bath at 30 °C for 24 h, after which, they were centrifuged for 4–6 min and the solvent filtered. This process was repeated once. The resulting residue was dried under a fume hood for 24–48 h to evaporate off the remaining solvent (Hussey et al., 2012a). For liver tissue, lipid extraction was repeated twice given the known high levels of lipid in this tissue (Hussey et al., 2012a). Following lipid extraction, urea was extracted in both tissues by the addition of 1.9 ml of deionized water and vortexed for 1 min. Cryovials were then placed in a water bath at 30 °C for 24 h, after which, they were centrifuged for 4–6 min and the water removed using a medical syringe. The water washing process was repeated three times and the samples dried (Li et al., 2015). Approximately 710–890 μg of muscle tissue for each sample was weighed into 5 mm × 3.5 mm tin capsules of known mass and $\delta^{15}N$ and $\delta^{13}C$ values determined using a Continuous Flow Isotope Ratio Mass Spectrometer (IR-MS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valencia, CA, USA). Stable isotope values are expressed in delta (δ) notation and are defined as parts per thousand (‰) in relation to a known standard, as follows:

$$\delta X = \left[\left(R_{\text{sample}} \Big/ R_{\text{standard}}\right) - 1\right] \times 1000,$$

where R_{Sample} and $R_{Standard}$ correspond to $^{13}C/^{12}C$ and $^{15}N/^{14}N$ values in the experimental and standard (control), respectively. Precision, assessed by the standard deviation of replicate analyses of four standards (NIST1577c, internal lab standard - tilapia muscle), USGS 40 and Urea; n = 68 for all), measured ≤0.18‰ for $\delta^{15}N$ and ≤0.14‰ for $\delta^{13}C$. Accuracy, based on the certified values of USGS 40 (n = 68 for $\delta^{15}N$ and $\delta^{13}C$) analysed throughout runs and not used to normalize samples showed a mean difference of −0.05‰ for $\delta^{15}N$ and −0.07‰ for $\delta^{13}C$ from the certified value. Instrumentation accuracy checked throughout sample runs was based on NIST standards 8573, 8547 and 8574 for $\delta^{15}N$ and 8542, 8573 and 8574 for $\delta^{13}C$ (n = 20 for all). The mean difference from the certified values were −0.17, −0.10, −0.14‰ for $\delta^{15}N$ and −0.10, −0.06 and 0.14‰ for $\delta^{13}C$.

2.5. Data analysis

The difference between mother trace element concentrations and their respective litters were determined for each tissue type (muscle and liver). Data were log transformed to meet assumptions of normality. One-sample t-tests were used to examine differences in trace element concentrations between each litter and their respective mother; individual mother provided the theoretical values.

The Se:Hg molar ratio was calculated by dividing the concentration in ppm ($\mu g.g^{-1}$) by the molecular weight. For each individual, we divided the Se concentration ($\mu g.g^{-1}$) by 78.96 and the Hg concentration ($\mu g.g^{-1}$) by 200.59, and then calculated the Se:Hg ratio separately for mothers and embryos and for both muscle and liver. Pearson's tests were performed to determine correlations between Hg concentrations and Se:Hg molar ratios.

Differences between mother and embryo $\delta^{13}C$ and $\delta^{15}N$ values for each tissue ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively), were calculated for litter–mother pairs (as a method of standardizing litters to facilitate among litter comparisons):

$$\Delta\delta^{13}C = \delta^{13}C_{embryo} - \delta^{13}C_{mother}$$

$$\Delta\delta^{13}N = \delta^{15}N_{embryo} - \delta^{15}N_{mother}$$

The $\delta^{13}C$ and $\delta^{15}N$ values of each litter were also compared to that of their respective mother using one-sample t-tests; individual mother provided the theoretical value. Differences in combined trace elements concentrations and $\delta^{13}C$ and $\delta^{15}N$ values between tissues were then tested using a univariate PERMANOVA run on Euclidean distances matrices with 9999 permutations (Anderson, 2001). Analysis was performed in the PRIMER-E software 6.0 (Anderson et al., 2008). Finally, we examined Pearson correlation values between total length and $\delta^{13}C$ and $\delta^{15}N$ values and trace element concentrations of embryos to examine the potential effects of maternal shifts in diet and the accumulation of isotopes/elements over gestation. The level of significance for all analysis was designated at $p < 0.05$.

3. Results

The mean total length (±standard error; SE) of pregnant *M. higmani* and their respective embryos was 50.6 cm ± 0.39 (range: 48–54 cm) and 16.9 cm ± 0.08 (range: 12.5–19 cm), respectively (Table 2). Embryos had higher concentrations of most trace elements in muscle tissue when compared to mothers (essential n = 8 [100%]; nonessential n = 4; [66.6%]; Table 3), with the exception of Hg. The mean Hg (±standard error) value in mothers ($0.15 \pm 0.09\ \mu g\ g^{-1}$) was almost four times higher than that recorded in the embryos. When considering the percent difference in muscle tissue trace element concentrations, embryos had 822% more Cu, 799% more Cd, and 782% more Pb, but 88% less Hg than their mothers. In contrast, liver concentrations of Al, As, Se, Hg and Cd were significantly higher in mothers compared to embryos (Table 3). Embryos had 35% less Al than mothers, 23% less As, 38% less Se, 89% less Hg, and 94% less Cd. Ba, Tl and U, were not detected in muscle, but were present at low doses in liver tissue, and at lower doses in embryos when compared to mothers. Embryos had 5% less Ba than mothers, 15% less Tl, and 31% less U. Overall, embryos concentration of trace elements (essential: Co, Fe and Se; nonessential: Al, As, Ba, Cd, Pb, Tl and U) were higher in liver than muscle (Table 4).

For all tissues, the Se:Hg ratio was >1, and embryo Se:Hg molar ratios were higher than their mothers. The mean mother muscle Se:Hg value was 8.99 ± 0.59 (ranging from 4.13 to 16.77), while the mean embryo muscle value was 706.17 ± 1.11 (ranging from 57.91 to 3397.5). In contrast, the mean mother Se:Hg ratio in liver was 50.9 ± 1.08 (range: 29.67 to 75.57) and 559.66 ± 1.08 in embryos (range: 214.10 to 1137.98). The coefficients of the Se:Hg molar ratio in muscle and liver were negatively correlated with Hg concentrations, indicated by the high Pearson's correlation coefficients (range of r^2: 0.72 to 0.98) (Fig. 2).

The fractionation of carbon between mother and embryos ($\Delta\delta^{13}C$) in muscle tissue ranged from −0.14–1.13‰, and was not significantly different. In liver, carbon fractionation values ranged from 0.12‰ to 1.45‰, with all litters significantly enriched in ^{13}C compared to mothers (Table 2). When considering $\delta^{15}N$ in muscle tissue, values were similar between mothers and embryos, with an observed fractionation value ($\Delta\delta^{15}N$) of −0.70–0.23‰. In contrast, 3 of 4 litter's livers were significantly enriched in ^{15}N relative to mothers (Table 2), with fractionation values ranging from 0.12 to 1.06‰. While the $\delta^{13}C$ values of embryo's muscle tissue were significantly enriched in ^{13}C (1.04‰; $t = 10.18$ $p < 0.001$) compared to liver (Fig. 3), there was a moderate, but significant ($t = 3.42$ $p = 0.002$) enrichment of ^{15}N (*ca.* 0.09‰) in embryo livers compared to muscle (Fig. 3).

For embryos, Al, Cu, Zn, As, Mn, Ni, Se, Pb and Co concentrations were negatively correlated with total length for muscle tissue, while Ni, Pb and Co concentrations showed a significant positive relationship in liver. One element, Cr, had a negative correlation with body size in liver tissue (Table 5). A strong negative correlation was observed between total length and muscle $\Delta\delta^{13}C$ values of embryos ($r = -0.90$, $p < 0.001$; Table 5). There was no observed relationship between $\Delta\delta^{13}C$ values of liver and body size and $\Delta\delta^{15}N$ values for both tissues.

4. Discussion

4.1. Comparison of maternal and embryo trace element concentrations

Since there is no direct contact between embryos and the external environment during gestation, all trace elements present in embryonic tissues can be considered to be derived through maternal offloading (Lyons and Lowe, 2013). Both essential (Cu, Fe, Se and Zn) and nonessential (Al, As and Pb) elements were transferred from *M. higmani* mothers to embryos, including known

Table 2
Total length (L_T) of four *M. higmani* mothers and their associated embryos (n = number of embryos in each litter) from Amazon Coast of Brazil in 2016. For embryos, $\delta^{13}C$ and $\delta^{15}N$ values for muscle and liver tissue are presented as the mean ± SE calculated for each litter, with levels of significance from one-sample t-tests shown by stars (*: $p < 0.05$, **: $p < 0.01$).

Sample	N	L_T (cm)	Muscle			Liver		
			$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	C:N	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	C:N
Mother A		50	−15.15	11.31	3.11	−15.72	10.52	3.75
Litter A	2	16	−14.62 ± 0.04	11.26 ± 0.08	3.14 ± 0.08	−15.59*	11.07**	3.47
Mother B		48	−15.42	11.36	3.16	−16.91	10.32	3.88
Litter B	3	13.1 ± 0.33	−14.29	10.66	3.10	−16.28 ± 0.14*	10.68 ± 0.19	3.73 ± 0.04
Mother C		50.5	−14.83	11.23	3.08	−16.24	10.66	3.52
Litter C	5	18.5 ± 0.31	−14.81 ± 0.05	11.18 ± 0.06	3.14 ± 0.02	−15.72 ± 0.11*	11.22 ± 0.05**	3.60 ± 0.07
Mother D		54	−14.72	11.34	3.12	−16.75	10.81	3.62
Litter D	8	17.5 ± 0.32	−14.70 ± 0.03	11.35 ± 0.05	3.13 ± 0.02	−15.59 ± 0.12**	11.59 ± 0.07**	3.45 ± 0.03

Table 3
Means ± SE (μg.g^{-1}) concentrations of 16 trace elements in muscle (A) and liver (B) tissue of four *Mustelus higmani* mothers caught off the Amazon Coast of Brazil in 2016 in comparison with their respective litters. With the exception of Cd, Ba, Tl and U (which are presented in ng.g^{-1} due to the low concentrations recorded), all values are in μg.g^{-1}. The values presented for Cd, Ba, Tl, and U in muscle tissue are below the detection limit of the equipment. Significance of the one-way *t*-test results between mother and litters are indicated by * except for Mother and Litter A where only two embryos were present. A – Muscle. B – Liver.

Element	Mother A	Litter A (N = 2)	One sampleT-test	Mother B	Litter B (N = 3)	One sample T-test	Mother C	Litter C (N = 5)	One sample T-test	Mother D	Litter D (N = 8)	One sample T-test
Essential												
Co	1.67	11.25	2.32	3.10	22.57 ± 9.67*	3.50	1.73	4.91 ± 2.20**	3.97	3.17	7.74 ± 3.17**	4.22
Cr	0.29	0.88 *	9.18	0.33	1.023 ± 0.43	2.73	0.26	0.54 ± 0.51	1.18	0.37	1.19 ± 0.57*	4.07
Cu	0.22	3.05	2.19	0.24	6.85 ± 1.79*	6.37	0.23	0.99 ± 0.14**	11.34	0.39	1.58 ± 0.60**	5.54
Fe	5.02	42.06	1.96	6.92	14.22 ± 24.49*	3.63	4.77	19.65 ± 15.16*	2.19	11.06	24.26 ± 5.63**	6.62
Mn	0.13	0.53	2.69	0.11	1.89 ± 1.04*	2.94	0.11	0.22 ± 0.12	1.93	0.22	0.26 ± 0.09	1.06
Ni	0.04	0.19	1.42	0.04	0.29 ± 0.10*	4.09	0.04	0.07 ± 0.01**	3.93	0.05	0.12 ± 0.08*	2.20
Se	0.21	1.05	2.08	0.27	2.60 ± 0.89*	4.48	0.38	0.80 ± 0.17**	5.24	0.86	0.89 ± 0.30	0.30
Zn	4.30	19.48	2.17	4.90	36.63 ± 15.58*	3.52	4.25	8.84 ± 1.13**	9.08	4.52	13.27 ± 3.81**	6.49
Nonessential												
Al	5.34	14.40	1.62	4.32	19.67 ± 8.02*	3.31	4.76	5.83 ± 0.96*	2.48	5.13	7.91 ± 1.58**	4.96
As	19.07	17.09	−0.29	23.78	37.40 ± 7.63*	3.09	17.88	16.90 ± 1.33	−1.63	30.62	22.45 ± 6.71*	−3.43
Hg	0.13	0.02	−15.45	0.07**	0.00 ± 0.002	−30.22	0.05**	0.00 ± 0.001	−167.93	0.37**	0.01 ± 0.005	−187.2
Pb	0.00	0.02	1.67	0.00	0.045 ± 0.02	0.59	0.00	0.01 ± 0.009*	2.392	0.00	0.01 ± 0.004**	8.29
Cd	<0.93	<0.93	–	<0.93	40.12 ± 38.91	1.75	<0.93	<0.93	–	<0.93	50.13 ± 83.36	1.13
Ba	<0.44	29.05	1.08	<0.44	33.08 ± 56.55	0.99	<0.44	9.97 ± 21.31	0.99	<0.44	10.1 ± 25.59	1.06
Tl	<0.003	<0.003	–	<0.003	<0.003	–	<0.003	<0.003	–	<0.003	<0.003	–
U	<0.004	<0.004	–	<0.004	<0.004	–	<0.004	<0.004	–	<0.004	<0.004	–

Element	Mother A	Litter A (N = 2 – pool)	Litter B (N = 3)	One sample T-test	Litter C (N = 5)	One sample T-test	Mother D	One sample T-test
Essential								
Co	61.70	38.01	20.93 ± 6.41	−3.90	13.27 ± 3.69	−26.90	43.32**	−32.72
Cr	1.40	1.32	0.83 ± 0.22	0.45	0.65 ± 0.04	−6.99	0.74	−1.04
Cu	1.91	14.08	4.59 ± 0.57	8.64	6.18 ± 5.23	1.18	2.62**	−10.00
Fe	123.88	282.89	93.17 ± 17.25	−0.75	60.94 ± 27.54	−1.95	63.06	0.62
Mn	0.74	4.71	0.36 ± 0.14	−1.54	0.43 ± 0.21	−5.38	0.66**	−39.82
Ni	0.20	0.24	0.14 ± 0.05	1.15	0.10 ± 0.02	0.24	0.12**	−3.25
Se	3.78	2.05	1.35 ± 0.19	−11.78	3.08 ± 1.90	−1.66	3.76**	−10.71
Zn	6.68	62.05	8.76 ± 1.05	5.43	15.89 ± 11.20	1.36	10.36**	−4.64
Nonessential								
Al	35.00	29.87	17.94 ± 4.75	−2.11	13.16 ± 0.56	−66.66	26.02**	−8.66
As	26.53	16.59	16.33 ± 0.02	−167.60	18.02 ± 11.46	−0.75	25.97**	−25.12
Hg	0.18	0.02	0.00 ± 0.00	−110.40	0.00 ± 0.00	−71.41	0.32**	−554.53
Pb	0.19	0.21	0.12 ± 0.03	0.83	0.08 ± 0.00	−5.82	0.09	−0.24
Cd	1039.02	<0.12	<0.12	–	<0.12 ± 0	–	3187.92**	−182.42
Ba	761.53	843.26	442.71 ± 136.96	0.32	314.97 ± 14.92	−15.62	425.72*	−2.629
Tl	0.41	0.31	0.35 ± 0.02	−5.34	0.42 ± 0.35	0.93	0.67**	−32.03
U	1.50	1.74	0.73 ± 0.26	−1.93	0.55 ± 0.09	−11.83	1.10**	−14.35

*p < 0.05.
**p < 0.01.

hazardous elements, such as Pb and Cd, that have no known biological function. As would be expected, Fe, Zn, Co and Cu, elements that are critical for successful embryonic growth and development (Tacon, 1987; Wood et al., 2012), were offloaded at the highest concentrations. Key constituents of metabolic enzymes (e.g., Cu, Co, Fe, Mn, Se, Zn), assisting oxygen transport (e.g. Fe), providing protection against free radical damage (e.g., Se, Zn), and aiding metabolism of carbohydrates (e.g., Cr) are physiological processes that are dependent on concentrations of these trace elements (Wood et al., 2012).

In contrast, four trace elements offloaded to *M. higmani* embryos such as Cd, Hg, and Pb are among the most toxic to organisms (ATSDR, 2017). While As concentrations in marine fish are higher (1–10 μg g^{-1}) than those in freshwater fish (<1 μg g^{-1}) (Ciardullo et al., 2010; Schaeffer et al., 2006), the high doses recorded in *M. higmani* are likely related to the characteristics of the study area; large amounts of total As is transported from the Andes to the ocean via sediment and dissolved in water discharged from the Amazon river basin (Scarpelli, 2005). High concentrations of As (up to 100 μg g^{-1}) have been recorded in some edible marine species in other locations, but in most cases, the values are total As concentrations, rather than that of the toxic inorganic form, Arsenite (ATSDR, 2007). Typically up to 95% of As in fish muscle is present in the non-toxic arsenobetane form (Zhang et al., 2016).

Unlike most elements analysed, Hg was found in higher concentrations in muscle and liver tissue of *M. higmani* mothers compared to embryo tissues. Hg muscle concentrations in pregnant female *M. higmani* were on average ~17 times higher than embryos, while liver concentration were ~19 times higher. These results are in agreement with those reported for the common thresher and leopard shark (Dutton and Venuti, 2019; Lyons and Lowe, 2013; van Hees and Ebert, 2017). While most contaminants preferentially accumulate in liver tissue, Hg, in particular methylmercury (which is the principal form recorded in most fish species with more than 98% recovery of total mercury; Souza-Araujo et al., 2016a; Watanabe et al., 2017; WHO, 1990, 2008), tends to associate with proteins (Mason et al., 1995), and accumulates in different tissue types (Lyons and Lowe, 2013; Mull et al., 2012). Given the liver is the primary energy storage organ from which females draw resources to nourish offspring, and most Hg is held in muscle, only a small proportion of the mother's Hg burden are offloaded to litters.

Despite the occurrence of low concentrations of Cd and Pb in pregnant female *M. higmani* muscle tissue (the Cd doses were below the LD), they were present in respective embryo tissues.

Table 4
Mean ± SE values of 16 trace elements in muscle and liver tissue of *M. higamni* embryos from Amazon Coast of Brazil in 2016. Statistical significance, and Pseudo-F values for comparisons between all muscle and liver samples of individuals sampled from the Northern Coast of Brazil are shown; *p < 0.05; **p < 0.01

Element	Muscle Mean ± SE	Liver Mean ± SE	Pseudo-F
Essential			
Co	0.00 ± 0.00	0.02 ± 0.00**	15.70
Cr	0.83 ± 0.11	0.81 ± 0.05	0.21
Cu	2.06 ± 0.47	4.16 ± 0.94	25.92
Fe	20.31 ± 3.28	94.70 ± 14.08**	29.45
Mn	0.48 ± 0.14	0.79 ± 0.27	0.26
Ni	0.12 ± 0.02	0.13 ± 0.01	<0.001
Se	1.03 ± 0.16	2.46 ± 0.26**	21.68
Zn	14.42 ± 2.45	14.62 ± 3.52	0.24
Nonessential			
Al	9.08 ± 1.26	18.89 ± 1.58**	49.66
As	22.81 ± 1.80	17.83 ± 1.26*	43.97
Ba	13.17 ± 6.00	0.43 ± 0.03**	136.05
Cd	0.02 ± 0.01	0.28 ± 0.15*	31.06
Hg	0.03 ± 0.01	0.04 ± 0.01	0.29
Pb	0.01 ± 0.00	0.11 ± 0.00**	96.93
Tl	0.00	0.34 ± 0.04**	62.61
U	0.00	0.82 ± 0.08**	6.60

Previous studies investigating maternal offloading of Cd and Pb in the Pacific sharpnose and common thresher shark also found that Cd and Pb accumulated in embryo muscle and liver tissue. For both species and including *M. higmani*, the doses of both elements were higher in liver compared to muscle tissue (Dutton and Venuti, 2019; Frías-Espericueta et al., 2014).

With the exception of Hg, liver tissue of embryos accumulated higher concentrations of all nonessential elements relative to muscle. This is to be expected given liver tissue is more metabolically active than muscle (Ardeshir et al., 2017). Hussey et al. (2010) reported that neonatal dusky sharks (*Carcharhinus obscurus*) had high hepatic lipid levels, inferred by high hepatosomatic index (HSI) immediately following birth and a decline in HSI values with increasing body size. This indicates maternal allocation of lipid reserves to developing offspring during gestation that facilitates the transfer of non-essential elements. Moreover, during the early phase of *M. higmani* gestation, when maternal allocation of lipid reserves is higher, a negative correlation between trace element concentrations and increasing embryo body size was found. This indicates that trace elements are diluted with growth, and are more concentrated in younger (mid gestation) than near term individuals (late gestation). This trend is identical to that observed for aplacental sharks (Le Bourg et al., 2014).

There are a growing number of studies examining Se:Hg molar ratios as a measure of toxicity, with the inclusion of this parameter in health risk assessments for the consumption of fish muscle (white) tissue. This is based on the fact that Se is known to neutralize the toxicity of Hg^{2+} (Pařízek and Ošťádalová, 1967), through high affinity binding to Hg that produces inert mercuric selenide (HgSe) compounds in the bloodstream (Burk et al., 1974). Se, if present at high relative concentrations (Se:Hg > 1), may consequently have a potential protective effect on reducing methylmercury toxicity for consumption of fish muscle, including shark meat (Burger et al., 2013; Dutton and Venuti, 2019; Kaneko and Ralston, 2007). In contrast, acute toxicity of methymercury can occur when the Se:Hg molar ratio is < 1 (Peterson et al., 2009; Ralston and Raymond, 2010; Yang et al., 2010). For *M. higmani*, the Se:Hg molar ratio was much higher than 1 in all tissues of mothers and embryos, suggesting that Se may play a protective role against Hg toxicity during embryonic developmental stages similar to that reported for the common thresher shark (Dutton and Venuti, 2019).

4.2. Maternal-offspring fractionation of stable isotopes

Our results indicate that the mean $\delta^{13}C$ and $\delta^{15}N$ levels (−15.04 ± 0.14 and 11.33 ± 0.05, respectively) for pregnant female *M. higmani* sampled off Northern Brazil are quite variable when compared to other species of the genus *Mustelus* previously studied ($\delta^{13}C$: 15.8 ± 1 and $\delta^{15}N$: 14.5 ± 1.2; Domi et al., 2005; $\delta^{13}C$: 14.1 ± 0.5 and $\delta^{15}N$: 15.68 ± 0.4; Borrell et al., 1995; $\delta^{13}C$: 16.3 ± 0.4 and $\delta^{15}N$: 12.5 ± 0.8; Endo et al., 2013). While baseline stable isotope values were not sampled in our study area, isotope values across studies suggest *M. higmani* is a secondary consumer (Cortés, 1999; Tagliafico et al., 2015). Neonatal elasmobranchs would be expected to have higher $\delta^{15}N$ and $\delta^{13}C$ values than mothers, as a result of isotopic discrimination of maternal resources throughout development (Pilgrim, 2007; Post et al., 2007). Our relatively small $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values, however, indicates minimal fractionation occurs between *M. higmani* mothers and embryos.

Embryos were more enriched in ^{13}C relative to mothers, but the $\Delta\delta^{13}C$ values was below the upper the limit of the 0–2‰ change expected to denote a difference in trophic level (Caut et al., 2008; McMeans et al., 2009). This suggests that the isotopic composition of *M. higmani* embryos (both $\delta^{15}N$ and $\delta^{13}C$) predominantly reflects maternal tissue composition and consequently their foraging patterns/habitat use (Pilgrim, 2007). Similar, but slightly higher $\Delta\delta^{13}C$ values have been reported between mother and embryos for other placentotrophic species including the Atlantic sharpnose ($\delta^{13}C$: 1.2 in both tissues and $\delta^{15}N$: 1.4 and 1.7‰ in muscle and liver,

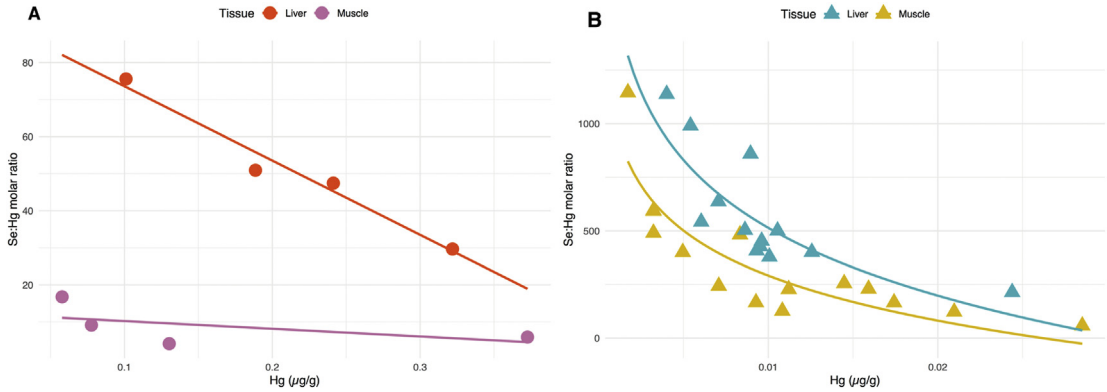

Fig. 2. Relationship between Se:Hg molar ratio and Hg concentration in muscle and liver of mothers (A) and embryos (B) of *Mustelus higmani* sampled from the Amazon Coast of Brazil in 2016.

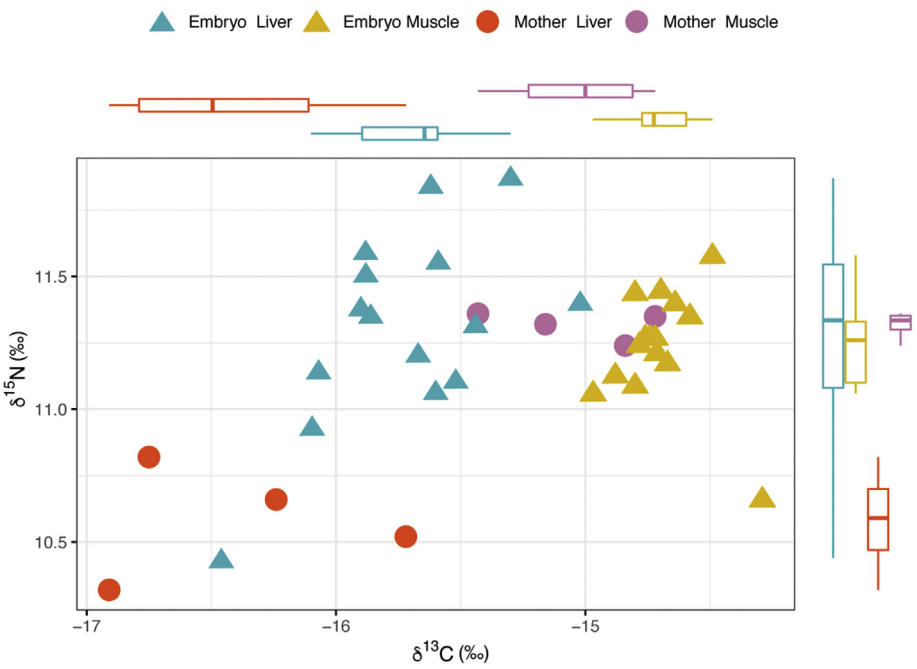

Fig. 3. Muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values for four pregnant female *Mustelus higmani* and associated embryos (n = 18) sampled from the Amazon Coast of Brazil in 2016.

Table 5
Pearson correlations between embryo total length and differences between mother and embryo $\delta^{13}C$ and $\delta^{15}N$ values ($\Delta\delta^{15}N$ and $\Delta\delta^{13}C$) and trace elements in muscle and liver tissue of *Mustelus higmani* sampled from the Amazon Coast of Brazil in 2016. Ba, Tl and U were excluded from this analysis due to values lower than the LD. * = p < 0.05

Variable	Muscle		Liver	
	r	P	r	p
$\Delta\delta\ ^{13}C$	−0.90	<0.001*	−0.014	0.96
$\Delta\delta\ ^{15}N$	0.30	0.25	0.24	0.36
Essential				
Co	−0.73	<0.001*	−0.57	0.008*
Cr	−0.091	0.73	−0.55	0.011*
Cu	−0.87	<0.001*	−0.14	0.55
Fe	0.055	0.83	−0.37	0.1
Mn	−0.81	<0.001*	−0.2	0.39
Ni	−0.66	0.004*	−0.56	0.01*
Se	−0.75	<0.001*	0.34	0.14
Zn	−0.75	<0.001*	−0.12	0.61
Nonessential				
Al	−0.74	<0.001*	−0.24	0.3
As	−0.58	0.014*	−0.22	0.35
Cd	−0.031	0.91	0.049	0.84
Hg	−0.11	0.67	−0.17	0.47
Pb	−0.64	0.005*	−0.51	0.02*

respectively; McMeans et al., 2009), scalloped hammerhead ($\delta^{13}C$: 1.01‰ and $\delta^{15}N$: 0.82‰ in muscle; Vaudo et al., 2010) and blacktip shark ($\delta^{13}C$: 0.26‰ and $\delta^{15}N$: 0.88‰ in muscle; Vaudo et al., 2010). In agreement with Olin et al. (2018), our results show that the degree of fractionation between embryos and mothers of placentatrophic sharks is variable, but typically positive fractionation occurs. Intraspecific variation among mothers could be attributed to physiological differences, if gestation occurs in the same environment and individuals consume the same resources (Barnes et al., 2008) or a result of variable resource use patterns.

As a more metabolically active organ, $\delta^{15}N$ and $\delta^{13}C$ values in liver are known to turn over faster compared to muscle tissue (Kim et al., 2012; MacNeil et al., 2006), allowing examination of recent shifts in diet/habitat use (Hussey et al., 2012b). Observed differences in the stable isotope values of both pregnant female and *M. higmani* embryo muscle and liver tissue may reflect shifts in diet/habitat use of mothers during the gestation period. Alternatively, this may be a result of physiological process driving variation among tissues or varying amino acid composition between tissue types (Lorrain et al., 2002; Pinnegar and Polunin, 1999).

The observed negative relationship between embryo L_T and $\Delta\delta^{13}C$ values could be due to initial yolk phase use as a nutritional source in the early stages of *M. higmani* gestation. This result and the non-significant relationship between $\Delta\delta^{15}N$ values and embryo L_T in muscle and liver, however contrasts with previous findings (McMeans et al., 2009). Alternatively, our results are similar to Le Bourg et al. (2014), whereby $\delta^{13}C$ values in muscle tissue of *Squalus megalops* embryos (an aplacental species) were negatively correlated with L_T. The authors suggested this could be due to higher incorporation rates of heavy isotopes in tissues such as cartilage or kidney in growing embryos. Further comparisons of $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values in embryo-mother pairs in multiple tissues of *M. higmani* as well as other shark species across a range of reproductive strategies is required to fully characterize these isotope dynamics.

5. Conclusions

We conclude that pregnant female *M. higmani* offload both essential and nonessential trace elements to their embryos during gestation and that isotopic fractionation between mother and embryo is minimal, indicating embryo tissues primarily reflect those of the mother's diet and habitat occupied during gestation. It is also evident that the liver accumulates more nonessential trace elements than muscle and that trace elements concentrations in embryos are diluted with growth. Finally, the Se:Hg molar ratio of *M. higmani* tissues suggest that Se may play a protective role against Hg toxicity during the early stages of embryonic development.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Juliana de Souza-Araujo: Conceptualization, Writing - original draft. **Ryan Andrades:** Data curation. **Marcelo de Oliveira Lima:** Formal analysis. **Nigel E. Hussey:** Formal analysis. **Tommaso Giarrizzo:** Conceptualization, Supervision.

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