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BRIEF COMMUNICATIONS

Mother–embryo isotope (δ^{15}N, δ^{13}C) fractionation and mercury (Hg) transfer in aplacental deep-sea sharks

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Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic values and total mercury (Hg) concentrations were analysed in muscle and liver of mothers and embryos of two aplacental shark species, *Squalus megalops* and *Centrophorus moluccensis*. Embryos of the two species had similar or lower isotopic values than their respective mothers, the only exception being for δ^{13}C, which was higher in the liver of *C. moluccensis* embryos than in their mothers. Hg concentrations were systematically lower in embryos compared with their mothers suggesting a low transfer of this element in muscle and liver.

Key words: elasmobranchs; isotopic differences; maternal influence; trace metal.

Stable isotope analysis and trace metal analyses have been used increasingly to investigate the trophic ecology, foraging habitats and heavy metal contamination of elasmobranchs over the last decade (Domi *et al.*, 2005; McMeans *et al.*, 2010; Pethybridge *et al.*, 2010). Ratios of nitrogen isotopes (^{15}N:^{14}N, denoted δ^{15}N) are commonly used to infer the trophic position of a species within a community, while carbon isotope ratios (^{13}C:^{12}C, denoted δ^{13}C) are used to infer the food webs used by that species (Hobson, 1999). These properties have allowed for the successful use of carbon and nitrogen stable isotope values to depict the feeding ecology of elasmobranchs, including trophic interactions and ontogenetic shifts of diet and habitat use (Hussey *et al.*, 2012*a*).

Mercury (Hg) concentrations provide a useful indicator of foraging habitats and trophic position of large marine predators because body burden concentrations have been found to be highly correlated with size and age, trophic position, environmental variables and geographic location (Rivers *et al.*, 1972; Atwell *et al.*, 1998; Power *et al.*, 2002; Colaço *et al.*, 2006). Hg is a non-essential metal that is released from both natural and anthropogenic sources (Fitzgerald *et al.*, 2007) and the consumption of

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marine products, including many shark species, represents an important pathway of human exposure to Hg (Buzina *et al.*, 1989; Svensson *et al.*, 1992). Consequently, it is important to monitor Hg concentrations because of the toxicity of this metal.

In order to correctly interpret stable isotope and Hg values in the tissues of young sharks, especially those tissues with long turnover rates such as muscle (Domi *et al.*, 2005; McMeans *et al.*, 2010; Pethybridge *et al.*, 2010), it is critical to understand the dynamics of maternal provisioning (Vaudo *et al.*, 2010). Mother–offspring differences in stable isotope values have been previously investigated in a few placentatrophic shark species (McMeans *et al.*, 2009; Vaudo *et al.*, 2010) but this issue has not been explored in aplacental species. In placentatrophic sharks, embryos tend to have enriched isotopic values relative to their mothers (McMeans *et al.*, 2009; Vaudo *et al.*, 2010). Differences in Hg concentrations between mothers and embryos and maternal transfer of this contaminant have been studied in placental (Hueter *et al.*, 1995; Adams & McMichael, 1998) and aplacental sharks (Childs *et al.*, 1973; Greig *et al.*, 1977; Hueter *et al.*, 1995; Pethybridge *et al.*, 2010). For example, Pethybridge *et al.* (2010) showed that the magnitude of Hg transfer to embryos was higher in placentatrophic sharks than in aplacental species and hypothesized that this was due to differences in the reproduction mode. Indeed, embryos of placentatrophic sharks are nourished by external yolk-sac reserves before switching to a placental resource (Hamlett, 1993). In contrast, embryos of aplacental sharks are successively nourished by external and internal yolk-sac reserves (lecithotrophy) with no supplementary maternal contribution (Guallart & Vicent, 2001; Braccini *et al.*, 2007; Kousteni & Megalofonou, 2011), apart from embryos of oophagous sharks which also feed on unfertilized eggs (Lyons *et al.*, 2013). Consequently, stable isotope dynamics and Hg transfers may differ between mothers and embryos of placentatrophic and aplacental shark species. In this study, differences of stable carbon and nitrogen isotope ratios and Hg concentrations were investigated between mothers and embryos of two species of aplacental sharks.

Five gravid shortspine spurdogs *Squalus megalops* (MacLeay 1881) and four gravid small-fin gulper sharks *Centrophorus moluccensis* Bleeker 1860 were caught off the south-east coast of La Réunion Island, western Indian Ocean (55° 33′ E; 21° 07′ S) between January and March 2012. Muscle and liver tissues were collected from each mother and their respective embryos and were then dried and ground into a fine powder. As lipids are highly depleted in ^{13}C relative to other tissue components (DeNiro & Epstein, 1977), lipids were removed from muscle and liver samples by three successive extractions prior to stable isotope analysis [1 h shaking in 4 cm^3 of cyclohexane at room temperature and subsequent centrifugation; Chouvelon *et al.* (2011)]. Lipid extraction is an important step to standardize data among individuals and across the two species sampled (Hussey *et al.*, 2012*b*). This process also removes the urea and trimethylamine oxide (TMAO) present in shark tissues, which can potentially affect $\delta^{15}N$ values (Hussey *et al.*, 2012*a*). After drying, lipid-free sub-samples were weighed (0·350 to 0·450 ± 0·001 mg) in tin cups and analysed with a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific; www.thermoscientific.com) coupled to an elemental analyser (Flash EA1112, Thermo Scientific) at the isotope facility of the University of La Rochelle (France). Reference gas was calibrated against international reference materials (IAEA-N1, IAEA-N2 and IAEA-N3 for nitrogen; NBS-21, USGS-24 and IAEA-C6 for carbon). Results are expressed in the δ notation relative to PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, according to the equation: $\delta X = [(R_{sample}\, R_{standard}{}^{-1}) - 1] \times 10^3$,

where X is ^{13}C or ^{15}N and R is the isotope ratio ^{13}C:^{12}C or ^{15}N:^{14}N. Replicate measurements of a laboratory standard (acetanilide) indicated that analytical errors were <0·1‰ for $\delta^{13}C$ and $\delta^{15}N$. Per cent C and N elemental composition of tissues were obtained using the elemental analyser and were used to calculate the sample C:N (mean ± S.D. C:N = 2·77 ± 0·16 for muscle and 3·28 ± 0·24 for liver in *S. megalops* and 2·50 ± 0·24 for muscle and 4·38 ± 1·72 for liver in *C. moluccensis*).

Total Hg measurements were calculated using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254; www.onlinecas.com). At least two aliquots of 5–15 mg of homogenized dry muscle and liver tissue subsamples for each individual were analysed. The analytical quality (*i.e.* accuracy and reproducibility) of the Hg measurements by the AMA-254 was assessed by the analyses of blanks and TORT-2 certified reference material (Lobster Hepatopancreas Reference Material from the National Research Council of Canada; www.nrc-cnrc.gc.ca) at the beginning and at the end of the analytical cycle, and by running controls for every 10 samples (Bustamante *et al.*, 2006). Results of quality controls showed a satisfactory precision with a relative S.D. of 6·0%. The accuracy was 93% of the assigned concentration ($n = 14$). The detection limit was 0·005 μg g^{-1} dry mass (dwt). All Hg concentrations in tissues reported are expressed in μg g^{-1} dwt.

The values of the embryos ($\delta^{13}C$ and $\delta^{15}N$ values and Hg concentrations) were compared to that of their mother using one-sample *t*-tests, individual mother isotopic values being the theoretical values. Levels of significance were determined by using sequential Bonferroni corrections (Rice, 1989) for each variable, in each species. Correlation coefficients between total body length (L_T) and $\delta^{13}C$, $\delta^{15}N$ and Hg concentrations were computed for muscle and liver in *S. megalops* embryos ($n = 21$), but not in *C. moluccensis* because of low sample size ($n = 8$) and the skewed distribution (7 cm for two embryos, and 20 cm for the other six embryos).

TABLE I. Differences in isotopic values between mothers and embryos. Levels of significance after sequential Bonferroni correction (one-sample *t*-tests) are shown in the table. The signs indicate that embryos have non-significantly different (=), higher (>) or lower (<) isotopic values than their mother

Squalus megalops					
Mother's identification	Embryos (n)	$\delta^{13}C$ muscle	$\delta^{13}C$ liver	$\delta^{15}N$ muscle	$\delta^{15}N$ liver
1	5	=	=	<**	=
2	3	=	=	=	>*
3	3	=	=	<*	=
4	4	=	<***	<***	=
5	6	<*	<***	<***	=
Centrophorus moluccensis					
1	2	=	>*	<*	<*
2	2	=	>**	=	<*
3	2	=	NA	=	NA
4	2	=	>*	=	<*

*, $P < 0·05$; **, $P < 0·01$; ***, $P < 0·001$; NA, not available.

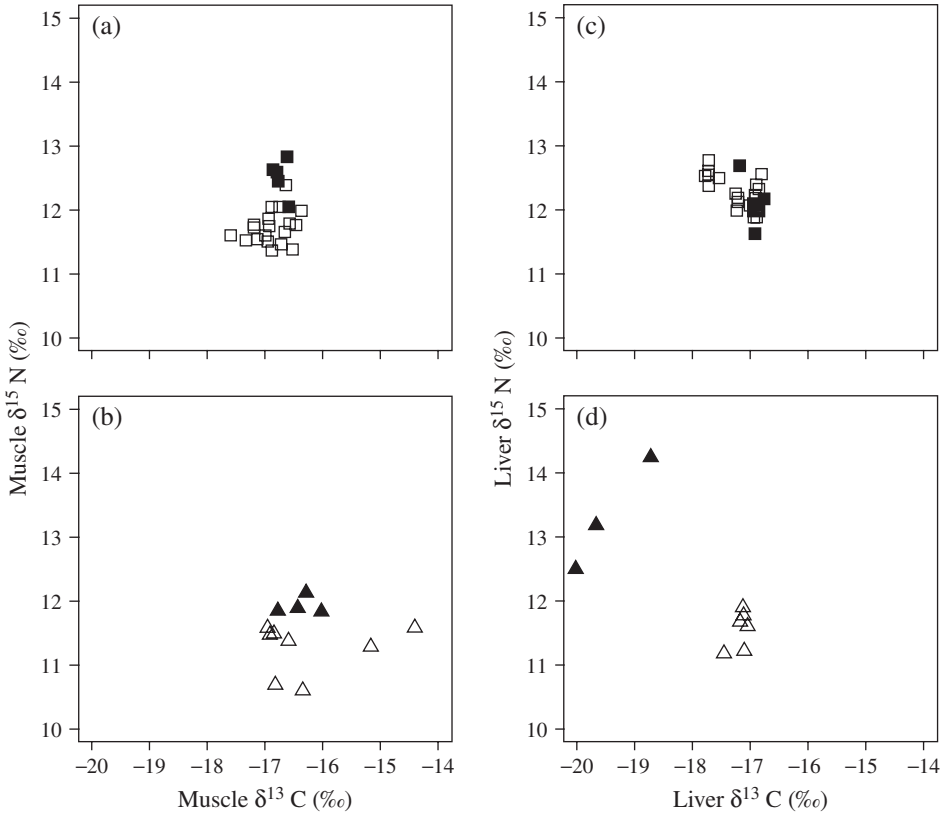

FIG. 1. Isotopic values from (a), (b) muscle and (c), (d) liver tissues of (a) *Squalus megalops* (■, mothers; □, embryos) and (b) *Centrophorus moluccensis* (▲, mothers; △, embryos).

For *S. megalops*, most embryos had δ^{13}C values similar to their mothers in both muscle and liver, but they had lower δ^{15}N values than their mothers in muscle and similar δ^{15}N values in liver (Table I and Fig. 1). All embryos had lower Hg concentrations than their mothers in muscle and liver tissues (Table II). Values for δ^{13}C and δ^{15}N, and Hg concentrations in muscle of *S. megalops* embryos were negatively correlated with their L_T (r being similar, *c.* −0·60), while δ^{13}C was the only variable correlated with L_T in liver ($r = -0{\cdot}84$; Fig. 2). For *C. moluccensis*, δ^{13}C and δ^{15}N values were generally not different between mothers and embryos in muscle tissues, whereas in liver, embryos had higher values for δ^{13}C and lower ones for δ^{15}N (Table I and Fig. 1). As in *S. megalops*, *C. moluccensis* embryos had lower Hg concentrations than their mothers in muscle and liver tissues (Table II).

Previous studies on mother–offspring differences of stable isotope ratios in placentatrophic sharks have shown that embryos are generally enriched in δ^{15}N, but fractionation of δ^{13}C is variable among species (McMeans *et al.*, 2009; Vaudo *et al.*, 2010). These results show that embryos of aplacental sharks tended to have similar or lower isotopic values when compared to their mothers. The exception, however, was δ^{13}C values in liver of *C. moluccensis* embryos, which were generally higher than in their mothers, suggesting a change in the feeding area of the mothers after the maturation

TABLE II. Hg concentration ($\mu g\ g^{-1}$ dry mass) in maternal and embryo's muscle and liver. For *Squalus megalops*, all differences between embryos and mothers are significant at $P < 0{\cdot}001$ level after sequential Bonferroni correction (one-sample *t*-tests) and, for *Centrophorus molluccensis* (levels of significance are shown in Table I)

Squalus megalops					
Mother			Embryos		
Identification	Muscle	Liver	*n*	Muscle (mean ± S.D.)	Liver (mean ± S.D.)
1	10·476	2·606	5	0·552 ± 0·181	0·041 ± 0·009
2	10·225	2·030	3	0·480 ± 0·059	0·038 ± 0·003
3	9·274	0·430	3	0·475 ± 0·028	0·026 ± 0·002
4	10·714	3·652	4	0·445 ± 0·026	0·028 ± 0·006
5	10·194	2·932	6	0·388 ± 0·035	0·010 ± 0·007
Centrophorus moluccensis					
1	3·727	0·941	2	0·409 ± 0·011 **	0·050 ± 0·001***
2	6·548	4·851	2	0·855 ± 0·097 *	0·059 ± 0·006 **
3	4·281	2·027	2	0·362 ± 0·088 **	NA
4	5·093	0·965	2	0·359 ± 0·071 **	0·036 ± 0·005 **

NA, not available.

of the eggs because isotopic values in liver are considered shorter-term, more recent indicators of diet than in muscle tissue (Domi *et al.*, 2005; Chouvelon *et al.*, 2012). Nevertheless, C:N in liver of *C. moluccensis* mothers are high ($6{\cdot}27 \pm 0{\cdot}91$, $n = 4$), indicating insufficient lipid extraction. Males and non-gravid females of this species, sampled and analysed at the same time for another purpose, did not display such a high mean C:N ($3{\cdot}25 \pm 0{\cdot}58$, $n = 5$ males and 6 females). High lipid contents are probably responsible for an underestimation of $\delta^{13}C$ values in liver of *C. moluccensis* mothers. Furthermore, there was no negative linear relationship in *S. megalops* muscle and liver of embryos between C:N and $\delta^{13}C$ (indicating depletion of $\delta^{13}C$ by lipids; computations were not done for *C. moluccensis* embryos, and adults of the both species because of low sample sizes). A lower $\delta^{15}N$ in embryos is most often observed in muscle of *S. megalops* and liver of *C. moluccensis*. Hg concentrations in embryos were always lower than in mothers, as observed in previous studies for placental and aplacental sharks (Childs *et al.*, 1973; Greig *et al.*, 1977; Hueter *et al.*, 1995; Adams & McMichael, 1998; Pethybridge *et al.*, 2010). Lower Hg concentrations in the embryos of aplacental sharks are probably the result of the absence of supplementary maternal transfer of nutrients.

Values of $\delta^{13}C$ and $\delta^{15}N$, and Hg concentrations in muscle of *S. megalops* embryos were negatively correlated with L_T. This is probably the result of the absence of supplementary maternal transfer of nutrients during development: heavy isotopes and Hg atoms become progressively diluted in the body of the growing embryos despite uptake coming from yolk consumption. This is supported by similar correlation coefficients which indicate that the dilution kinetics are similar for isotopes and Hg. As embryos of aplacental sharks that receive no supplemental nourishment cannot have a higher dry mass than the initial eggs, global isotopic values and total Hg content may be the same in the initial eggs and in the fully developed embryos (if changes due to metabolic

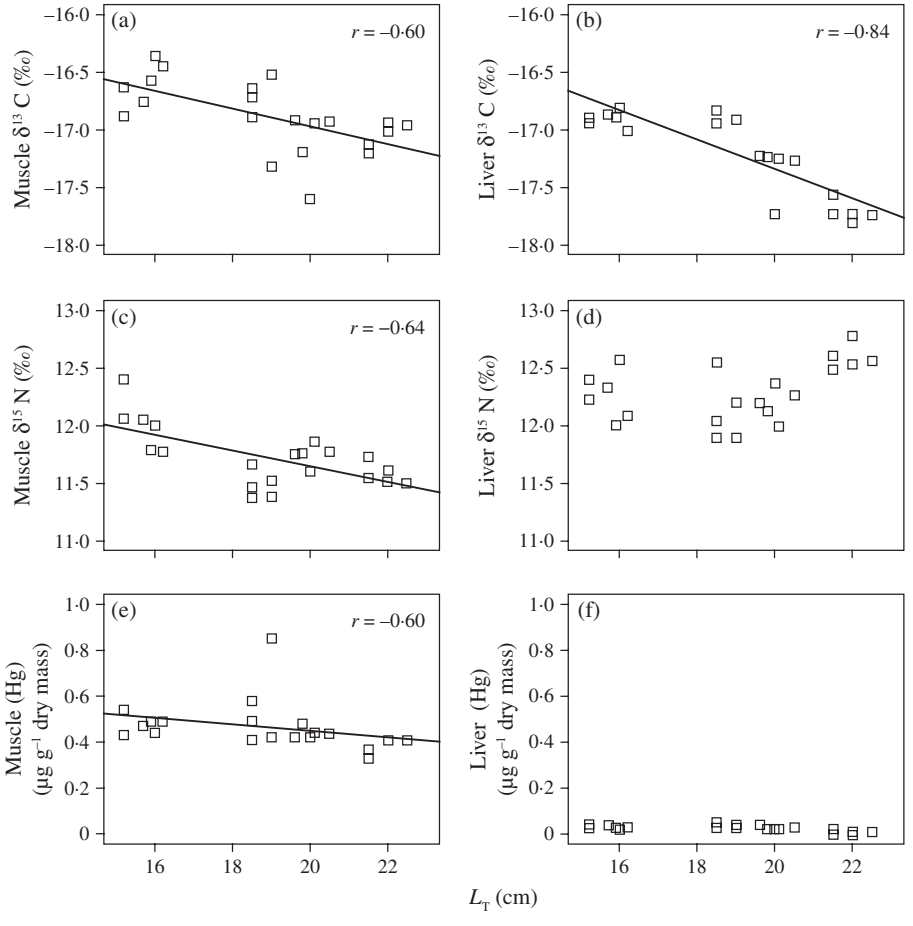

FIG. 2. Correlation coefficients between total body length (L_T) and (a), (b) δ^{13}C, (c), (d) δ^{15}N and (e), (f) Hg concentrations in (a), (c), (e) muscle and (b), (d), (f) liver of *Squalus megalops* embryos. Correlation coefficients are shown only if $P < 0.05$. The curves were fitted by: (a) $y = -0.0754x - 15.4575$, (b) $y = -0.1269x - 14.8017$, (c) $y = -0.0680x + 13.0054$ and (e) $y = -0.0134x + 0.7164$.

processes and waste removal are excepted). Consequently, dilution in muscle may occur because of incorporation of heavy isotopes and Hg in another tissue (such as cartilage or kidney) at a faster rate. In contrast, isotopic values in muscle increase with increasing body length in the embryos of the placentatrophic Atlantic sharpnose shark *Rhizoprionodon terraenovae* (Richardson 1836) because they switch from yolk to placental nourishment (McMeans *et al.*, 2009). Maternal transfer of Hg has not previously been observed in other species of aplacental sharks (Childs *et al.*, 1973; Greig *et al.*, 1977) except maybe for New Zealand lanternshark *Etmopterus baxteri* Garrick 1957 (Pethybridge *et al.*, 2010) and for lamniforms (Lyons *et al.*, 2013). This last exception could be explained by oophagy in lamniforms. Gravid females continue to produce unfertilized eggs, which the embryos consume as supplemental nourishment. The δ^{13}C values of liver were negatively correlated with L_T in *S. megalops* embryos while δ^{15}N values and Hg concentrations showed no relationship with L_T.

In conclusion, the present results show that the transfer mechanisms of nutrients, as inferred from stable isotope values, and Hg differ between placental and aplacental sharks. The correlations of isotopic values and Hg concentrations in muscle with L_T of *S. megalops* embryos also suggest that muscle is not the primary tissue where heavy isotopes and Hg are incorporated during development. Further studies on other tissues would be necessary to confirm that other tissues accumulate heavy isotopes and Hg in embryos of *S. megalops*. Unfortunately, the low number of *C. moluccensis* embryos and the skewed distribution of L_T did not allow for the study of correlations of L_T with isotopic values and Hg concentrations in this species.

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