# Mother-embryo isotope ($\delta$15N, $\delta$13C) fractionation and mercury (Hg) transfer in aplacental deep-water sharks

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Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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 $\delta^{13}C$, which was higher in the liver of *C. moluccensis* embryos than in their mothers. Hg concentrations were systematically lower in embryos compared to their mothers suggesting a low transfer of this element in muscle and liver.

Key words: sharks; stable isotopes; trace metal; maternal influence.

Stable isotope analysis and trace metal analyses have been increasingly used 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 $\delta^{15}N$) are commonly used to infer the trophic position of a species within a community, while carbon isotope ratios ($^{13}C$:$^{12}C$, denoted $\delta^{13}C$) 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 isotopes values to depict the feeding ecology of elasmobranchs, including trophic interactions and ontogenetic shifts of diet and habitat use (see Hussey *et al.*, 2012 for a review).

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/age, trophic position, environmental parameters 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 marine products including many shark species represents an important pathway of human exposure to Hg (Buzina *et al.*, 1989; Svensson *et al.*, 1992). Consequently, Hg concentrations are important to monitor 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 of stable isotope values have been previously investigated in a few placentatrophic shark species (McMeans *et al.*, 2009; Vaudo *et al.*, 2010). However, to the best of our knowledge, this issue has not been explored in aplacental species. In placentatrophic sharks, embryos tended to have enriched isotopic values relative to their mothers (McMeans *et al.*, 2009; Vaudo *et al.*, 2010). Differences of Hg concentrations between mothers and

embryos and maternal transfer of this contaminant have been studied in placental (Hueter *et al.*, 1995; Adams and 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 the magnitude of Hg transfer to embryos was higher in placentatrophic sharks than in aplacental species and hypothesised 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 and Vicent, 2001; Braccini *et al.*, 2007; Kousteni and Megalofonou, 2011), if we except oophagous sharks whose embryos also feed on unfertilised 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 the present study, we investigated differences of stable carbon and nitrogen isotope ratios and Hg concentrations between mothers and embryos of two species of aplacental sharks.

Five gravid shortspine spurdogs *Squalus megalops* (MacLeay) and four gravid smallfin gulper sharks *Centrophorus moluccensis* (Bleeker) 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 then were dried and ground into a fine powder. As lipids are highly depleted in $^{13}C$ relative to other tissue components (DeNiro and 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 standardise data among individuals and across the two species sampled (Hussey *et al.*, 2012b). This process should also remove the urea and trimethylamine oxide (TMAO) present in shark tissues, which can potentially affect $\delta^{15}N$ values (Hussey *et al.*, 2012a). 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, Germany) coupled to an elemental analyser (Flash EA1112 Thermo Scientific, Italy) 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 $\delta^{15}N$, respectively, according to the equation: $\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$, where $X$ is $^{13}C$ or $^{15}N$ and $R$ is the isotope ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Replicate measurements of a laboratory standard (acetanilide) indicated that analytical errors were <0.1‰ for $\delta^{13}C$ and $\delta^{15}N$. Percent C and N elemental composition of tissues were obtained using the elemental analyser and used to calculate the sample C:N ratio (mean C:N ± S.D.: 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 performed using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254 from Altec©). At least two aliquots of 5 to 15 mg of homogenised 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) at the beginning and at the end of the analytical cycle, and by running controls every 10 samples (Bustamante *et al.*, 2006). Results of quality controls showed a satisfactory precision with a relative standard deviation of 6.0%. The accuracy was 93% of the assigned concentration (n = 14). The detection limit was 0.005 µg $g^{-1}$ dry weight (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 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 of lengths (7 cm for two embryos, and 20 cm for the other six).

For *S. megalops*, most embryos had $\delta^{13}C$ values similar to their mothers in both muscle and liver, but they had lower $\delta^{15}N$ values than their mothers in muscle and similar $\delta^{15}N$ values in liver (Table 1 and Fig. 1). All embryos had lower Hg concentrations than their mothers in muscle and liver tissues (Table 2). Values for $\delta^{13}C$ and $\delta^{15}N$, and Hg concentrations in muscle of *S. megalops* embryos were negatively correlated with their total body length (r being similar, *ca* - 0.60) while $\delta^{13}C$ was the only variable correlated with total length in liver (r = - 0.84, Fig. 2). For *C. moluccensis*, $\delta^{13}C$ and $\delta^{15}N$ values were generally not different between mothers and embryos in muscle tissues whereas, in liver, embryos had higher values for $\delta^{13}C$ and lower ones for $\delta^{15}N$ (Table 1 and Fig. 1). As in *S. megalops*, *C. moluccensis* embryos had lower Hg concentrations than their mothers in muscle and liver tissues (Table 2).

Previous studies on mother-offspring differences of stable isotopes ratios in placentatrophic sharks have shown that embryos are generally enriched in $\delta^{15}N$ but fractionation of $\delta^{13}C$ is variable among species (McMeans *et al.*, 2009; Vaudo *et al.*, 2010). The present results show that embryos of aplacental sharks tended to have similar or lower isotopic values when compared to their mothers. The exception, however, was $\delta^{13}C$ 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 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 ratios in liver of *C. moluccensis* mothers are high (6.27 ±0.91, n = 4), indicating insufficient

lipid extraction. Males and non-gravid females of this species, sampled and analyzed at the same time for another purpose, had not such a high mean C:N ratio (3.25 ± 0.58, n = 5 males and 6 females; these data will be published elsewhere). High lipid contents are likely 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 (data not shown) between C:N ratios 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 and McMichael, 1998; Pethybridge *et al.*, 2010). Lower Hg concentrations in the embryos of aplacental sharks is likely 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 total length. This is likely 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 weight 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 we except changes due to metabolic processes and waste removal). 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 shark *Rhizoprionodon terraenovae* because they switch from yolk to placental nourishment (McMeans *et al.*, 2009). Maternal transfer of Hg has not

been previously observed in other species of aplacental sharks (Childs *et al.*, 1973; Greig *et al.*, 1977) except maybe for *Etmopterus baxteri* (Pethybridge *et al.*, 2010) and for lamniform sharks (Lyons *et al.*, 2013). This last exception could be explained by oophagy in lamniforms. Gravid females continue to produce unfertilised eggs, which the embryos consume as supplemental nourishment. The $\delta^{13}C$ values of liver were negatively correlated with total length in *S. megalops* embryos while $\delta^{15}N$ values and Hg concentrations showed no relationship with total length.

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 total length 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 lengths did not allow for the study of correlations of total length with isotopic values and Hg concentrations in this species.

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species of placentatrophic sharks. *Journal of Fish Biology* **77,** 1724-1727.

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 by stars (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). The signs indicate that embryos have non-significantly different (=), higher (>), or lower (<) isotopic values than their mother.

| *Squalus megalops* | | | | | |
|---|---|---|---|---|---|
| mother's id | n embryos | $\delta^{13}$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 | = | >* | = | <* |

Table II. Hg concentration (µg $g^{-1}$ dry weight) in maternal and embryo's muscle and liver. For *S. megalops*, all differences between embryos and mothers are significant at $p < 0.001$ level after sequential Bonferroni correction (one-sample t-tests) and, for *C. molluccensis*, levels of significance are shown in the Table I.

| *Squalus megalops* | | | | | |
|---|---|---|---|---|---|
| Mother | | | Embryos | | |
| Id | muscle | liver | n | muscle (mean ± SD) | liver (mean ± SD) |
| 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 ** |

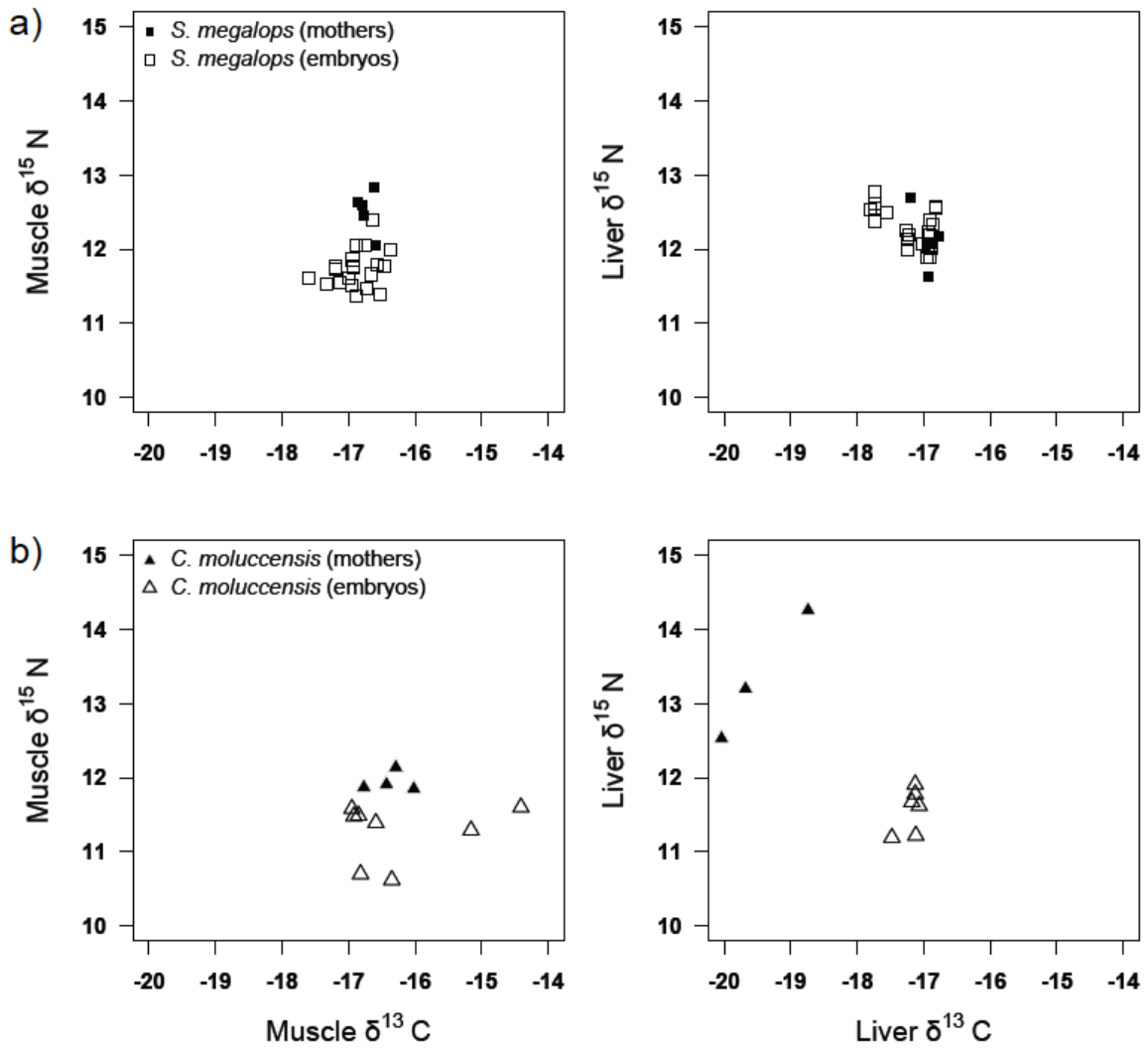


Fig. 1. Isotopic values (‰) from muscle and liver tissues in mothers and embryos of a) *S. megalops* and b) *C. moluccensis*.

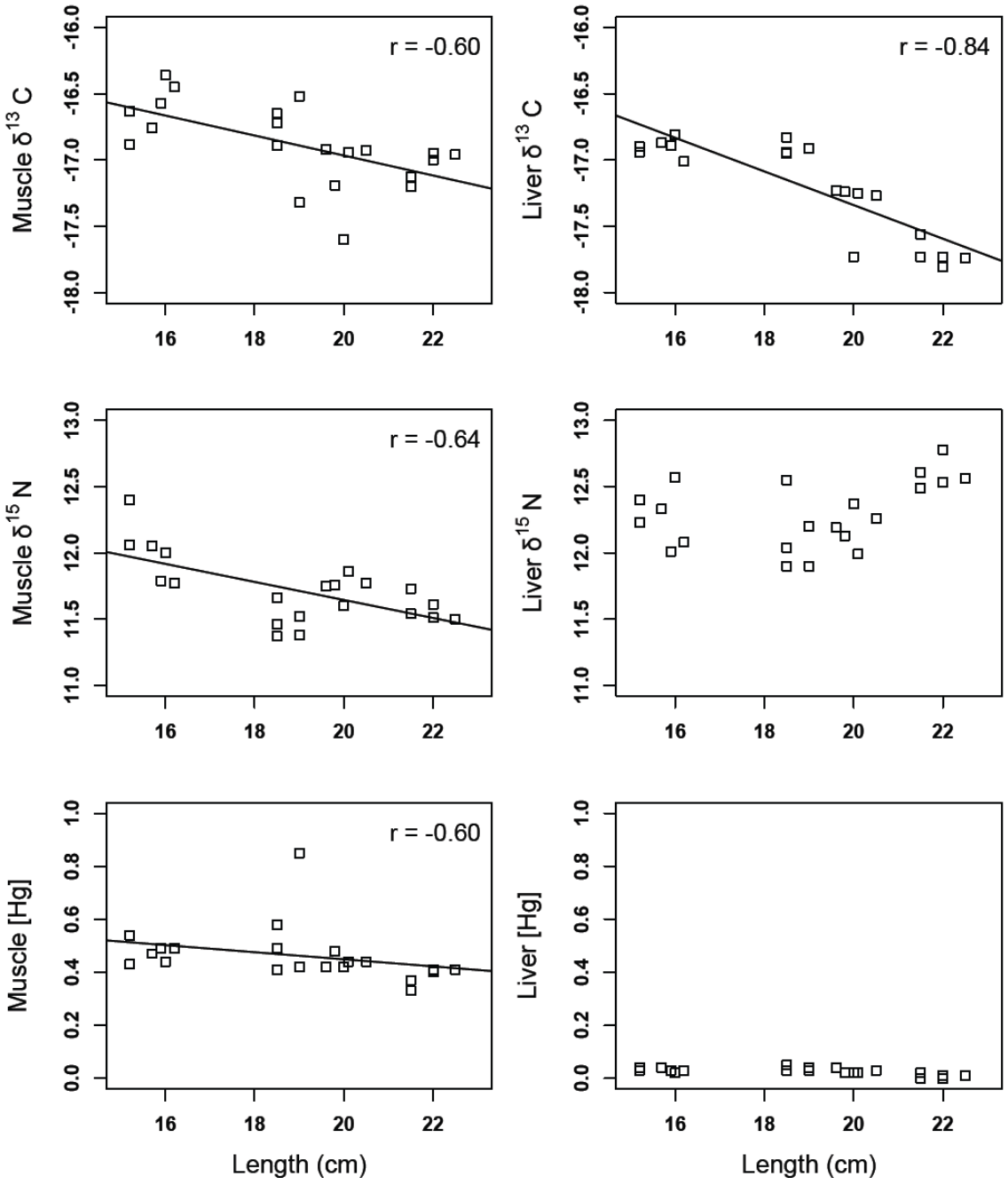


Fig. 2. Correlation coefficients between total body length and $\delta^{13}C$ (‰), $\delta^{15}N$ (‰), and Hg concentrations (µg $g^{-1}$ dry weight) in muscle and liver of *S. megalops* embryos. Correlation coefficients are shown only if $p < 0.05$.