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Stable-isotope comparisons between embryos and mothers of a placentatrophic shark species

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Stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotopes of Atlantic sharpnose shark *Rhizoprionodon terraenovae* embryos and mothers were analysed. Embryos were generally enriched in ^{15}N in all studied tissue relative to their mothers' tissue, with mean differences between mother and embryo δ^{15}N (i.e. $\Delta\delta^{15}$N) being 1·4‰ for muscle, 1·7‰ for liver and 1·1‰ for cartilage. Embryo muscle and liver were enriched in ^{13}C (both $\Delta\delta^{13}$C means = 1·5‰) and embryo cartilage was depleted ($\Delta\delta^{13}$C mean = −1·01‰) relative to corresponding maternal tissues. While differences in δ^{15}N and δ^{13}C between mothers and their embryos were significant, muscle δ^{15}N values indicated embryos to be within the range of values expected if they occupied a similar trophic position as their respective mothers. Positive linear relationships existed between embryo total length (L_T) and $\Delta\delta^{15}$N for muscle and liver and embryo L_T and $\Delta\delta^{13}$C for muscle, with those associations possibly resulting from physiological differences between smaller and larger embryos or differences associated with the known embryonic nutrition shift (yolk feeding to placental feeding) that occurs during the gestation of this placentatrophic species. Together these results suggest that at birth, the δ^{15}N and δ^{13}C values of *R. terraenovae* are likely higher than somewhat older neonates whose postpartum feeding habits have restructured their isotope profiles to reflect their postembryonic diet.

Key words: Atlantic sharpnose shark; carbon isotopes; multiple tissues; nitrogen isotopes; *Rhizoprionodon terraenovae*; shark neonate.

INTRODUCTION

Fisheries management, conservation and ecological concerns have prompted considerable efforts to better understand the early life history of sharks (McCandless *et al.*, 2007). Overall, these efforts seem designed to gather information regarding shark nursery area delineation and habitat use, shark reproduction, feeding, growth, population trends, trophic interactions and anthropogenic impacts. Stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) can be useful indicators of fish food habits, trophic interactions and habitat use (Hobson *et al.*, 1995; Herzka, 2005; Leakey *et al.*, 2008). While no published report exists regarding similar analysis applied directly to neonate sharks, several studies have used stable-isotope analysis to investigate

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the diet and movement of sharks (Fisk *et al*., 2002; Estrada *et al*., 2003, 2006; Kerr *et al*., 2006).

Based on theoretical considerations, an interesting possibility exists regarding the interpretation of results from stable-isotope analysis of shark neonates. The isotope value of an organism is expected to be higher than that of its diet by 0–2‰ for δ^{13}C and 2–5‰ for δ^{15}N (DeNiro & Epstein, 1978; Minigawa & Wada, 1984; Post, 2002). Hence, if a shark embryo and its mother are physiologically similar and if no remodelling or other biasing of nutrients occurs between both organisms, neonate tissues would be expected to exhibit enriched stable-isotope values relative to maternal tissues for some period after gestation, *i.e.* until postpartum feeding habits restructure and reduce the neonate's stable-isotope value. The potential implication of this regarding the assignment of trophic position is obvious and possibly significant, and thus an investigation into the matter might best precede conventional studies of shark neonates using δ^{13}C and δ^{15}N analysis. One challenge to such an investigation is that opportunities to sample tissues from shark neonates and their respective mothers in the wild are generally rare and to pointedly do so would probably be time consuming and expensive. It seems, however, that the potential issue of neonate isotope enrichment might be addressed by studying embryos and their mothers. With the above in mind, comparisons of stable isotopes of carbon and nitrogen between sets of embryos and mothers of a viviparous shark species were made.

MATERIALS AND METHODS

The Atlantic sharpnose shark *Rhizoprionodon terraenovae* (Richardson) (Carcharhinidae), was chosen as the focus of this study because it was readily available, it was not imperilled (Cortés, 2000) and mature females were expected to be pregnant during the period of the field operations. Twelve pregnant *R. terraenovae* and all embryos present in each female's left uterus (all embryos from one fish hereafter referred to as a litter; the left uterus was arbitrarily chosen as sample uterus) were collected from 27 September to 20 October 2004 in the western Gulf of Mexico during two scientific surveys (30° 00′ N; 88° 11′ W and 26° 17′ N; 96° 20′ W). *Rhizoprionodon terraenovae* were opportunistically sampled for stable-isotope analysis, *i.e.* only fish that died during survey operations were available for study. The total length (L_T) of each adult and embryo was measured and each fish was assigned an identification number (ID; from 1 to 12 for adults based on the mean L_T of their embryos (ascending order); the embryo IDs facilitated later recognition of litter mates and mothers). Approximately 2 g of muscle (from above the spine), one vertebra and 2 g of liver (from the left liver lobe) were collected from each adult and 0·2 g samples of muscle and liver, and several vertebrae were collected from similar locations in each embryo. Tissues were individually placed in ID-coded Whirl Pack® bags and immediately frozen until analysis.

Prior to δ^{13}C and δ^{15}N analysis, muscle, liver and cartilage from all adults and embryos were freeze dried for 48 h. Tissue was then pulverized using a ball-mill grinder (SPEX CertiPrep 8000-D ball milling unit, SPEX CertiPrep; www.spexcsp.com) and lipids were extracted from muscle and liver (to decrease the influence of the different lipid profiles expected among the samples; Post *et al*., 2007) using a modified method outlined by Bligh & Dyer (1959) as follows: 5 ml of 2:1 chloroform to methanol solution was added to each vial prior to vortexing vials for 30 s, vials were allowed to sit for 24 h, each vial was decanted through filter paper and the solution was collected in a pre-weighed aluminium tray, an additional 5 ml of 2:1 chloroform to methanol solution was added to each vial and vials were vortexed for 30 s before decanting each tissue sample and solution through filter paper to isolate the study samples. Cartilage was not lipid extracted due to the difficulty of pulverizing it to thoroughly expose it to the extraction solution. Approximately 1 μg of each tissue was loaded into tin capsules and stable carbon and nitrogen isotope ratios were determined on a continuous-flow

isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron; www.thermo.com). Replicate analyses of a bovine internal laboratory standard ($n = 16$) yielded a precision of $\pm 0{\cdot}16‰$ and $\pm 0{\cdot}04‰$ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Stable isotopes were expressed as a delta (δ) value where $\delta X = 1000\ [(R_{sample}\ R_{standard}^{-1}) - 1]$, with $X = {}^{15}N$ or ${}^{13}C$ and $R =$ the ratio ${}^{15}N : {}^{14}N$ or ${}^{13}C : {}^{12}C$ (Peterson & Fry, 1987). The conventional standard reference materials used were NIST sucrose for CO_2 and NIST ammonium sulphate for N_2.

Values of $\delta^{13}C$ and $\delta^{15}N$ in all tissues and the L_T of *R. terraenovae* were normally distributed (Shapiro–Wilk's test) and hence data transformation was not performed. Means ± S.E. and corresponding 95% CI were calculated for $\delta^{13}C$ and $\delta^{15}N$ values for muscle, liver and cartilage for adults and 11 of 12 litters (those that were comprised of greater than one embryo). The adult mean for $\delta^{13}C$ and $\delta^{15}N$ for each tissue was considered significantly different (relative to the others) if its CI was not overlapped by the CI of either of the two remaining tissue means, and similarly, litter mean values (11 litters total) for $\delta^{13}C$ and $\delta^{15}N$ for each tissue were considered significantly different using the same technique. Differences between mother and embryo $\delta^{15}N$ and $\delta^{13}C$ for each tissue are indicated by $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$, respectively, and were calculated for each embryo–mother pair (as a method of standardizing litters to facilitate among litter comparisons) as follows: $\Delta\delta^{15}N = \delta^{15}N_{embryo} - \delta^{15}N_{mother}$ and $\Delta\delta^{13}C = \delta^{13}C_{embryo} - \delta^{13}C_{mother}$. Note that the use of the symbol Δ to indicate the difference in $\delta^{15}N$ and $\delta^{13}C$ between mother and embryo *R. terraenovae* differs from its previous usage to indicate the difference in $\delta^{15}N$ and $\delta^{13}C$ between an animal and its diet (Caut *et al*., 2009). Mean ± S.E. $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ values were calculated for each of the three study tissues for each of the 11 litters comprising greater than one embryo and *t*-tests with Bonferroni pair-wise corrections were used to test whether values were significantly different from zero ($P < 0{\cdot}05$; *i.e.* whether litter $\delta^{15}N$ and $\delta^{13}C$ values were significantly higher or lower than those of adults). Two-way ANOVA was performed to determine the influence of litter and tissue on: (1) $\Delta\delta^{15}N$ and (2) $\Delta\delta^{13}C$, using data for the 11 litters containing greater than one embryo and all three tissues. To test whether a relationship existed between adult L_T and stable isotope values, simple linear regressions (SLR) were fitted to adult L_T and $\delta^{15}N$ and $\delta^{13}C$ data for each of the three tissues studied. Possible associations between embryo L_T and $\Delta\delta^{15}N$ values and embryo L_T and $\Delta\delta^{13}C$ values were also investigated using SLR fit to data for each of the three tissues studied. For SLR analysis, the slope of the associations were considered significant if $P < 0{\cdot}05$ for t values. All analyses were conducted using SYSTAT® Version 11.0 (Systat Software Inc.; www.systat.com).

RESULTS

Mean ± S.E. $\delta^{15}N$ values for each of the three study tissues collected from adult sharks were: muscle, $14{\cdot}2 \pm 0{\cdot}2‰$ (range 12·4–14·7‰); liver, $14{\cdot}0 \pm 0{\cdot}1‰$ (13·6–14·5‰); cartilage, $13{\cdot}6 \pm 0{\cdot}2‰$ (12·1–14·3‰). Mean ± S.E. $\delta^{13}C$ values for adults were: muscle, $-16{\cdot}3 \pm 0{\cdot}0‰$ (range −16·5 to −16·1‰); liver, $-17{\cdot}6 \pm 0{\cdot}3‰$ (−19·8 to −16·4‰); cartilage, $-13{\cdot}8 \pm 0{\cdot}1‰$ (−14·4 to −12·8‰). Differences among adult tissue means (determined by CI overlap) were not significant: $\delta^{15}N$ values in any tissue muscle and liver were similar but higher than adult cartilage and $\delta^{13}C$ values were significantly different among all three tissues, with cartilage being highest (*i.e.* least negative) followed by muscle and then liver (Fig. 1).

While variations in $\delta^{15}N$ and $\delta^{13}C$ within litters were generally low, some litters displayed considerably more variation than others (Fig. 1). Differences among the tissue means of each litter (determined by CI overlap) were as follows: for $\delta^{15}N$, litters 5, 10 and 11 were higher in liver and muscle than cartilage, litters 8 and 9 were higher in muscle than cartilage and litters 3 and 6 were higher in liver and cartilage; for $\delta^{13}C$, litters 2, 6 and 7 were lower in liver than muscle, litters 1, 4, 5, 8 and 11 were lower in liver than muscle or cartilage and litters 3 and 9 was lower in

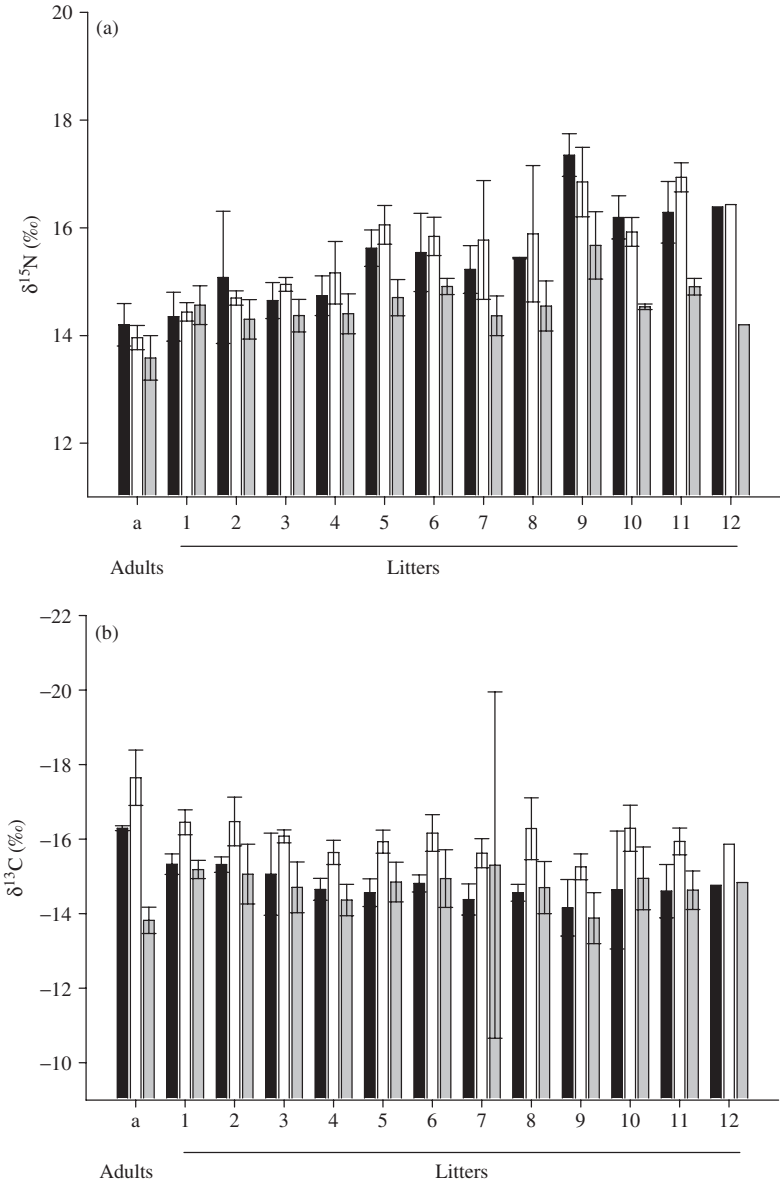

FIG. 1. Means ± 95% CI for (a) δ^{15}N and (b) δ^{13}C for muscle (■), liver (□) and cartilage (▒) for 12 *Rhizoprionodon terraenovae* mothers (adults) and embryos comprising 11 left uterus litters (numbers 1–11) and corresponding information for one left uterus litter comprised by a single embryo (number 12). Note that comparisons between mean adult and litter δ^{15}N and δ^{13}C are not valid using this figure because the isotope levels of each litter were probably biased by those of its mother.

liver than cartilage (Fig. 1). Relative to their mothers, mean $\Delta\delta^{15}$N values indicated that litters were enriched in ^{15}N by 1·4, 1·7 and 1·1‰ in muscle, liver and cartilage, respectively, and mean $\Delta\delta^{13}$C values indicated that litters were enriched in ^{13}C by 1·2‰ in muscle and liver, and depleted in ^{13}C by −1·0‰ in cartilage (Table I).

When considered individually, however, not all litters followed these trends (Table I). Based on *t*-test comparisons, values of $\Delta\delta^{15}$N and $\Delta\delta^{13}$C for each of the studied tissues were significantly different from zero for 45·5–81·1% of all litters for which variance data were available, with all significantly different $\Delta\delta^{15}$N and $\Delta\delta^{13}$C values representing enrichments except for those of cartilage $\Delta\delta^{13}$C (Table I). The two-way ANOVA showed that litter, tissue and their interaction were each significantly influential regarding $\Delta\delta^{15}$N and $\Delta\delta^{13}$C variation (Table II).

No linear relationship existed between adult L_T and δ^{15}N or δ^{13}C for any adult tissue ($P > 0{\cdot}05$). Values of $\Delta\delta^{15}$N, however, were positively correlated with embryo L_T regarding muscle [$r^2 = 0{\cdot}381$, $P < 0{\cdot}001$; Fig. 2(a)] and liver [$r^2 = 0{\cdot}265$, $P < 0{\cdot}001$; Fig. 2(c)], but not cartilage [$P > 0{\cdot}05$; Fig. 2(e)], albeit low amounts of the variation in $\Delta\delta^{15}$N for muscle was explained by L_T (38%). Values of $\Delta\delta^{13}$C were positively correlated with embryo L_T in muscle [$r^2 = 0{\cdot}400$, $P < 0{\cdot}001$; Fig. 2(b)], but not in liver [$P > 0{\cdot}05$; Fig. 2(d)] or cartilage [$P > 0{\cdot}05$; Fig. 2(f)].

DISCUSSION

The significant differences in ^{15}N and ^{13}C among adult *R. terraenovae* muscle, liver and cartilage in the present study corresponded with the results of MacNeil *et al.* (2005) that δ^{15}N was lower in cartilage than in muscle or liver and δ^{13}C was higher in cartilage relative to muscle or liver in adult shortfin makos *Isurus oxyrinchus* Rafinesque, thresher sharks *Alopias vulpinus* (Bonnaterre) and blue sharks *Prionace glauca* (L.); however, all trends among these three tissues were not similar between the two studies. Differences in δ^{15}N and δ^{13}C among fish tissues could have been attributed to physiological composition or lipid concentration (for δ^{13}C) (Pinnegar & Polunin, 1999), although lipids were extracted from muscle and liver. Values of δ^{15}N among adult *R. terraenovae* for each tissue were generally similar and below the upper limit of the above 2–5‰ change expected to denote a trophic step difference. Values of δ^{13}C of muscle and cartilage, but not liver, were likewise generally similar among adult *R. terraenovae* and below the upper limit of the above 0–2‰ change expected to denote a trophic difference. Typically, the trophic position of fishes is assigned based on muscle δ^{15}N values (Hobson *et al.*, 1995). Using data compiled by Caut *et al.* (2009), a fish-specific value of $3{\cdot}2 \pm 1{\cdot}4$‰ (muscle mean ± S.D.) can be calculated for the expected increase in muscle δ^{15}N between successive trophic steps. Based on the fact that all of the differences in muscle δ^{15}N values among the studied adult *R. terraenovae* were below this fish-specific mean, the above conclusion that these fish occupied a similar trophic position is further and probably more robustly supported. Variations in δ^{15}N and δ^{13}C that did exist among adult *R. terraenovae* for each study tissue (Fig. 1) may have been associated with minor diet or physiological variations or both (Pinnegar & Polunin, 1999; Sweeting *et al.*, 2005).

Based on mean muscle $\Delta\delta^{15}$N values (calculated for each mother–litter pair) that ranged from 0·0 to 2·7‰ (Table I), the embryos comprising the litters studied occupied similar trophic positions relative to their respective mothers. The intra-tissue variation in δ^{15}N and δ^{13}C present among litter mates could have been attributed to differences in embryo growth, metabolism or both, *i.e.* factors known to sometimes produce δ^{15}N and δ^{13}C variations among individuals even when they are reared in the same environment and are consuming the same resources (Gannes *et al.*, 1997;

TABLE I. Total length (L_T) of 12 mother (adult) *Rhizoprionodon terraenovae* and mean ± S.E. L_T of embryos comprising their left uterus litters (n = number of embryos per left uterus litter). $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ are the differences in $\delta^{15}N$ and $\delta^{13}C$, respectively, between each embryo and its mother for muscle, liver and cartilage and are reported as the mean ± S.E. calculated for each litter

			Muscle		Liver		Cartilage	
Sample	n	L_T (cm)	$\Delta\delta^{15}N$ (‰)	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	$\Delta\delta^{13}C$ (‰)	$\Delta\delta^{15}N$ (‰)	$\Delta\delta^{13}C$ (‰)
Adult 1		77·4	0·1 ± 0·1	0·9 ± 0·1*	0·5 ± 0·1*	3·2 ± 0·1*	0·9 ± 0·1*	−0·6 ± 0·1*
Litter 1	3	6·5 ± 0·0						
Adult 2		84·0	0·3 ± 0·3	1·1 ± 0·0	0·8 ± 0·0*	0·5 ± 0·2	0·4 ± 0·1	−0·7 ± 0·2
Litter 2	2	8·5 ± 0·0						
Adult 3		81·4	0·0 ± 0·1	1·3 ± 0·3	1·4 ± 0·0*	0·8 ± 0·1*	0·6 ± 0·1*	−0·6 ± 0·2
Litter 3	3	10·4 ± 0·1						
Adult 4		83·5	2·4 ± 0·1*	1·6 ± 0·1*	1·5 ± 0·2*	1·8 ± 0·1*	2·3 ± 0·1*	−0·9 ± 0·2*
Litter 4	4	11·5 ± 0·0						
Adult 5		80·0	1·0 ± 0·1*	1·8 ± 0·1*	1·7 ± 0·1*	2·4 ± 0·1*	0·4 ± 0·1*	−1·6 ± 0·2*
Litter 5	3	14·1 ± 0·3						
Adult 6		79·0	1·1 ± 0·2*	1·5 ± 0·1*	2·3 ± 0·1*	0·8 ± 0·2	1·3 ± 0·0*	−1·3 ± 0·4*
Litter 6	3	14·3 ± 0·2						
Adult 7		70·0	1·0 ± 0·1	1·9 ± 0·1*	2·1 ± 0·3	1·2 ± 0·1	1·0 ± 0·1	−1·6 ± 1·1
Litter 7	2	14·5 ± 0·0						
Adult 8		71·0	1·2 ± 0·1*	1·8 ± 0·1	1·6 ± 0·3	1·1 ± 0·2	0·6 ± 0·1	−0·9 ± 0·2
Litter 8	2	14·8 ± 0·0						
Adult 9		86·6	2·7 ± 0·1*	2·0 ± 0·0*	2·3 ± 0·2*	1·9 ± 0·1*	1·4 ± 0·2*	−1·1 ± 0·2
Litter 9	3	15·0 ± 0·1						
Adult 10		74·0	1·9 ± 0·1	1·5 ± 0·4	2·4 ± 0·1	2·4 ± 0·1	0·9 ± 0·0*	−0·9 ± 0·2
Litter 10	2	15·2 ± 0·0						
Adult 11		81·5	2·3 ± 0·2	1·6 ± 0·2*	2·5 ± 0·1*	0·8 ± 0·1*	1·5 ± 0·0	−1·1 ± 0·2*
Litter 11	3	15·4 ± 0·1						
Adult 12		72·0	2·3	1·7	2·3	4·0	1·1	−0·6
Litter 12	1	18·6						
Mean ± S.E.			1·4 ± 0·2	1·5 ± 0·1	1·7 ± 0·1	1·5 ± 0·2	1·1 ± 0·1	−1·0 ± 0·1

*, the $\Delta\delta^{15}N$ or $\Delta\delta^{13}C$ for a mother–litter pair is significantly different ($P < 0·05$) from zero, an indication that the litter's embryo v. mother differences were significant.

TABLE II. Results of two-way ANOVA used to test the effect: (1) litter and (2) tissue on $\Delta\delta^{15}$N and $\Delta\delta^{13}$C, which are the differences in δ^{15}N and δ^{13}C, respectively, between *Rhizoprionodon terraenovae* embryos and mothers

	$\Delta\delta^{15}$N				$\Delta\delta^{13}$C			
	d.f.	M.S.	*F*	*P*	d.f.	M.S.	*F*	*P*
Litter	11	3·14	55·38	<0·001	11	0·90	7·77	<0·001
Tissue	2	4·08	71·83	<0·001	2	60·34	524·2	<0·001
Litter × tissue	22	0·68	11·92	<0·001	22	1·13	9·77	<0·001
Error	57	0·06			57	0·12		

Sweeting *et al.*, 2005). A larger sample size of embryos would have been useful to better describe the variations and comparisons between embryos sampled from both uteri would likewise have been valuable. Nevertheless, significant δ^{15}N enrichment in all three tissues studied and δ^{13}C enrichment in muscle and liver of embryos relative to their respective mothers were observed for embryos comprising 63·6–81·8% of all litters for which variance data were available, as was significant ^{13}C depletion in embryonic cartilage in 45·5% of the same litters (Table I).

Although embryos generally did not exhibit the expected increase in δ^{15}N of 2–5‰ relative to their mothers, it is interesting that positive linear relationships existed between embryo L_T and $\Delta\delta^{15}$N for muscle and liver and embryo L_T and $\Delta\delta^{13}$C for muscle [Fig. 2 (a), (c)]. The relationships could have been associated with physiological change or change in nutrient source or nutrient composition throughout the course of development. Studies have indicated that δ^{15}N of fast-growing fishes is driven by an increased allocation of incoming amino acids to growth (*i.e.* increase in body mass) as opposed to catabolism (*i.e.* tissue turnover), resulting in a lower $\Delta\delta^{15}$N relative to slower growing adults (Paterson *et al.*, 2006). In addition, and possibly important to the present study, as a placentatrophic species (Hamlett *et al.*, 2005*a*), the embryos of *R. terraenovae* switch their nutrient source during the course of development from yolk synthesized by the mother (*i.e.* lecithotrophy) to nourishment supplied by a placental connection (Hamlett, 1993). While specifics regarding the isotopic composition of *R. terraenovae* egg yolk and the placental nutrition pathway are few (Hamlett *et al.*, 2005*b*), yolk is expected to be isotopically light due to the preferential selection of lighter isotopes during lipid synthesis (DeNiro & Epstein, 1977). Therefore, the larger embryos in this study may have had higher $\Delta\delta^{15}$N and $\Delta\delta^{13}$C relative to smaller embryos because they had made the switch from yolk feeding to placental feeding, while the smaller embryos had not. The placental connection is formed in *R. terraenovae* when embryos are *c.* 7–10 cm L_T and is definitely established in October or November when embryos are *c.* 18 cm (Hamlett, 1993). The stable isotopes of the smaller embryos (6·5–11·5 cm L_T; embryos of litters 1–4) in this study may have better reflected yolk feeding relative to the larger embryos (14·1–18·6 cm L_T; embryos of litters five to 12), which probably had been feeding placentally for a longer period. As the overall study objective was to compare homologous embryonic and adult tissues, unfortunately, neither yolk nor placenta was sampled. Nevertheless, the results corroborate the conclusion of Estrada *et al.* (2006) that in some instances stable isotopes may be useful for tracing the nutrition

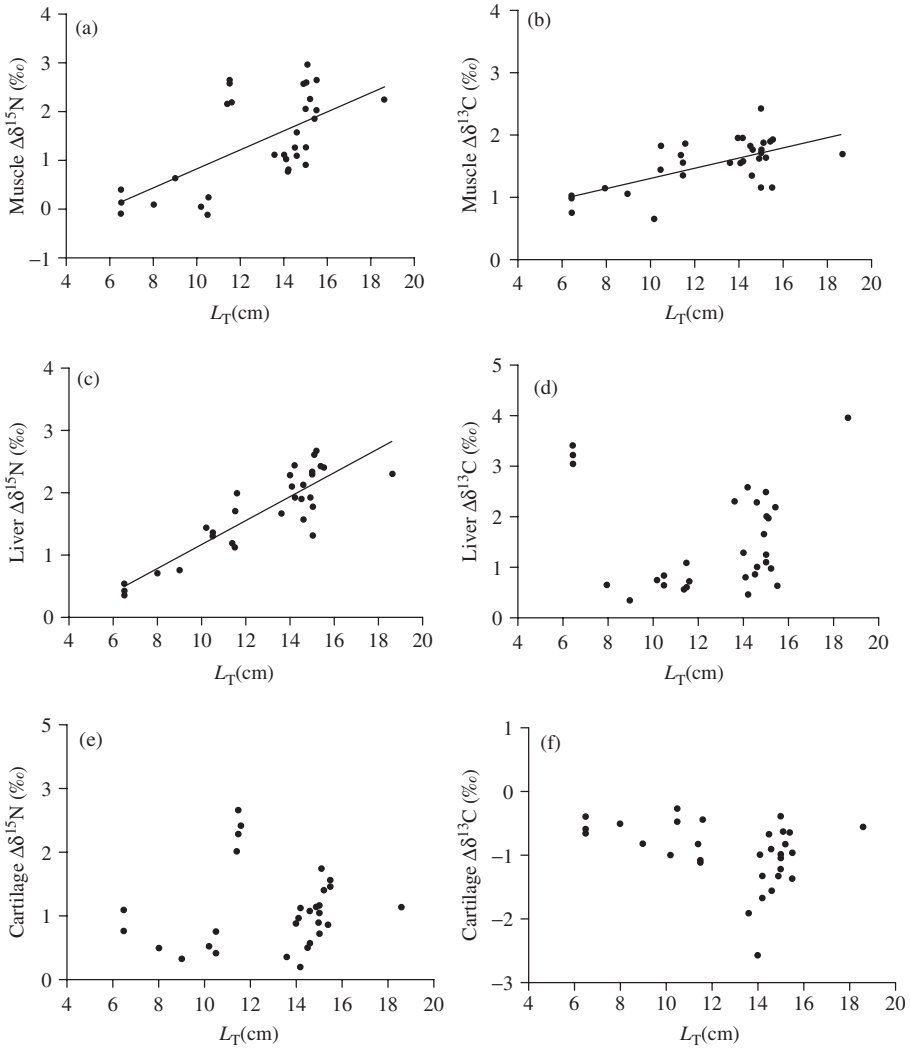

FIG. 2. Total length (L_T) and (a), (c), (d) $\Delta\delta^{15}$N and (b), (d), (f) $\Delta\delta^{13}$C values for (a), (b) muscle, (c), (d) liver and (e), (f) cartilage of *Rhizoprionodon terraenovae* embryos and corresponding linear regressions, where $\Delta\delta^{15}$N and $\Delta\delta^{13}$C are the difference in δ^{15}N and δ^{13}C between each embryo and its mother. The curves were fitted by: (a) $y = 0{\cdot}20x - 1{\cdot}13$, (b) $y = 0{\cdot}08x + 0{\cdot}52$ and (c) $y = 0{\cdot}19x - 0{\cdot}71$. In (d), (e) and (f) the slopes did not deviate significantly from zero ($P > 0{\cdot}05$).

sources of developing shark embryos, and in the present case, a larger sample of more developed embryos might have resulted in higher values of $\Delta\delta^{15}$N. Of further importance regarding the present study is that *R. terraenovae* are typically born at *c*. 29–37 cm L_T (Compagno, 2002) and hence even the larger embryos in this study had considerable growth ahead of them prior to birth.

The significant differences in $\Delta\delta^{15}$N among the studied embryonic tissues, may have been attributed to differences in tissue physiology and composition (Pinnegar & Polunin, 1999) or isotopic routing (Gannes *et al*., 1997; Miller, 2006). Although

significant differences were observed in $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ among the three embryonic tissues studied, muscle might be considered to be the most informative tissue regarding the nutritional resources of embryos based on its relationship with embryo L_T in both $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$. Evaluation of $\delta^{15}N$ and $\delta^{13}C$ in fish tissue with slower (*e.g.* cartilage and cornea) relative to faster (*e.g.* liver) turnover has been suggested to provide temporal information about diet switches in fishes (MacNeil *et al.*, 2005; Miller 2006), although the mechanisms governing the uptake and elimination of ^{15}N and ^{13}C among fish tissue are not yet fully understood (Gannes *et al.*, 1997; Caut *et al.*, 2009).

Because this was the first study to compare stable isotopes of shark embryos and their specific mothers as well as the only study to report $\delta^{15}N$ and $\delta^{13}C$ values based on tissue sampled from shark embryos, it is impossible to know if the results can be applied to other shark species, and in particular, to those that are placentatrophic. In their study of the ontogenetic feeding ecology of white sharks *Carcharodon carcharias* (L.), Estrada *et al.* (2006) reported embryonic values for $\delta^{15}N$ and $\delta^{13}C$ based on cartilage harvested from what they surmised to be embryonic locations on vertebrae of *C. carcharias* ranging from 128 to 526 cm L_T (*i.e.* all postpartum specimens when vertebrae were collected). In that study, comparisons of $\delta^{15}N$ and $\delta^{13}C$ at two embryonic locations on vertebrae with postpartum locations on the same vertebrae revealed the embryonic locations to be higher in $\delta^{15}N$ relative to each of the five postpartum locations studied but similar for the most part regarding $\delta^{13}C$. In addition, differences in $\delta^{15}N$ between the two embryonic locations (*i.e.* with the location representing older embryos being depleted by 0·5‰ or more relative to that representing younger embryos) were noted for 26 of the 27 studied vertebrae. Estrada *et al.* (2006) interpreted the first of those results as a possible indication that the isotopic composition of *C. carcharias* yolk may be substantially different than that of postpartum tissue, an interpretation supported by isotopic studies of bird egg constituents *v.* adult bird tissue (Hobson *et al.*, 2000). Regarding the difference in $\delta^{15}N$ between the two studied embryonic locations, Estrada *et al.* (2006) suggested that the change may have been an indication of maternal or embryonic changes in physiology, metabolism or ecology during gestation [Fuller *et al.* (2004) in support of maternal change and Vander Zanden *et al.* (1998) in support of embryonic change]. It is possible that the different trends reported by Estrada *et al.* (2006) for $\delta^{15}N$ and $\delta^{13}C$ from cartilage of embryo *v.* postpartum *C. carcharias* and those reported here for cartilage of *R. terraenovae* embryos and mothers may reflect species differences, including the fact that *C. carcharias* is an ovatrophic rather than a placentatrophic species (Gilmore *et al.*, 2005). Regardless of the reasons for these differences, however, this study corroborates the results of Estrada *et al.* (2006) that significant isotopic differences can exist between embryonic and postpartum sharks and stable isotope analysis may be useful regarding the identification of changes in embryo metabolism and nutrition occuring during gestation. In addition, based on the present results, it also appears likely that at birth, the $\delta^{15}N$ and $\delta^{13}C$ signatures of *R. terraenovae* are higher than somewhat older neonates whose postpartum feeding habits have restructured their isotope profiles to reflect their postembryonic diet.

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