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Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals

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Stable isotopes of neonatal vertebrates reflect those of their mother's diet and foraging location. Evaluating feeding strategies and habitat use of neonates is consequently complicated by the maternal isotopic signal and its subsequent elimination with growth. Thus, methods that measure the loss of the maternal signal, i.e. when the isotopic signal of a neonate reflects its own diet, are needed. Values of $\delta^{13}C$ and $\delta^{15}N$ were measured in liver and muscle tissues of <1 year old bull (*Carcharhinus leucas*) and Atlantic sharpnose (*Rhizoprionodon terraenovae*) sharks and related to age using, total length, date sampled and umbilical scar stage (USS). We observed a decline in $\delta^{13}C$ and $\delta^{15}N$ values with age that was different among species, similar among isotopes, and greater in liver than in muscle; highlighting that retention of the maternal signal is dependent on species-specific life history and tissue characteristics. USS was most effective for assessing the loss of the maternal isotopic signal in the faster growing Atlantic sharpnose shark, but was less effective for the slower growing bull shark. Total length and date sampled were overall less effective and may be more informative for slower growing species when coupled with USS, as variable size at birth and misclassification of animals >1 year old, which remain in nursery habitats, increase the variability of the isotopic values. Consideration of the maternal signal and measuring its loss are thus necessary when analyzing the stable isotopes of young animals, as there is potential to misinterpret feeding strategies, overestimate trophic position and incorrectly assign carbon source. Copyright © 2011 John Wiley & Sons, Ltd.

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in different animal tissues provide a tool to examine species trophic interactions as they are dietary integrators across variable time scales.[1] Enrichment of isotopes within tissues of a consumer over that of its diet arises as a result of the greater retention of the heavier over the lighter isotope during the process of protein amination and deamination for ^{15}N and respiration for ^{13}C, respectively.[2,3] This produces ratios in a consumer's tissues, between approximately 0 and 2‰ for $\delta^{13}C$, and 2 and 5‰ for $\delta^{15}N$, higher than those of its diet,[2,4,5] but see the recent review.[6]

Size and season-based shifts in diet that reflect the changing role of an organism within a community are common and often explain variation in stable isotope composition between species and among individuals within a population. However, changes in diet are not instantly manifested in the isotopic composition of a consumer's tissues but require a period of time to achieve equilibrium.[7,8] A consumer's tissue will reflect a combination of effects apart from diet (i.e. metabolism, growth, isotopic routing, and tissue protein composition) thereby potentially masking other factors that can cause a shift in isotopic composition as an animal grows.[9] When considering newborn animals, interpreting stable isotope values is further complicated by (i) the mother-young transfer of maternal resources and hence isotopic signature, either during gestation and/or through post-parturition survival on maternal reserves;[10] and (ii) known isotopic discrimination between placental-connected young and their mothers.[11,12]

In light of the documented declines in some predator populations, raising concerns over ecosystem effects,[13] understanding the trophic role of young age classes of sharks, assumed to be top predators within coastal habitats,[14] is important. Carcharhinid sharks bear live young and although parental care is absent, young are provisioned with maternal resources in the form of an enlarged liver.[15,16] Although neonatal sharks begin to feed soon after parturition, it is expected that the stable isotope composition of their tissues will reflect that of the mother and/or provisioned reserves. It has been observed that the embryos of the placentatrophic Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) were enriched in both ^{15}N and ^{13}C in muscle and liver tissues relative to their respective mothers' tissues.[12] At birth, the $\delta^{15}N$ and $\delta^{13}C$ values of neonates are therefore higher than those of young-of-year sharks whose postpartum feeding

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habits would have restructured their stable isotope profiles to reflect that of their postembryonic diet. Similar to that of other placental species (e.g. pinnipeds, ursids and viperids), stable isotope analysis of neonatal sharks is therefore confounded by variable mixtures of mother and own diet signals,[17–19] which, if not accounted for, will distort the true nitrogen and carbon sources, leading to misinterpretation of data.

Values of $\delta^{13}C$ and $\delta^{15}N$ were determined for liver and muscle tissue of two species of sharks, the bull (*Carcharhinus leucas*; Valenciennes, 1839) and the Atlantic sharpnose (*Rhizoprionodon terraenovae*; Richardson, 1836), to measure the loss of the maternal isotopic signal on the stable isotope values of growing neonate (<4 weeks) and young-of-year (<1 year old) sharks. Three measures, considered to be proxies for age, were used to quantify this relationship: total length, date sampled, and umbilical scar stage. Umbilical scar stage is a unique characteristic among fishes, and was included in these analyses as it affords advantages over date sampled and total length by providing a quantifiable measure of the age of young animals.[20] Inter- and intra-species variation in birth date and size at birth are well documented.[21,22] Here we tested the prediction that the isotopic values of neonatal/young-of-year sharks would decline with increasing total length and date sampled, and reduced umbilical scar presence, until they reached equilibrium with their diet, i.e., when the isotopic values of a young shark reflect its own diet. This prediction was based on (i) the known enrichment in ^{15}N and ^{13}C of neonates relative to their mothers,[12] and (ii) the premise that the young sharks of both study species inhabit isotopically distinct habitats from adults; bull sharks remain in low-salinity estuaries for several years[23] and Atlantic sharpnose sharks inhabit nearshore coastal environments.[24] The tissues of both bull and Atlantic sharpnose sharks will therefore adopt a more ^{13}C- and ^{15}N-depleted estuarine diet than that of their mothers' marine signature, which will result in the predicted decline in $\delta^{13}C$ and $\delta^{15}N$ values over time. Moreover, because variable tissue turnover and growth rates influence isotopic values, we predicted that $\delta^{13}C$ and $\delta^{15}N$ values in neonatal/young-of-year sharks would decline (i) at a faster rate in liver than muscle, and (ii) more quickly in the faster growing Atlantic sharpnose shark.

EXPERIMENTAL

Liver and muscle (~5 g) were sampled from 39 bull and 42 Atlantic sharpnose sharks collected from nursery habitats of the Caloosahatchee and Myakka Rivers of Florida (USA) between May and October of 2006–2008, and from Georgia (USA) estuaries between May and August of 2005, respectively. The total length (TL), date sampled and umbilical scar stage (USS) were recorded for all individuals. A qualitative six-point USS scale was devised where (i) open wound with umbilical remains attached (USS1); (ii) open wound without remains (USS2); (iii) wound partially open (USS3); (iv) wound completely closed (USS4); (v) faint scar present (USS5); and (vi) no scar present (USS6). A limited amount of information is available on the time required for the umbilical scar to heal completely, but the majority of estimates range from 4 to 6 weeks.[25] Duncan and Holland[20] estimated ~2 weeks for the umbilical scar of neonate scalloped hammerhead (*Sphyrna lewini*) to be healed, which corresponds to our USS4 descriptor. Furthermore, Duncan and Holland[20] suggest ~1 year for complete disappearance of the scar. Only sharks estimated to be ≤1 year old were included in the statistical analyses.

Tissues were sub-sampled (~1.0 g), freeze-dried for 48 h, pulverized and lipid extracted by twice agitating the pulverized tissue in 2:1 chloroform/methanol solution for 24 h and decanting the solvent (modified method outlined by Bligh and Dyer[26]). The relative abundances of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined on ~0.5–1.0 mg sub-samples on a ThermoFinnigan Delta^{Plus} mass spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) at the Chemical Tracers Laboratory, Great Lakes Institute for Environmental Research, University of Windsor (Windsor, Canada), and at the Odum School of Ecology, University of Georgia (Athens, GA, USA). The results are expressed in standard delta notation (δ), defined as parts per thousand as follows:

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 10^3,$$

where R is the ratio of heavy to light isotopes in the sample and standard, respectively.[1] The standard reference material was Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen for N_2. The analytical precision based on the standard deviation of two standards (NIST 8414 (NIST, Gaithersburg, MD, USA) and internal lab standard; $n = 76$) for $\delta^{13}C$ ranged from 0.06‰ to 0.09‰ and for $\delta^{15}N$ ranged from 0.10‰ to 0.21‰. The accuracy of analysis based on NIST standards (sucrose (NIST 8542) and ammonium sulphate (NIST 8547); $n = 3$ for each) that were analyzed in conjunction with the shark tissue samples were within 0.01‰ and 0.07‰ of the certified values for $\delta^{13}C$ and $\delta^{15}N$, respectively.

The stable isotope data were found to be normally distributed based on probability plots. Consequently, no data transformations were performed. We ruled out the possible effects of sex, season, sampling location and year on the stable isotope values of both shark species (see Supporting Information). The data were therefore grouped per species for all following analyses.

To test the prediction that the $\delta^{13}C$ and $\delta^{15}N$ values of neonatal/young-of-year sharks of each species declined with age; (1) the relationships between date sampled and tissue $\delta^{13}C$ and $\delta^{15}N$ values; and (2) the relationship between total length and tissue $\delta^{13}C$ and $\delta^{15}N$ values of both liver and muscle tissue were fitted with polynomial models (*lm* in R). This was based on the premise that polynomial models often produce the best fit for determining the relationship between stable isotope values and either total length and date sampled, as isotope assimilation of new diet into an individual's tissues is expected to experience a lag-time with the loss of maternal isotopic signal.[27] In addition, polynomial models best fit our prediction that the isotopic values of neonate/young-of-year sharks will decline (e.g. representing the loss of the maternal signal), reach an asymptote or equilibrium with their diet (i.e. complete turnover of the maternal isotopic signal), and subsequently respond to the new diet (e.g. remain stable, increase or decrease). We did consider using an exponential decay model[28] to characterize

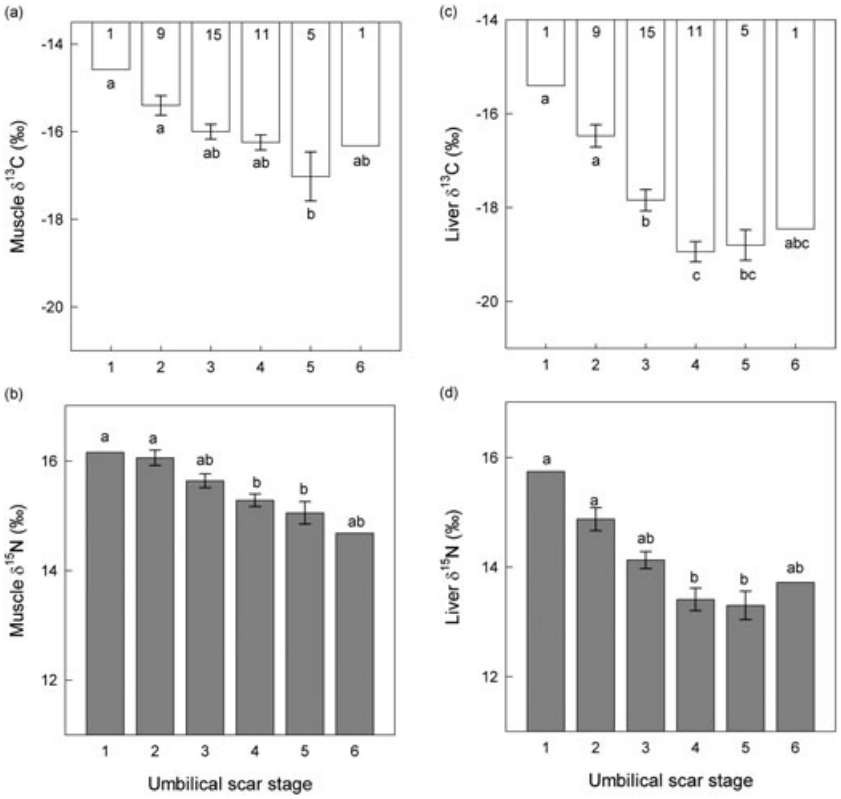

Figure 1. Relationships between USS and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE) for (a, b) muscle and (c, d) liver of the Atlantic sharpnose (*Rhizoprionodon terraenovae*). Letters displayed above a given USS indicate the USS(s) for which pair-wise comparisons revealed significant differences. Numbers in plots (a) and (c) represent the sample size of sharks per USS.

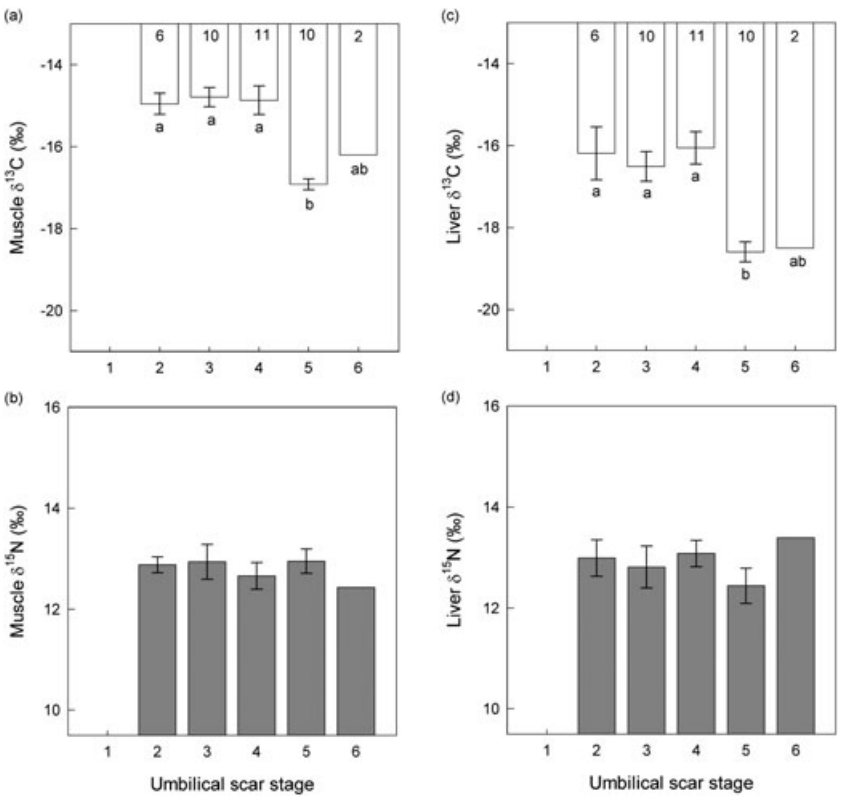

Figure 2. Relationships between USS and $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE) for (a, b) muscle and (c, d) liver of the bull shark (*Carcharhinus leucas*). Letters displayed above a given USS indicate the USS(s) for which pair-wise comparisons revealed significant differences. Numbers in plots (a) and (c) represent the sample size of sharks sampled per USS.

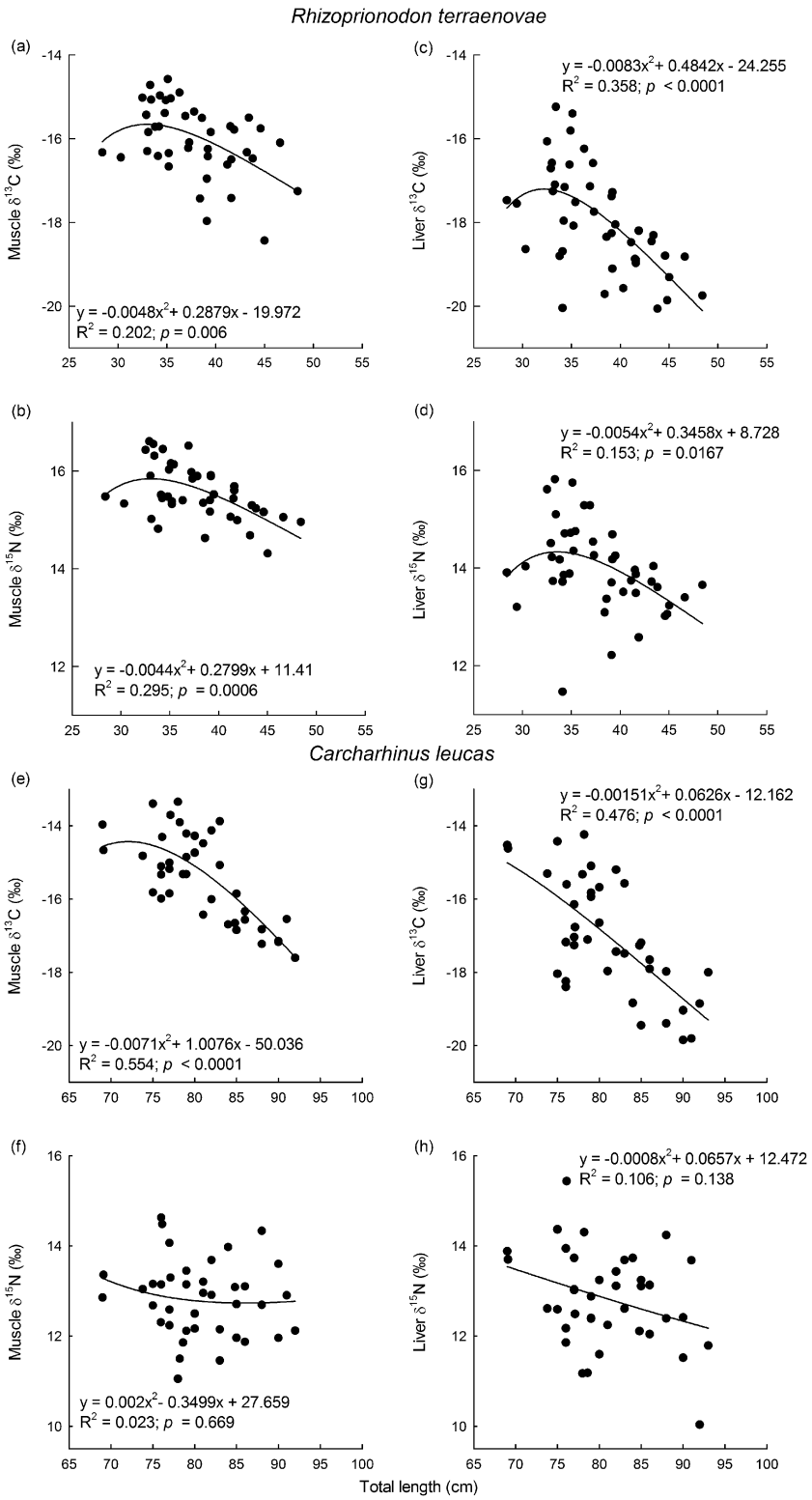

Figure 3. Relationships between total length (TL) and $\delta^{13}C$ and $\delta^{15}N$ values for (a, b) muscle and (c, d) liver tissues of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) and for (e, f) muscle and (g, h) liver tissues of the bull shark (*Carcharhinus leucas*); curves were fitted with polynomial models.

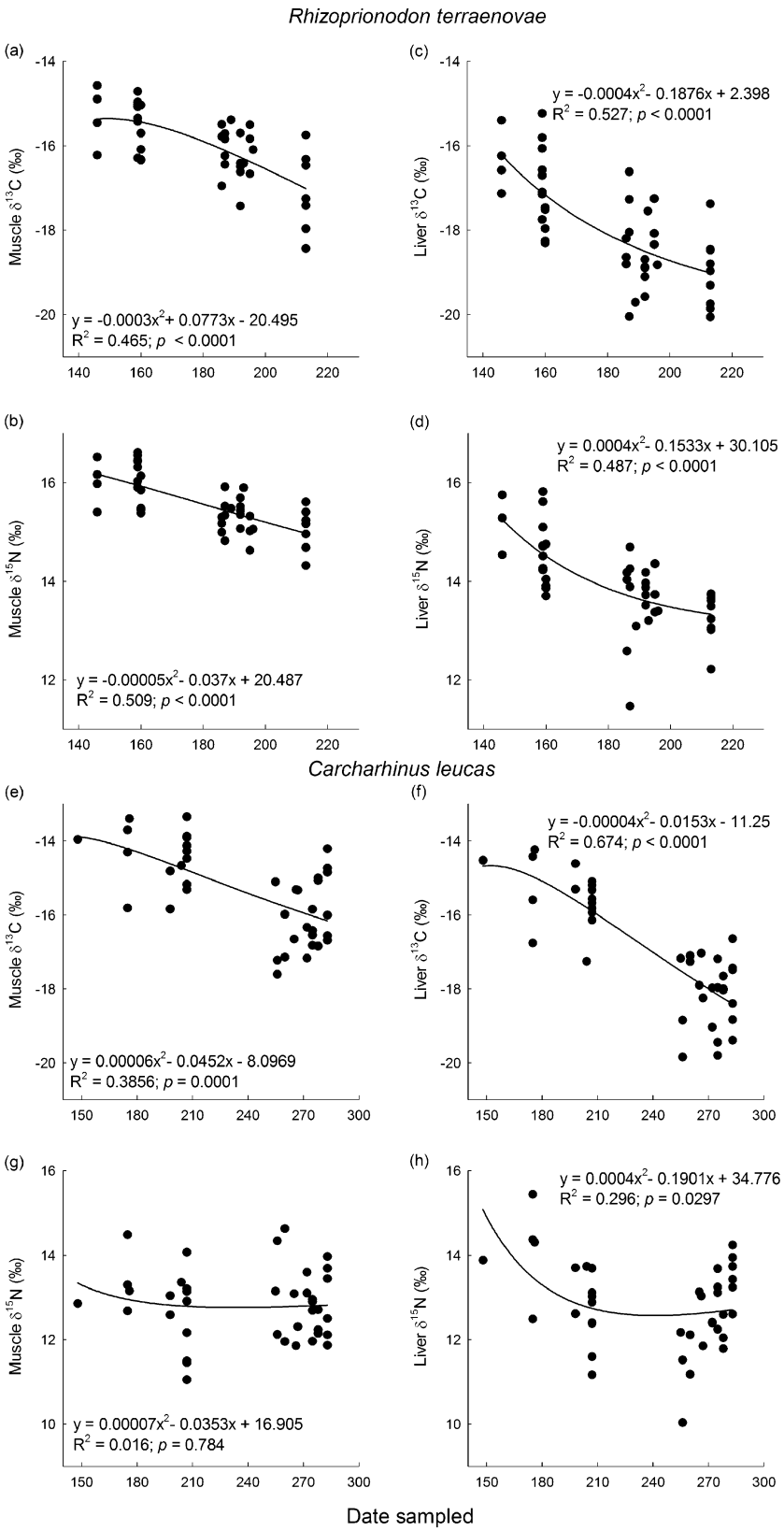

Figure 4. Changes in $\delta^{13}C$ and $\delta^{15}N$ values in regard to date sampled for (a, b) muscle and (c, d) liver tissues of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) and for (e, f) muscle and (g, h) liver tissues of the bull shark (*Carcharhinus leucas*); curves were fitted with polynomial models.

our predictions; however, we were unable to sample the required endpoints (i.e. mothers and new diet) (see Discussion). Because USS is an ordinal variable, one-way analysis of variance (ANOVA) was used to test for differences among umbilical scar stages. As the sample sizes were unbalanced, the significance of pair-wise comparisons was tested using adjusted Bonferroni tests. Statistical analyses were conducted using program R,[29] with a criterion for significance of $p < 0.05$ being used for all analyses. All mean values are presented ± one standard error.

RESULTS

For the Atlantic sharpnose, there was a significant decline in muscle $\delta^{13}C$ values between USS1 and USS5 (−14.9‰ to −17.0‰; $F_{5,36} = 4.178$, $p = 0.004$; Fig. 1(a)). In liver tissue, the $\delta^{13}C$ decline was more pronounced between USS1 and USS4 (−15.4‰ to −18.8‰; $F_{5,36} = 13.868$, $p < 0.0001$; Fig. 1(c)). In agreement with this, the $\delta^{15}N$ values for the Atlantic sharpnose showed that both muscle and liver values decreased with USS (16.2‰ to 14.7‰; $F_{5,36} = 5.612$, $p = 0.001$ and 15.8‰ to 13.7‰; $F_{5,36} = 8.427$, $p < 0.0001$, respectively; Figs. 1(b) and 1(d)). Pair-wise comparisons found that USS4 and USS5, $\delta^{13}C$ and $\delta^{15}N$ muscle and liver tissue values were significantly lower than USS1 and USS2 (Figs. 1(a)–1(d)). For liver and muscle tissue, pair-wise comparisons indicated that the stable isotope values of the Atlantic sharpnose do not continue to decline beyond USS4 and USS5, respectively (Figs. 1(c) and 1(d)). However, the low sample size of USS6 limits the interpretation of this result.

For the bull shark, the decline in $\delta^{13}C$ with USS was significant for both muscle and liver tissue (−15.0‰ to −16.2‰; $F_{4,34} = 11.120$, $p < 0.0001$ and −15.4‰ to −18.5‰; $F_{4,34} = 8.450$, $p < 0.0001$, respectively; Figs. 2(a) and 2(c)). The USS5 muscle and liver $\delta^{13}C$ values were significantly lower than all other USSs, accepting limited data for USS6. The range of $\delta^{15}N$ values for both bull shark tissues was narrow and no significant $\delta^{15}N$-USS relationships were detected (muscle: $F_{4,34} = 0.299$, $p = 0.876$; liver: $F_{4,34} = 0.675$, $p = 0.614$; Figs. 2(b) and 2(d)).

For the Atlantic sharpnose, there was a significant decline in $\delta^{13}C$ and $\delta^{15}N$ values in muscle and liver tissue with increasing total length (Figs. 3(a)–3(d)) and consecutive sampling date (Figs. 4(a)–4(d)), yet the date sampled exhibited stronger relationships than total length, based on the coefficients of determination. Despite the stronger $\delta^{13}C$ and $\delta^{15}N$ relationships, only the $\delta^{13}C$ and $\delta^{15}N$ relationship between Atlantic sharpnose liver and date sampled suggested that sharks were approaching the point when the maternal stable isotope signal was no longer influencing the values seen in young-of-year sharks. Muscle $\delta^{13}C$ and $\delta^{15}N$ values vs. total length and sampling date indicated that the stable isotope values of sharks were still declining, and thus still potentially influenced by the mother's isotope signal. In contrast for the bull shark, only the declines in $\delta^{13}C$ with increasing total length (Figs. 3(e) and 3(g)) and consecutive sampling date (Figs. 4(e) and 4(g)) were significant but neither tissue showed evidence of approaching the point where the stable isotope values in the young-of-year were not influenced by maternal isotopes. Unlike the Atlantic sharpnose, the bull shark total length exhibited a stronger relationship with muscle $\delta^{13}C$ value, whereas date sampled exhibited a stronger relationship with the liver $\delta^{13}C$. The bull shark $\delta^{15}N$ values of liver tissue showed a small depletion with increasing total length and consecutive sampling date (Figs. 3(h) and 4(h)), while for muscle tissue there was no change (Figs. 3(f) and 4(f)). Neither date sampled nor total length was a strong predictor of the $\delta^{15}N$ relationships of either bull shark tissue.

DISCUSSION

Our results revealed the distinct loss of enriched isotopic values commensurate with increasing total length, consecutive sampling date and healing of the umbilical scar in neonate to young-of-year Atlantic sharpnose and bull sharks. These trends, with the exception of bull shark $\delta^{15}N$, affirm the prediction that neonates of both study species have higher isotopic values than young-of-year, confirming that the interpretation of stable isotopes in young sharks is complicated as a result of the maternal isotopic signal. The expression of maternal isotopic signals in offspring has been documented in a number of non-elasmobranch species,[10,18,30] but this is the first study to adopt multiple age measures to document the rate of maternal isotopic signal loss of neonatal sharks as they progress through their first year. In addition, the loss of maternal isotopic signal was variable between species and tissues, highlighting the potential implications for using stable isotope data from multiple tissues to characterize diet and habitat use of <1 year old animals.

For both the Atlantic sharpnose and bull shark, the $\delta^{13}C$ and $\delta^{15}N$ muscle and liver values declined with total length and consecutive sampling date, but most relationships did not reach an asymptote as predicted. This would suggest that both these age measures are problematic for estimating when the complete loss of the maternal isotopic signal occurs in young sharks. Overall, date sampled was a stronger predictor of maternal signature loss than total length. However, date sampled can be difficult to quantify, specifically for the species in this study, as the two species pup at various times throughout the spring and early summer.[31] Consequently, if a species utilizes or revisits nursery habitat for an extended period of time (i.e., >1 year), similar to the bull shark, second-year cohorts could be misclassified as neonates or young-of-year although they would have already lost their maternal isotopic signal and their tissue isotope values would be reflecting their own diet. If these >1 year old sharks were categorized based on sampling date, they would likely increase the variability in isotopic values in early age classes and complicate interpretation of these relationships.

The length of sharks at birth (i.e. total length (TL)) is also highly variable and the size ranges of early age classes overlap.[21,22] Size at birth of Atlantic sharpnose has been reported in the range of 25–41 cm TL.[21,32,33] In this study, Atlantic sharpnose sharks collected in June had overlapping TLs but represented three umbilical scar stages and bull sharks from USS2 to USS4 included individuals ranging between 69 and 86 cm TL. In addition, three bull sharks not included in these analyses exhibited characteristics of ≥1 year old individuals (lack of scar and different isotope values), but were of a similar length to the young-of-year sharks sampled here. It is therefore necessary to couple TL and date sampled with USS to provide a more reliable estimate of the true age

of the shark to assess if the maternal isotopic signal is still present.

Atlantic sharpnose liver $\delta^{13}C$ and $\delta^{15}N$ values exhibited a significant change at USS4 from earlier scar stages, suggesting these young sharks had replaced the maternal isotopic signal with that of their own diet. Reported $\delta^{15}N$ turnover rates in liver tissue of freshwater stingrays (*Potamotrygon motoro*) of ~166 days[8] provide further support that USS4 of the Atlantic sharpnose shark was at or near equilibrium, considering the USS timeline of Duncan and Holland.[20] Accurate inferences on the diet and trophic ecology of young Atlantic sharpnose using stable isotopes of liver would therefore seem permissible at stages later than USS4. Muscle $\delta^{13}C$ and $\delta^{15}N$, however, did not indicate a diet switch until USS5 and USS6, respectively, which is expected as the muscle tissues of juvenile sandbar sharks (*Carcharhinus plumbeus*) reached equilibrium at >500 days for $\delta^{13}C$ and >300 days for $\delta^{15}N$.[34] However, reported mother-embryo muscle tissue discrimination values for Atlantic sharpnose of 1.3‰ for $\delta^{13}C$ and 1.1‰ for $\delta^{15}N$[12] would suggest that the USS6 shark was approaching complete maternal signal replacement and assimilation of new diet, based on the difference between USS1 and USS6, but a larger range of sizes including adults would be required to confirm this.

Bull shark muscle and liver $\delta^{13}C$ values indicated loss of maternal isotopic signal and assimilation of new diet at USS5; however, the limited data ($n = 2$) for USS6 warrants caution with the interpretation. The lack of a $\delta^{15}N$-USS relationship limits any inferences made about maternal isotopic influence on $\delta^{15}N$ in this species. If indeed we consider the estimates of turnover rates of muscle $\delta^{15}N$ and $\delta^{13}C$ detailed above, young bull sharks would not be predicted to reach equilibrium with their diet, i.e. complete turnover of the maternal isotopic signal, until they were >1 year old or reached a TL of approximately 90–100 cm.[22,32] However, based on liver turnover rates, we would have expected bull shark liver tissue to have reached equilibrium prior to USS6. Therefore, the reported turnover rates for elasmobranch liver tissue, in conjunction with the fact that neither the $\delta^{15}N$ nor the $\delta^{13}C$ of bull shark tissues approached equilibrium, may suggest that USS is not appropriate for this slow growing species and that sampling older individuals is necessary to fully document loss of maternal signature. Likewise, both whole blood and plasma have been shown to assimilate the stable isotope ratios of a new diet within shorter time frames than muscle tissue, ranging from several days in the case of plasma[28,35] to several weeks[36] or months[34] in the case of whole blood. These tissues may be more easily quantified using USS, as they will show the loss of the maternal isotopic signal more definitively in slow growing species. Hence, a combined approach, USS and TL, and/or possibly the use of blood plasma, would be an appropriate method to determine when newborn animals are in equilibrium with their own diet.

The rate of loss of maternal isotopic signal was quicker in Atlantic sharpnose than in bull sharks, based on the USS estimations for when the maternal isotope signal was lost. This was likely a result of the faster growth rate reported for this species and associated rate of tissue turnover. The growth coefficient (K) for bull sharks of 0.08-0.09 $year^{-1}$[22,32] is much lower than that for Atlantic sharpnose (K = 0.42–0.50 $year^{-1}$).[21,33] The faster turnover in liver stable isotope values of Atlantic sharpnose as opposed to muscle, is consistent with trends seen for liver and muscle in fishes, birds and marine mammals.[7,8,28,34,37,38] Thus, the length of time for which the maternal isotopic signal will influence the stable isotope values of a young shark is inversely related to the growth rate of the species and the metabolic activity of the tissues.

In contrast to our expectations, the mean $\delta^{13}C$ USS2–USS4 values in both bull shark tissues were similar and did not decline until later stages. In addition, ANOVAs revealed that three USS4 bull sharks collected furthest from the mouth of the river (26.5 km upstream) had the most enriched ^{13}C liver and muscle signatures (~13‰; see Supporting Information). Marine food webs are typically enriched in ^{13}C compared with terrestrial or freshwater food webs due to differing contributions from C_3 and C_4 production sources among these habitats.[30] Therefore, the neonate isotopic values were expected to diverge from those of their mothers as they assimilate a more $\delta^{13}C$-depleted estuarine diet (mean consumer taxa $\delta^{13}C \sim -20.8 \pm 0.19$ in the Caloosahatchee and Myakka Rivers; J. Olin, unpublished data). The lack of ^{13}C depletion in the youngest sharks would suggest feeding in marine as opposed to estuarine environments, yet this would seem counterintuitive as bull sharks pup in estuarine environments and inhabit riverine systems for ~1–2 years.[23] It is more probable that the constant $\delta^{13}C$ values observed in the youngest bull sharks reflect the use of liver reserves provisioned by the mother.[16] Considering that the Atlantic sharpnose showed depletion in both ^{13}C and ^{15}N from birth, this may suggest greater maternal investment in bull sharks, relative to the Atlantic sharpnose. Nevertheless, apart from variable growth rates between species, it is likely that variation in maternal investment across shark species may also complicate the establishment of a single scar stage for all species at which the stable isotopes reflect the actual diet of young sharks.

For the bull shark, the lack of a decline in $\delta^{15}N$ values with age could result from (1) young sharks feeding on a diet with $\delta^{15}N$ values that are comparable with those of their mothers or (2) equivalent source $\delta^{15}N$ values between young/mother habitats. Given the trend of increasing body size-trophic level relationships in large predatory fish and sharks,[39,40] mother-young feeding at the same trophic level would seem unlikely. A more probable explanation is equivalent source $\delta^{15}N$ values between young/mother habitats. Baseline estuarine $\delta^{15}N$ values in developed areas, like the Caloosahatchee River estuary, are reportedly higher than coastal values;[41] therefore, the $\delta^{15}N$ values of young individuals would be artificially inflated.

An unexpected result was the lack of difference in the rate of maternal isotopic signal loss among liver and muscle tissues of the bull shark. If we consider the previous argument that baseline $\delta^{15}N$ signatures are similar between neonate and mother habitat, it is probable that we can extend this point to explain the similar $\delta^{15}N$ values for the liver and muscle tissue of the bull shark. Liver tissue $\delta^{15}N$ turns over significantly faster than muscle tissue $\delta^{15}N$.[8,34] Therefore, bull shark liver would reflect a diet representative of the enriched ^{15}N baseline, producing similar $\delta^{15}N$ values to the slow turnover muscle tissue which would reflect maternal reserves.

How the maternal stable isotope signal in near-term sharks and rays varies between species or families adopting different

reproductive strategies (i.e. oviparous, ovoviviparous) is unknown. In teleost fishes, embryos are often depleted in ^{13}C, as a result of feeding on lipid-rich yolk, and the isotopic values increase post-hatch with the assimilation of new dietary resources.[42,43] Clearly, consideration of the maternal influence through the mother-young transfer of maternal resources is thus necessary in any study using stable isotopes to assess diet, foraging behaviour and/or habitat use of young animals.

Future research should focus on determining tissue-specific turnover rates of the maternal signal in neonate to young-of-year sharks by applying exponential decay models.[28,44] Through sampling pregnant females (and associated newborn pups) and principal prey items in the diet of neonate/young-of-year sharks within the nursery habitat, exponential decay models would facilitate an examination of the rate of isotopic change or loss of maternal signature. This type of model would provide a predictive framework for investigators to determine when stable isotope values in tissues represent true diet and which juvenile animals could be sampled without the influence from maternal reserves. Defining the maternal and dietary endpoints of large sharks, however, may be challenging when considering that (i) sampling large pregnant females within a nursery ground is inherently difficult, (ii) defining the dietary endpoint of neonatal sharks may be complex as many species undergo a rapid diet shift with size, which may overlap the dietary endpoint of interest, and (iii) for certain shark species, juvenile and adult habitat overlap and therefore nursery habitat will not be isotopically distinct, which complicates the definition of neonatal/young-of-year dietary endpoints. Furthermore, the maternal isotopic signal is inherently variable,[45] both within a species and among species, and is influenced by whether the species is a generalist or specialist feeder and/or if mothers forage in the same/variable habitat. A single estimate of maternal isotopic tissue turnover would therefore not be applicable to all species, but would guide field-sampling protocols.

It is difficult to draw definitive conclusions over the precise timing of tissue $\delta^{13}C$ and $\delta^{15}N$ values achieving equilibrium with diet (i.e. exact USS or TL) when considering that growth and maternal investment are species-specific. The declining trend of $\delta^{13}C$ values of both species for all three age measures, however, supports the hypothesis that the maternal isotopic influence on stable isotope values of young sharks is evident for an extended period of time after birth. Regardless of determining the exact stage of stable isotope diet-equilibrium, our data provide the first practical approach to understanding and measuring the loss of the maternal signal in stable isotope values of young sharks. Until a comprehensive timeline for stable isotope tissue turnover in these age classes and across species can be determined, we suggest that a combination of USS and TL will enable investigators to effectively sample animals that will provide accurate data for dietary and food web studies.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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