

USE OF ISOTOPIC ANALYSIS OF VERTEBRAE IN RECONSTRUCTING ONTOGENETIC FEEDING ECOLOGY IN WHITE SHARKS

JAMES A. ESTRADA,¹ AARON N. RICE,^{2,3} LISA J. NATANSON,⁴ AND GREGORY B. SKOMAL^{5,6}

¹Department of Biological Sciences, University of New England, Biddeford, Maine 04004 USA

²Department of Organismal Biology and Anatomy, University of Chicago, Chicago, Illinois 60637 USA

³Division of Fishes, Department of Zoology, Field Museum of Natural History, Chicago, Illinois 60605 USA

⁴National Marine Fisheries Service, National Oceanographic and Atmospheric Administration, Narragansett, Rhode Island 02882 USA

⁵Massachusetts Division of Marine Fisheries, Martha's Vineyard Research Station, Vineyard Haven, Massachusetts 02568 USA

Abstract. We conducted stable ¹³C and ¹⁵N analysis on white shark vertebrae and demonstrated that incremental analysis of isotopes along the radius of a vertebral centrum produces a chronological record of dietary information, allowing for reconstruction of an individual's trophic history. Isotopic data showed significant enrichments in ¹⁵N with increasing sampling distance from the centrum center, indicating a correlation between body size and trophic level. Additionally, isotopic values verified two distinct ontogenetic trophic shifts in the white shark: one following parturition, marking a dietary switch from yolk to fish; and one at a total length of >341 cm, representing a known diet shift from fish to marine mammals. Retrospective trophic-level reconstruction using vertebral tissue will have broad applications in future studies on the ecology of threatened, endangered, or extinct species to determine life-long feeding patterns, which would be impossible through other methods.

Key words: *Carcharodon carcharias*; conservation tool; diet history; elasmobranch; feeding ecology; ontogeny; stable isotope analysis; trophic shift; vertebrae; white shark.

INTRODUCTION

Many organisms exhibit ontogenetic changes in feeding patterns that reflect changing roles within ecological communities, and this trait had been documented in several species of sharks (Tricas and McCosker 1984, Klimley 1985, Lowe et al. 1996, Yamaguchi and Taniuchi 2000, Ebert 2002). Identifying trophic shifts has traditionally involved the sampling of stomach contents from several hundred individuals at various stages of maturity, each representing a temporal datum point (Cortés 1997). However, extensive individual trophic histories are impossible to elucidate because of the inherent temporal limitations of stomach contents analysis, SCA (Cortés 1997, 1999). Additionally, there are several distinct caveats associated with the use of SCA for trophic level determination, including the regurgitation of prey items upon capture, differential digestion rates of prey, distinguishing between ingested vs. assimilated prey, and the large number of individuals required for study (Cortés 1997, Estrada et al. 2005). The latter is of special concern when dealing with rare, threatened, or protected species or those that are relatively expensive to sample (Cortés 1997). Here we use stable isotope analysis to describe ontogenetic changes in trophic level for a rare and threatened species, the white shark (*Carcharodon carcharias*), as an alternative to traditional SCA.

Tissues typically used for isotopic diet reconstruction, such as hair (Schwertl et al. 2003), teeth (Walker and Macko 1999), scales (Perga and Gerdeaux 2003), and baleen (Best and Schell 1996, Hobson et al. 2004), do not span the entire lifetime of the organism and are vulnerable to external or environmental disturbances (Klevezal 1996). However, the vertebrae of elasmobranchs may be unique in that they are not susceptible to wear, reflect life history parameters of the individual organism (Cailliet et al. 1986, Campana et al. 2002), are metabolically inert, and have a high organic composition (Campana et al. 2002). Hence, shark vertebrae may retain dietary information and reveal life-long trophic histories.

Stable isotope analysis is a reliable technique for examining the trophic level of groups of animals because ratios of carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N) isotopes in a predator's tissues directly reflect the isotopic composition of its prey (DeNiro and Epstein 1978, 1981, Schoeninger and DeNiro 1984, Fry 1988, Hobson et al. 1994). Carbon isotope ratios are conservative from phytoplankton to apex predators, with an enrichment of <1‰ per trophic level, whereas nitrogen isotope ratios undergo a predictable enrichment of 3–4‰ per trophic position (e.g., Rau et al. 1983, Schoeninger and DeNiro 1984, Fry et al. 1999). Thus, the carbon isotope is commonly used as an indicator of a consumer's primary prey or food chain from which it is foraging (DeNiro and Epstein 1978, Peterson and Fry 1987), whereas the nitrogen isotope is used to assess relative trophic level within the food web (DeNiro and

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⁶ Corresponding author. E-mail: gregory.skomal@state.ma.us

Epstein 1978, Minagawa and Wada 1984, Schoeninger and DeNiro 1984, Peterson and Fry 1987, Walker and Macko 1999). In metabolically inert and sequentially deposited tissue, isotope ratios would be expected to quickly and accurately reflect shifts in trophic level (Walker and Macko 1999). Incorporation of isotopic signatures into different tissue is rapid (Pinnegar and Polunin 1999) and reflects a time-integrated feeding history (Fry et al. 1999). Metabolically inert tissue, such as cartilage, does not show a turnover of isotopes (Pinnegar and Polunin 1999), thus reflecting and retaining the isotopic signature for the time period during which the vertebral band is formed. Recent studies have shown that the analysis of stable isotopes is reliable for examining trophic level in large pelagic fishes, including tunas (Estrada et al. 2005) and sharks (Rau et al. 1983, Estrada et al. 2003, MacNeil et al. 2005). However, no study has examined the entire trophic history of a single animal (Dufour and Gerdeaux 2001).

The white shark is the one of the largest living elasmobranchs and an apex predator of the world's oceans (McCosker 1985). Stomach contents analysis shows that white sharks exhibit an ontogenetic dietary shift from a fish-based diet to one dominated by marine mammals (Tricas and McCosker 1984, Klimley 1985). With that in mind, the purpose of this study was to compare isotopic ratios in white shark vertebrae to an established trophic shift, thereby investigating the use of vertebral tissue as a life-long record of trophic ecology in elasmobranchs.

MATERIALS AND METHODS

White shark vertebral samples ($n = 27$) were obtained from the archived collection of the NMFS (National Marine Fisheries Service) Apex Predators Program (Narragansett, Rhode Island, USA) and represented individuals captured in continental shelf waters of the western North Atlantic. We included vertebral samples spanning much of the size range of this species (128–526 cm total length; 23.1–1441.5 kg) to validate the stability of vertebral tissue. All vertebrae were excised from just above the gill arches; prior to this study, vertebrae had been air-dried or kept in alcohol for long-term preservation. For stable isotope analysis, a single vertebral centrum from each shark was subsampled, using a flexible-shaft hand-held drill fitted with a 1.6-mm bit, in 5-mm increments along its radius from center to the edge. We removed ~3–4 mg of powdered vertebral tissue for isotope analysis. Methods for sample preparation, isotopic analysis, and trophic position estimation follow previous studies (Estrada et al. 2003, Estrada et al. 2005). Because the organic matrix of shark vertebral cartilage is composed largely of collagen (Moss 1977, Rama and Chandrakasan 1984), an assumed fractionation rate of 3–4‰ for collagen was used (Schoeninger and DeNiro 1984). The vertebral radius/total length relationship was used to back-calculate mean size at

each subsampling point (Natanson 2002). Tissues were analyzed for isotopic composition using a mass spectrometer (Finnigan MAT Delta Plus; Thermo Electron, West Palm Beach, Florida, USA).

An ANOVA was used to test for differences of isotope fractionation vs. vertebral radius, and a Tukey's multiple-comparisons test was used (post hoc) to determine specific differences between vertebral sampling locations. To produce a more accurate reconstruction of ontogenetic enrichment patterns, it is essential to eliminate the effects of individual variability at the centrum center (or origin). Thus, analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ enrichment relative to isotope values at the 0-mm sampling location was analyzed using the following equations:

$$\begin{aligned} \text{nitrogen enrichment} &= \left(\frac{{}^{15}\text{N}_{x\text{mm}} - {}^{15}\text{N}_{0\text{mm}}}{{}^{15}\text{N}_{0\text{mm}}} \right) \\ \text{carbon enrichment} &= \left(\frac{{}^{13}\text{C}_{x\text{mm}} - {}^{13}\text{C}_{0\text{mm}}}{{}^{13}\text{C}_{0\text{mm}}} \right). \end{aligned}$$

The trophic level of the white shark was estimated using the following equation:

$$\text{trophic level} = \lambda + \frac{\delta^{15}\text{N}_{\text{cons}} - \delta^{15}\text{N}_{\text{base}}}{\Delta_n}$$

where λ is the trophic level of the organism used to estimate $\delta^{15}\text{N}_{\text{base}}$, $\delta^{15}\text{N}_{\text{cons}}$ is the nitrogen fractionation of the consumer, and Δ_n is the enrichment in ^{15}N (subscript n) per trophic level (Post 2002). To ensure that comparisons with co-occurring species were based on equivalent isotopic scales, data for $\delta^{15}\text{N}_{\text{base}}$ were obtained from previously published data (Estrada et al. 2003). The mean terrestrial and aquatic trophic enrichment of 3.4‰ was assumed (Post 2002).

RESULTS

In vertebrae of white sharks, we found a general increasing trend for $\delta^{15}\text{N}$ values with increasing sampling distance from the centrum center (Fig. 1). There were significant differences in mean $\delta^{15}\text{N}$ values among the vertebral tissue samples ($F = 21.3845$, $df = 6$, 106 , $P < 0.0001$) (Fig. 1). Specifically, 0- and 5-mm samples were significantly lower than the other sampling locations, whereas the 30-mm samples were significantly higher than all others. These findings are indicative of a general dietary change associated with increasing vertebral radius and, thus, increasing body size. The overall increasing trend in $\delta^{15}\text{N}$ was also apparent in individual vertebrae (Appendix), supporting the use of the technique in tracing the ontogeny of a single animal.

Although there was a general trend of increasing $\delta^{15}\text{N}$ values with vertebral radius in the white shark, individual variability was observed, particularly at the 0-mm location (Fig. 1). Additionally, 26 of the 27 specimens showed a depletion in $\delta^{15}\text{N}$ values at the 5-mm sampling location, regardless of body size or the

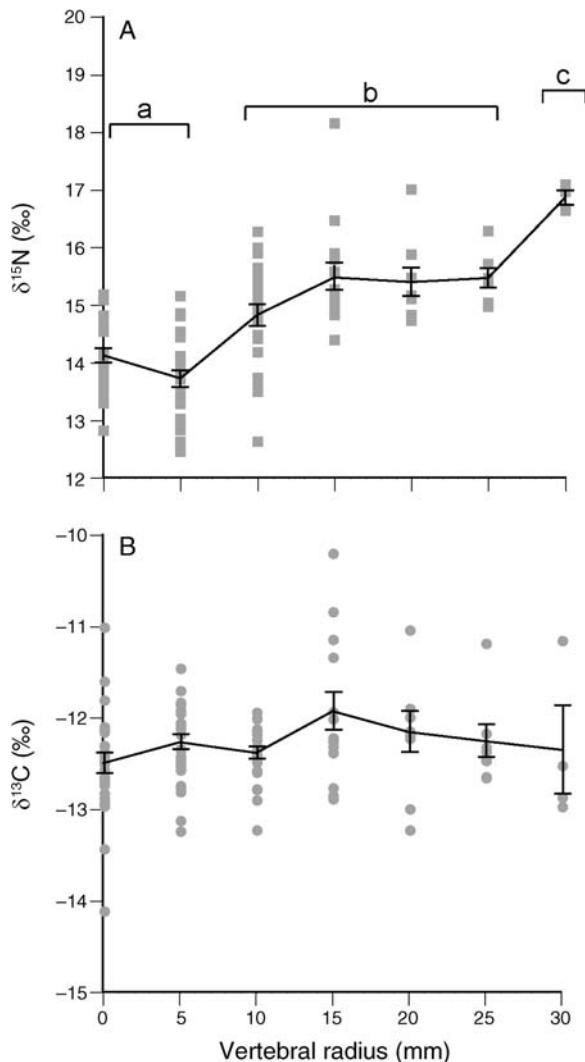


FIG. 1. Stable isotope fractionation during growth in the white shark ($n = 27$). (A) $\delta^{15}\text{N}$ fractionation (‰) vs. vertebral radius (mm). Significantly different groups are indicated by lowercase letters and corresponding spanner lines above the symbols. (B) $\delta^{13}\text{C}$ fractionation vs. vertebral radius. Squares (A) and circles (B) indicate individual values; the solid line connects means (\pm SE).

initial (0-mm) $\delta^{15}\text{N}$ measurement (Fig. 2). Of the 26 showing this depletion, 24 showed a drop of $\geq 0.5\%$. One exception, an adult female (Appendix), did not show this decrease between 0 and 5 mm, nor did it show the rate of increase in $\delta^{15}\text{N}$ as seen in the other plotted individuals.

Analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ enrichment in relation to the 0-mm location showed significant differences for both nitrogen ($F = 15.3574$, $df = 5, 80$, $P < 0.0001$) and carbon ($F = 2.484$, $df = 5, 80$, $P = 0.0382$) (Fig. 2). For nitrogen, the 5-mm sampling location was significantly lower than all other sampling locations, whereas 30 mm was significantly more enriched (Tukey's hsd post hoc test, $P < 0.05$). For carbon, enrichment at 5 mm was

significantly different than at 15 mm ($P < 0.05$), but there were no differences between other sampling locations.

When estimated trophic level for the white shark was plotted against body length (Fig. 3), results showed an increase of a full trophic level during ontogeny. Comparisons with other adult shark species co-occurring in the Northwest Atlantic Ocean (data from Estrada et al. [2003]) indicated that the trophic level of the white shark is similar to those of other piscivorous sharks immediately after birth. However, at total lengths of 150–200 cm, the white shark has a trophic level greater than those of all other species.

DISCUSSION

The sequential isotopic analysis of white shark vertebrae showed an overall trend of $\delta^{15}\text{N}$ enrichment with increasing distance from the centrum center in both species- and individual-level analyses (Fig. 1 and Appendix). These results suggest that vertebrae from elasmobranchs may be used to examine the trophic history of a single animal over time. For the pooled data, the 0- and 5-mm sampling locations were significantly more depleted than all others, whereas the 30-mm location was significantly enriched (Fig. 2). Back-calculated size estimates indicated that the 0- and 5-mm samples coincided with pre-birth lengths of 28 cm

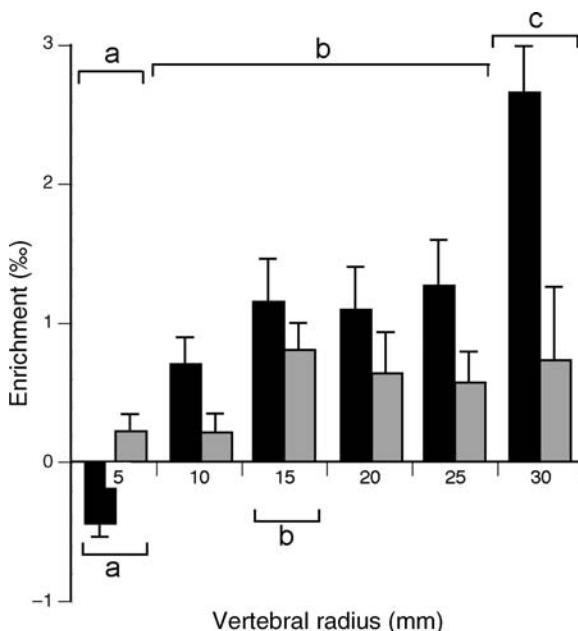


FIG. 2. Isotopic enrichment (mean + SE) of ^{15}N (black) and ^{13}C (gray) in the white shark vs. vertebral radius, relative to values at the 0-mm sampling location ($n = 27$). Enrichment was calculated as $(^{15}\text{N}_{x\text{mm}} - ^{15}\text{N}_{0\text{mm}}) / ^{15}\text{N}_{0\text{mm}}$ for nitrogen and $(^{13}\text{C}_{x\text{mm}} - ^{13}\text{C}_{0\text{mm}}) / ^{13}\text{C}_{0\text{mm}}$ for carbon. Different letters and spanners at the top of the panel indicate statistical differences in nitrogen enrichment ($P < 0.05$). Letters and spanners below the x-axis indicate statistical differences in carbon enrichment ($P < 0.05$).

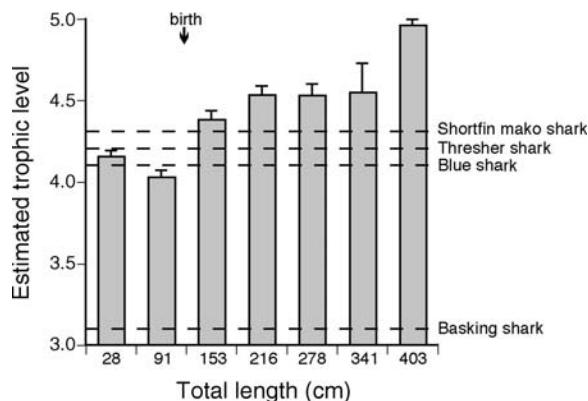


FIG. 3. Change in the estimated trophic level (mean + SE) of the white shark vs. total length, as calculated using the vertebral radius/total length relationship in Natanson (2002). Dashed lines indicate the trophic position of other adult shark species found in the same geographic region, determined by stable isotope analysis (Estrada et al. 2003). The arrow between 90 cm and 153 cm indicates white shark size at birth.

and 91 cm total length, respectively. White sharks reproduce through aplacental viviparity, in which intrauterine development involves embryonic oophagy (Francis 1996, Uchida et al. 1996, Saïdi et al. 2005); evidence of embryophagy is lacking (Francis 1996). After birth (vertebral radius >5 mm), juvenile white sharks are known to be piscivorous (Klimley 1985, McCosker 1985). Therefore, significant differences in $\delta^{15}\text{N}$ values are to be expected between pre- and post-birth vertebral samples, and $\delta^{15}\text{N}$ enrichment from 5 to 10 mm (91 to 153 cm) (Figs. 1 and 2) is probably due to a dietary switch from yolk to fish. At these locations, $\delta^{15}\text{N}$ values (Fig. 1) for the white shark are consistent with typical trophic-level enrichment due to predation on fishes when compared to previously published ^{15}N ratios of potential white shark fish prey (Estrada et al. 2003, 2005). These findings are supported by estimated trophic-level calculations, which indicate that the mean trophic level for white shark young-of-the-year and juveniles is very similar to other adult sharks (Estrada et al. 2003) that feed primarily on fish, such as the shortfin mako (*Isurus oxyrinchus*), common thresher (*Alopias vulpinus*), and blue (*Prionace glauca*) sharks (Cortés 1999) (Fig. 3). Additionally, $\delta^{13}\text{C}$ enrichment values at the 15-mm sampling location were found to be significantly different from those at 5 mm (Fig. 2), providing additional evidence that a dietary change was recorded in the vertebral tissue shortly after birth.

The significant enrichment in $\delta^{15}\text{N}$ values evident at all 30-mm sampling locations coincides with another white shark trophic shift occurring between the mean back-calculated sizes of 341 and 403 cm (Figs. 2 and 3). This is consistent with previous gut contents studies indicating that white sharks >300 cm long shift from a diet principally of fish to marine mammals (Klimley 1985, McCosker 1985). Published data on $\delta^{15}\text{N}$ values of odontocetes (Abend and Smith 1995) and seals (Smith et

al. 1996) range from $13.32\% \pm 0.065\%$ to $16.2\% \pm 3.4\%$, respectively, both below the values reported herein for large white sharks. Moreover, this shift coincides with ontogenetic changes in tooth morphology of white sharks (Tricas and McCosker 1984, McCosker 1985). Small white sharks (<300 cm) possess narrow, sharply pointed teeth with coarse or no serration that are well suited for grasping and feeding on fishes (McCosker 1985). At 300–400 cm in length, white sharks develop teeth that are basally broader, finely serrated, and well adapted to cutting through the thick skin of marine mammals (McCosker 1985). However, not all the sharks sampled in this study exhibited this profound dietary shift. A single vertebra with a radius of 25 mm from a 340-cm female showed no substantial enrichment relative to the 0-mm sample (Appendix). Some natural variability should be expected in large, opportunistic predators because relative prey availability and habitat usage could greatly affect changes in prey preference and, thus, foraging on larger prey such as marine mammals. Without more extensive tag recapture or catch data, it is difficult to test further hypotheses regarding this anomaly.

A surprising result was that $\delta^{15}\text{N}$ values in pre-birth samples (0 and 5 mm) were not enriched in relation to adult tissues. We expected to find typical trophic enrichments in neonate ^{15}N values (i.e., 3–4‰) due to feeding on intrauterine yolk from the mother. However, the isotopic composition of the white shark yolk is entirely unknown and may be substantially different than expected. In birds, egg constituents are not consistently enriched in either ^{13}C or ^{15}N in relation to adult fat or muscle tissue (Hobson et al. 2000). Therefore, it is possible that isotopic routing or fluctuations in protein supply during vertebrate pregnancy and development may alter the isotopic composition of yolk and other egg proteins, producing unanticipated values for fetal tissue (Fuller et al. 2004).

Another unexpected finding was the highly consistent (26 of 27 vertebrae) ^{15}N depletion between the 0- and 5-mm samples (Figs. 1 and 2). Twenty-four of the vertebrae showed a depletion of 0.5‰ or more between these two sampling locations, with three individuals showing a depletion >1.0‰ (Fig. 1). Additionally, the presence of this depletion in both small and large vertebrae validates the inert nature of the vertebral tissue and suggests a broad, pre-birth phenomenon (Fig. 2). We hypothesize that this uniform depletion at 5 mm corresponds to maternal (Fuller et al. 2004) or embryonic (Vander Zanden et al. 1998) changes in physiology, metabolism, or ecology. Because lamnoid embryos pass through 5–6 different nourishment stages during development (Gilmore et al. 2005), it is possible that various nutritional sources are isotopically heterogeneous. Additionally, later in development, many lamnoid embryos go through a period of substantially reduced growth and metabolism (Gilmore et al. 2005), which also may affect the isotope fractionation. Other plausible explanations include

perturbations in nitrogen homeostasis during pregnancy (Fuller et al. 2004), differences in protein utilization during development (Needham and Needham 1930), the onset of fetal urea production for osmotic balance during late gestation (Kormanik and Evans 1986, Kormanik 1993), or migration-related shifts in isotopic values (Best and Schell 1996) associated with maternal movement to habitat that is consistently depleted in ^{15}N and enriched in ^{13}C (Boustany et al. 2002, Bonfil et al. 2005).

For ^{13}C , relative enrichment values (in relation to the 0-mm location) at 5 mm were significantly different than at 15 mm (Fig. 2), but no differences were found between other sampling locations. The lack of more general trends in ^{13}C may be due to substantial variability at the 0-mm sampling location, possibly a result of differential maternal diet, and the prevalence of opportunistic feeding across habitats that vary in isotopic composition. In species that exploit a broad and highly variable prey base, such as the white shark, temporal and spatial resolution of the carbon isotope may not be sufficient for identifying patterns or changes in diet (Estrada et al. 2005). This would be especially true if little or no ^{13}C enrichment were occurring between trophic levels, or if inshore-offshore movements were frequent and transitory (Boustany et al. 2002, Bonfil et al. 2005). The $\delta^{13}\text{C}$ values were also considerably heavier than expected, ranging from -14.1% to -10.2% (Fig. 1). This marks a substantial enrichment from other published $\delta^{13}\text{C}$ values for white shark muscle, at -15.8% to -16% (Rau et al. 1983), and muscle from other shark species, at -17.7% to -15.9% (Estrada et al. 2003). Isotopic studies on fish scales have found similar ^{13}C enrichments over muscle (Perga and Gerdeaux 2003); this has been attributed to the differential amino acid composition of tissues (Winters 1971). Future isotopic analysis of individual amino acids present in vertebral tissue may help to clarify this issue.

In conclusion, we present strong evidence that incremental stable isotope analysis of vertebral tissue in elasmobranchs may be used to retrace the ontogenetic trophic history of an individual animal over its life history. We validated the use of this methodology in the white shark, a large predatory shark species that exhibits a known, conspicuous ontogenetic dietary shift. Our application of stable isotopes to preserved tissues exemplifies the potential for using specimens from museum collections to elucidate the ecology of species in need of conservation.

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APPENDIX

$\delta^{15}\text{N}$ fractionation in 13 individual white sharks plotted vs. vertebral radius, showing individual enrichment patterns as well as an overall species trend (*Ecological Archives* E087-048-A1).