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Short-term shifts of stable isotope ($\delta^{13}C$, $\delta^{15}N$) values in juvenile sharks within nursery areas suggest rapid shifts in energy pathways

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ABSTRACT

We quantified temporal changes in blood plasma $\delta^{13}C$ and $\delta^{15}N$ values collected from recaptured juvenile blacktip reef sharks (*Carcharhinus melanopterus*, n = 14) and sicklefin lemon sharks (*Negaprion acutidens*, n = 4) at liberty in Moorea, French Polynesia for 10-50 days, and juvenile bull sharks (*Carcharhinus leucas*, n = 7) at liberty in the Florida Coastal Everglades for 34–127 days to investigate shifts in assimilated biomass from energy reserves and consumed biomass. Blacktip reef and bull sharks exhibited significant changes in plasma $\delta^{13}C$ as they grew, with a mean $\Delta\ \delta^{13}C$/cm total length ± SD of 0.41‰/cm ± 0.72 and–0.82‰/cm ± 0.67. While low sample sizes precluded statistical analyses, sicklefin lemon exhibited a change of 0.49‰/cm ± 0.77. Blacktip reef sharks and bull sharks also exhibited significant shifts in $\delta^{15}N$ values — mean $\Delta\ \delta^{15}N$/cm TL ± SD = −0.23‰/cm ± 0.59, and −0.24‰/cm ± 0.20; shifts in $\delta^{15}N$ values for sicklefin lemon sharks averaged 0.19‰/cm ± 0.52. When data were normalized across species (accounting for species-specific difference in $\delta^{13}C$ and $\delta^{15}N$ ranges), no significant difference were found in the rate of $\delta^{15}N$ change between bull and blacktip reef sharks, but mean changes in $\delta^{13}C$/day among blacktip reef and sicklefin lemon sharks (~1%/day) were twice as fast as bull sharks (~0.5%/day). Comparisons between plasma and muscle isotope values in bull sharks yielded similar results to comparisons of plasma isotope values — rapid changes in $\delta^{13}C$ and $\delta^{15}N$. The magnitude and direction of changes in $\delta^{13}C$ and $\delta^{15}N$ values, however, were not uniform among individuals within each species, suggesting intraspecific variation in trophic interactions within the shark nurseries studied. Further studies quantifying shifts in energy pathways may contribute to elucidating the factors that shape foraging development in sharks and variation in trophic interactions within shark nurseries.

1. Introduction

Immediately after birth, vertebrates often rely on energy provided by parents, either from food or from energy reserves (e.g. yolk sacs, fat tissues), before they successfully develop foraging skills (e.g. Marteinsdottir and Steinarsson, 1998; Szabo and Duffus, 2008; Wallace et al., 2007). When newborn animals begin feeding, biomarkers (e.g. stable isotopes, fatty acids) can be used to quantify the speed at which the shift from relying on parental care to feeding independently occurs (e.g. Belicka et al., 2012; Dale et al., 2011; Meissner et al., 2012). For example, samples sequentially taken from the teeth of adult western Atlantic bottlenose dolphins (*Tursiops truncatus*) exhibit a gradual depletion of $\delta^{15}N$, and indicate that calves nurse for several years before becoming self-sufficient foragers (Knoff et al., 2008). Quantifying temporal variation in biomarkers of juveniles that are indicative of trophic interactions may therefore provide insight into the speed of foraging development and the factors that shape trophic interactions in juvenile animals. In light of environmental change and natural resource depletion by humans, investigating the trophic interactions of juvenile populations is especially important for threatened and/or endangered species that may be particularly vulnerable during early stages of their life history if food availability and other biotic factors that affect foraging development vary in response to predictable and unpredictable extrinsic drivers (reviewed by Yang and Rudolf, 2010).

Stable isotopes are naturally occurring biomarkers that provide tools to quantify temporal changes in animal diets (Hobson, 1999). Tissues with relatively fast stable isotope turnover rates are often most effective in detecting temporal variability in trophic interactions and ontogenetic dietary shifts, which are particularly useful when investigating short-term and/or rapid changes in trophic interactions that may occur shortly after birth (Bearhop et al., 2004). Recent advances in analytical methods also enable individual-level investigations of diet changes that can provide complementary information to population-level studies, providing insight into individual differences in the speed at which foraging develops in juvenile animals (see Layman et al., 2012 for a review). For example, comparing plasma and red blood cell $\delta^{13}C$ values, Rosenblatt et al. (2015) found that American alligators (*Alligator mississippiensis*) in the southeastern United States have very stable mixtures of prey taxa in their diets, but individuals can exhibit considerable

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intraspecific dietary differences, creating individual variation in the top-down effects alligators exert. Further developing methods to quantify individual-level changes in trophic interactions provides a means to test hypotheses generated from population-level studies and gain greater insight at the level of individuals, which is critical for understanding the role phenotypic variability plays in population dynamics and the ecological niches species fill (Bolnick et al., 2011; Sih et al., 2012).

Using stable isotope analysis to quantify shifts in energetic pathways and trophic interactions, however, can be challenging because of the lag time between consumption and assimilation into tissues (see Martinez del Rio et al., 2009 for a review), especially in slow growing taxa like sharks. Stable isotope analysis is increasingly being used to investigate the trophic interactions of large-bodied marine predators, including sharks (e.g. Hussey et al., 2012; Kinney et al., 2011; McMeans et al., 2010), yet relatively slow turnover times (e.g. muscle >250 days, Kim et al., 2012; fin >500 days, MacNeil et al., 2006) and few lab studies quantifying turnover rates and discrimination rates (Caut et al., 2013; Hussey et al., 2010a; Kim et al., 2012; Logan and Lutcavage, 2010; MacNeil et al., 2006; Malpica-Cruz et al., 2012) hinders our ability to interpret stable isotopes of elasmobranchs, especially among juveniles in which "maternal meddling" can greatly affect $\delta^{13}C$ and $\delta^{15}N$ values (Olin et al., 2011). Indeed, in placentatrophic sharks, embryos tend to have enriched isotopic values relative to their mothers, which persists into at least their first few months after birth, and may indicate that sharks are reliant on maternal energy reserves at the time of capture, despite feeding independently for weeks or even months prior to capture (e.g. McMeans et al., 2009; Olin et al., 2011; Vaudo et al., 2010). Isotopic lag times in elasmobranchs attributed to slow turnover rates also affects studies investigating ontogenetic niche shifts, which are common among cartilaginous fishes (Grubbs, 2010). As such, understanding how turnover rate and maternal provisioning affect our interpretation of stable isotope values from the tissues of elasmobranchs is important for studies of their trophic ecology, especially among newborn individuals.

Various methods are employed to study temporal change in trophic interactions using stable isotope analysis – longitudinal sampling of inert tissues, longitudinal sampling of metabolically active tissues, and comparing tissues with different turnover rates – each providing different, but complimentary diet information (Bearhop et al., 2004). Here we take advantage of recaptured juvenile individuals from three different shark species (bull shark, *Carcharhinus leucas*; blacktip reef shark, *Carcharhinus melanopterus*; and sicklefin lemon shark, *Negaprion acutidens*) sampled during long-term studies within coastal nurseries in Florida, USA (*C. leucas*) and Moorea, French Polynesia (*C. melanopterus* and *N. acutidens*) to investigate changes in stable isotope values of blood plasma and intra-individual temporal shifts in trophic interactions. Blood plasma collected during each sampling event has a much faster isotopic half-life ($\delta^{13}C$ = ~22 days, $\delta^{15}N$ = ~33 days) than other tissues in elasmobranchs (Kim et al., 2012; Malpica-Cruz et al., 2012), enabling us to detect changes in energy pathways (i.e. from maternal resources to self-feeding or among food webs) over the short time frames sharks were at liberty for (e.g. weeks to months). We also compare the changes in plasma isotope values of individual bull sharks from samples collected during multiple events to the difference between $\delta^{13}C$ and $\delta^{15}N$ values in plasma and muscle tissues collected during one sampling event to investigate the use of multi-tissue stable isotope analysis (comparing stable isotope values across tissues) to study temporal changes in shark trophic interactions.

2. Methods

2.1. Moorea, French Polynesia

Moorea, French Polynesia (17°30 S, 149°51 W) is part of the Windward Islands, west of Tahiti, and is surrounded by lagoons bordered by fringing reef that serve as nurseries for juvenile blacktip reef sharks and sicklefin lemon sharks (Mourier and Planes, 2013; Mourier et al., 2013a). Juvenile sharks of each species (14 blacktip reef sharks and four sicklefin lemon sharks) were recaptured (10–50 days at liberty) using small gillnets during sampling efforts in 2012. Sharks were externally tagged using a numbered spaghetti identification tag implanted next to the dorsal fin upon first capture, shark total length was measured to the nearest 0.5 cm during each capture to quantify change in length, and an 18 gauge needle was used to collect 3 mL of blood from the caudal vein during each capture. Blood samples were placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and immediately separated into components, including plasma, using a centrifuge spun for one minute at 3000 rpm. Plasma samples were put on ice and frozen before laboratory preparations. All samples were dried and homogenized prior to stable isotopic analysis at Florida International University's Stable Isotope Laboratory, during which variation among standards was 0.07‰ and 0.08‰ ± SD for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.2. Florida coastal everglades

The Shark River Estuary, FL, USA (25°25 N, 80°59 W) extends from the Gulf of Mexico to freshwater marshes in Everglades National Park, and serves as a year-round nursery for juvenile bull sharks (see Matich and Heithaus, 2012 for description of study area). Within the estuary, two geographically and isotopically distinct food webs (marine and freshwater-estuarine; Matich et al., 2011) provide prey for bull sharks, and sharks predominantly feed from freshwater-estuarine taxa at smaller sizes and incorporate marine taxa in their diets as they grow (Matich et al., 2010). Juvenile bull sharks ($n = 7$) were recaptured (34–127 days at liberty) using bottom-set longlines during long-term sampling efforts from 2008 to 2013 (see Heithaus et al., 2009 for a description of the sampling protocol). Sharks were tagged, measured (Curtis et al., 2011; Neer et al., 2005), and blood plasma was collected, preserved, and analyzed the same as for blacktip reef sharks and sicklefin lemon sharks in Moorea. Muscle samples (0.5 cm^3 of tissue) were also collected from five bull sharks (71% of individuals) using a biopsy punch *ca.* 5 cm lateral to the first dorsal fin upon recapture, and preserved and analyzed at Florida International University's Stable Isotope Laboratory. Variation among standards was 0.12‰ and 0.10‰ ± SD for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.3. Quantitative analysis

Stable isotope data from plasma collected during each capture were plotted ($\delta^{13}C$ and $\delta^{15}N$ separately) against shark total length for each individual of each species. Within each scatter plot ($n = 2$ per species), vectors connecting initial capture data (tail) and recapture data (head) were created for each individual to visually display changes in $\delta^{13}C$ or $\delta^{15}N$ values similar to Schmidt et al. (2007). The slopes of each vector (rate of change in plasma $\delta^{13}C$ or $\delta^{15}N$ with shark length) were measured, and mean slopes for each species were calculated. To account for differences in growth rates of species (bull sharks grow 10–20 cm TL/year, Neer et al., 2005; blacktip reef sharks grow *ca.* 6 cm TL/year, Mourier et al., 2013b; sicklefin lemon sharks grow 12–15 cm TL/year, Ebert et al., 2013), we also plotted plasma isotope values against the duration of time (days) between capture and quantified data. Two-tailed t-tests at $\alpha = 0.05$ were used to test if the magnitude of the slopes of vectors were significantly different from zero for each species. We conducted power analyses for each species (for $\delta^{13}C$ and $\delta^{15}N$) to determine if our sample sizes were adequate considering the variability of plasma $\delta^{13}C$ and $\delta^{15}N$ values, and to determine the minimum sample size for each species for power of 0.8.

To account for differences in dietary endpoints at each study site, plasma isotope values were converted to proportional values (i.e. $\delta^{13}C_{prop}$ and $\delta^{15}N_{prop}$; Newsome et al., 2007) based on the maximum and minimum $\delta^{13}C$ and $\delta^{15}N$ values of each species, respectively — plasma values were taken for each species and adjusted to values of 0–1 based on their proximity to the minimum or maximum $\delta^{13}C$ and $\delta^{15}N$ values for each species.

After data were converted to proportional values, isotope vectors were quantified. To account for differences in $\delta^{13}C$ trophic shifts (ecosystem differences lead bull sharks in the Shark River Estuary become more depleted in $\delta^{13}C$ as they grow (Matich et al., 2010) and blacktip reef and sicklefin lemon sharks in Moorea become more enriched in $\delta^{13}C$ as they grow (J. Kiszka *unpublished data*)), the absolute values of $\delta^{13}C_{prop}$ were taken, and Kruskal-Wallis analysis of variance at $\alpha = 0.05$ was used to assess differences in isotopic slopes of $\delta^{13}C_{prop}$ and $\delta^{15}N_{prop}$ across species. A post hoc Mann-Whitney test at $\alpha = 0.05$ was used to test for significant differences across species.

Differences in plasma isotope values of bull sharks collected during initial captures and recaptures of sharks were compared to differences in plasma and in muscle isotope values collected upon the second capture of the same individuals to assess the information provided by each approach. To account for differences in discrimination values of muscle and plasma stable isotope values (Hussey et al., 2012; Kim et al., 2012), bull shark $\delta^{13}C$ and $\delta^{15}N$ muscle values were adjusted based on differences in $\Delta^{13}C$ (muscle more enriched than plasma by 1.2‰) and $\Delta^{15}N$ (muscle more depleted than plasma by 0.5‰). A Mann-Whitney test at $\alpha = 0.05$ was used to compare the differences in plasma isotope values ($\delta^{13}C$ and $\delta^{15}N$ separately) upon capture and recapture of bull sharks with the differences in plasma isotopes and muscle isotopes collected from each bull shark upon being captured for the second time (i.e. samples collected during the recapture of each individual). JMP 10 was used for all statistical analyses.

3. Results

3.1. Moorea, French Polynesia

Fourteen juvenile blacktip reef sharks 55–70.5 cm total length (TL; mean ± SD = 64.2 cm ± 3.7) were recaptured from 25 Jan 2012 to 4 May 2012 (Table 1). All sharks were young-of-the-year based on the presence of umbilical scars. Mean time between captures was 23 ± 12 days (range: 10–50 days). Plasma $\delta^{13}C$ values among individuals ranged from −8.90 to −13.34‰ and plasma $\delta^{15}N$ values ranged from 9.91 to 16.91‰ (mean $\delta^{13}C$ = −11.60‰ ± 1.06, mean $\delta^{15}N$ = 13.46‰ ± 1.77; Fig. 1a & b). Slopes for change in plasma isotope values with shark length were significantly different from zero (mean $\delta^{13}C/TL$ = 0.41‰/cm ± 0.72 ($t = 4.50$, $p < 0.01$), mean $\delta^{15}N/TL$ = −0.22‰/cm ± 0.59 for $\delta^{13}C$ ($t = 4.62$, $p < 0.01$); Fig. 1a & b, Table 1). Slopes for plasma isotope values with days between captures (i.e. days at liberty) were also significantly different from zero (mean $\delta^{13}C$/days at liberty = 0.03‰/day ± 0.05 ($t = 4.39$, $p < 0.01$), mean $\delta^{15}N$/days at liberty = −0.01‰/day ± 0.03 ($t = 7.06$, $p < 0.01$); Fig. 2a & b, Table 1). Power analysis revealed analytical power was between 0.4 and 0.8.

Four juvenile sicklefin lemon sharks 62–78 cm TL (mean ± SD = 71.1 cm ± 5.8) were recaptured from 15 Feb 2012 to 18 Apr 2012 (Table 1). Power analysis revealed analytical power was between 0.1 and 0.6, with a minimum sample size of 7 to achieve a power of 0.8, so we include only descriptive data for this species. All sharks were young-of-the-year based on the presence of umbilical scars. Mean time between captures was 22 ± 5 days (range: 14–25 days). Plasma $\delta^{13}C$ values among individuals ranged from −11.34 to −12.82‰ and plasma $\delta^{15}N$ values ranged from 13.08 to 14.28‰ (mean $\delta^{13}C$ = −12.1‰ ± 0.54, mean $\delta^{15}N$ = 13.74‰ ± 0.39; Fig. 1c & d). Mean slopes for change in isotope values with shark length were 0.49‰/cm ± 0.77 for $\delta^{13}C/TL$, and 0.19‰/cm ± 0.52 for $\delta^{15}N/TL$ (Fig. 1c & d, Table 1). The slopes for plasma isotope values with days between captures were 0.02‰/day ± 0.03 for $\delta^{13}C$ and −0.01‰/day ± 0.02 for $\delta^{15}N$ (Fig. 2c & d, Table 1).

3.2. Florida coastal everglades

Seven juvenile bull sharks 68–85 cm TL (mean ± SD = 77.9 cm ± 5.0) were recaptured from 7 Nov 2008 to 13 Aug 2013 (five individuals in 2008–2009, one individual in 2010, one individual in 2013; Table 1). Mean time between captures was 83 ± 36 days (range: 34–127 days). Plasma $\delta^{13}C$ values among individuals ranged from −16.36 to −25.19‰ and plasma $\delta^{15}N$ values ranged from 11.33 to 13.28‰ (mean $\delta^{13}C$ = −21.99‰ ± 2.91, mean $\delta^{15}N$ = 12.18‰ ± 0.56; Fig. 1e & f). Slopes for change in plasma with shark length were significantly different from zero (mean $\delta^{13}C/TL$ = −0.82‰/cm ± 0.67 ($t = 5.13$, $p < 0.01$), mean $\delta^{15}N/TL$ = −0.24‰/cm ± 0.20 ($t = 3.18$, $p =$

Table 1
Change (Δ) in $\delta^{13}C$ and $\delta^{15}N$ values (in ‰) with shark total length (TL; in cm) and days at liberty with dates of capture and total lengths upon capture and recapture (TL (C/R)) for blacktip reef sharks (*C. melanopterus*), sicklefin lemon sharks (*N. acutidens*), and bull sharks (*C. leucas*).

	ID	Initial capture	Recapture	TL (C/R)	Δ $\delta^{13}C$	Δ $\delta^{15}N$	Δ $\delta^{13}C/TL$	Δ $\delta^{15}N/TL$	Δ $\delta^{13}C$/days	Δ $\delta^{15}N$/days
C. melanopterus	*Cm*1	9-Feb-12	27-Feb-12	62/62.5	0.06	0.66	0.12	1.32	<0.01	0.04
	*Cm*2	20-Mar-12	15-Apr-12	63.5/65	−0.03	−0.75	−0.02	−0.50	>−0.01	−0.03
	*Cm*3	8-Apr-12	28-Apr-12	69/70.5	0.63	−0.98	0.42	−0.66	0.03	−0.05
	*Cm*4	25-Jan-12	5-Mar-12	62/69.5	0.86	0.99	0.12	−0.25	0.02	0.04
	*Cm*5	27-Mar-12	9-Apr-12	66/99	0.43	−0.57	0.15	−0.19	0.03	−0.04
	*Cm*6	24-Feb-12	3-Apr-12	66.69	1.78	−1.12	0.59	−0.37	0.05	−0.03
	*Cm*7	9-Mar-12	23-Apr-12, 4-May-12	65/67.5	0.76	−0.54	1.52	−1.08	0.02	−0.01
	*Cm*8	7-Feb-12	22-Feb-12	55/56	1.51	0.28	1.51	0.29	0.10	0.02
	*Cm*9	7-Feb-12	23-Feb-12, 23-Mar-12	60/64	1.40	−0.63	0.70	−0.32	0.03	−0.01
	*Cm*10	24-Mar-12	19-Apr-12	59/62	1.19	<0.01	0.40	<0.01	0.05	<0.01
	*Cm*11	23-Feb-12	13-Apr-12	65/67	0.65	−1.69	0.32	−0.85	0.01	−0.03
	*Cm*12	23-Feb-12	8-Mar-12	62/63	−0.67	−0.66	−0.67	−0.66	−0.05	−0.05
	*Cm*13	23-Feb-12	8-Mar-12	62.5/63.5	−0.85	0.02	−0.85	0.02	−0.06	<0.01
	*Cm*14	24-Mar-12	3-Apr-12	66/67	1.42	0.22	1.42	0.22	0.14	0.02
N. acutidens	*Na*1	15-Feb-12	11-Mar-12	75/75.5	0.80	0.47	1.59	0.94	0.03	0.02
	*Na*2	15-Feb-12	29-Feb-12	62/64	0.51	−0.41	0.26	−0.20	0.04	−0.03
	*Na*3	29-Feb-12	25-Mar-12	68.5/71	0.79	−0.31	0.31	−0.13	0.03	−0.01
	*Na*4	25-Mar-12	18-Apr-12	75/78	−0.58	0.42	−0.19	0.14	−0.02	0.02
C. leucas	*Cl*1	10-Jul-13	13-Aug-13	83/85	−0.90	−1.18	−0.45	−0.59	−0.03	−0.03
	*Cl*2	7-Nov-08	14-Mar-09	75/77	−3.00	−0.31	−1.50	−0.15	0.02	>−0.01
	*Cl*3	7-Nov-08	11-Jan-09	80/82	−1.87	−0.34	−0.94	−0.17	−0.03	−0.01
	*Cl*4	14-Mar-09	12-Jun-09	75/79	−6.02	−0.25	−1.50	−0.06	−0.07	>−0.01
	*Cl*5	14-Mar-09	12-Jun-09	70/75	−5.80	−1.27	−1.16	−0.26	−0.06	−0.01
	*Cl*6	8-May-09	24-Jun-09	81/83	0.75	−0.85	0.38	−0.43	0.02	−0.02
	*Cl*7	23-Jun-10	28-Oct-10	68/81	−7.30	−0.55	−0.56	−0.04	−0.06	−0.01

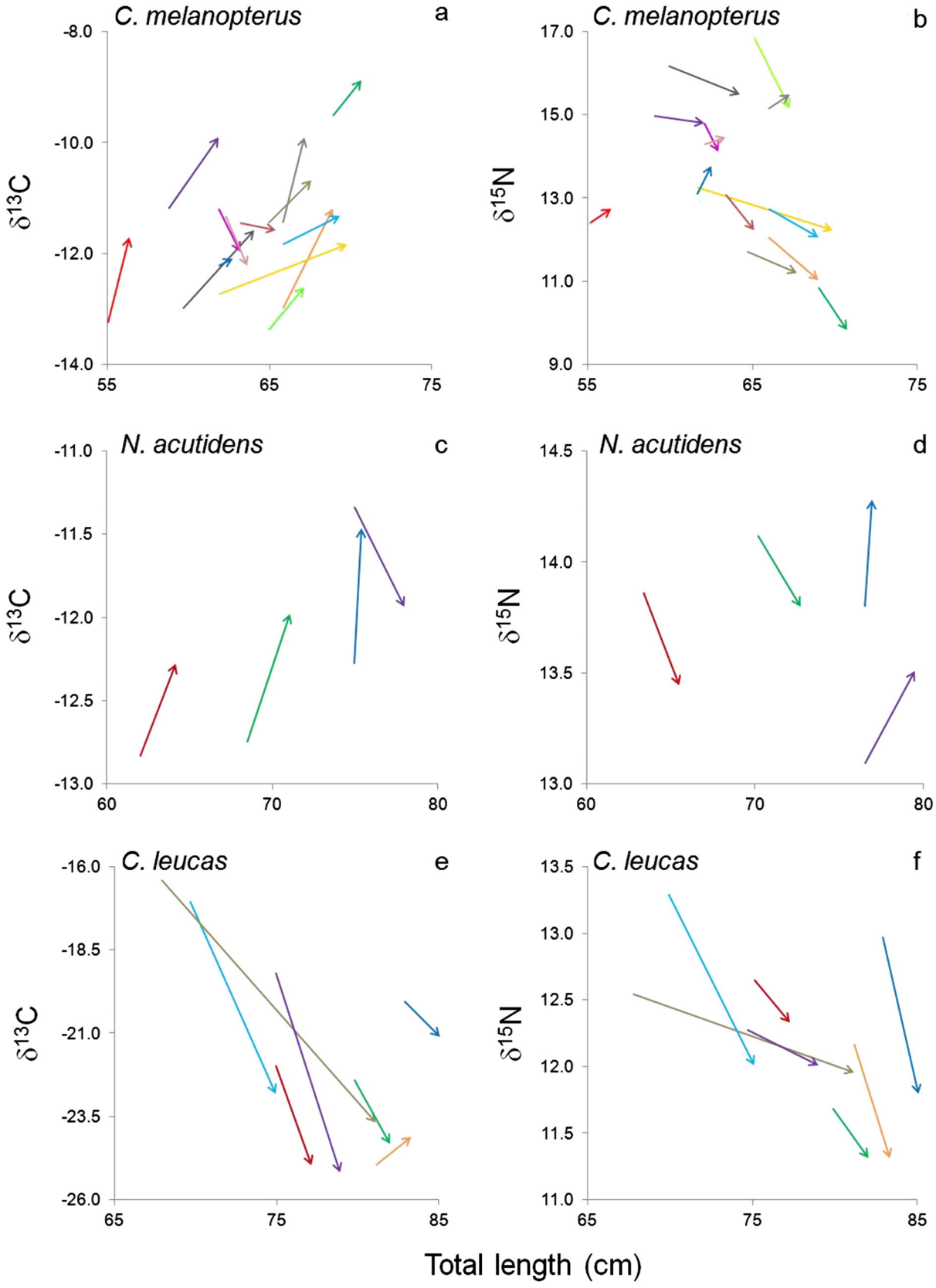

Fig.1. Change in $\delta^{13}C$ (a, c, & e) and $\delta^{15}N$ values (b, d, & f) with shark total lengths (in cm) for blacktip reef sharks (a & b), sicklefin lemon sharks (c & d), and bull sharks (e & f). Vectors represent plasma stable isotope data at size of initial capture (tail) and recapture (head) for each shark. Vectors of the same color in concurrent panels (i.e. a & b, c & d, e & f) are data from the same individual for each species.

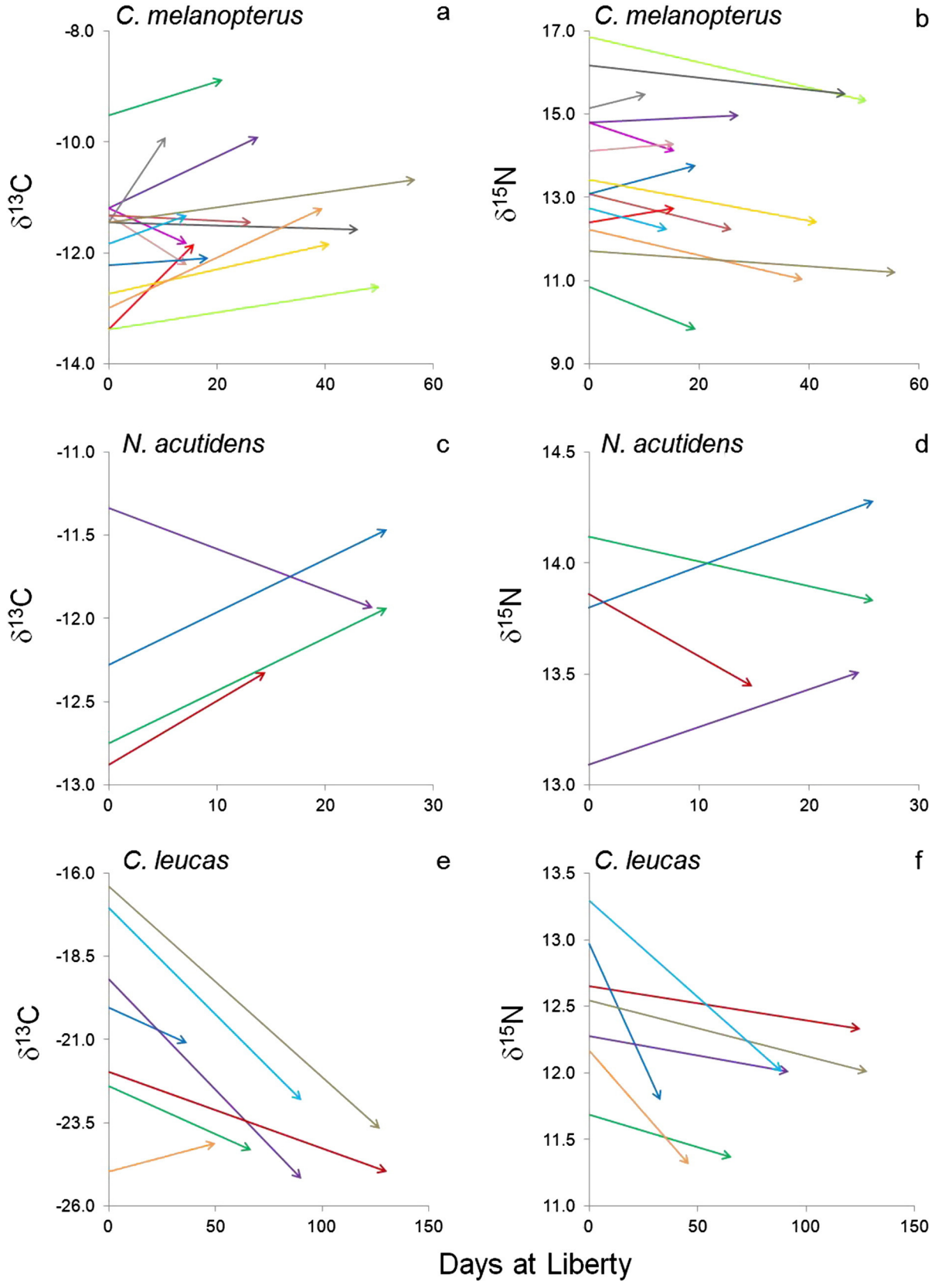

Fig. 2. Change in $\delta^{13}C$ (a, c, & e) and $\delta^{15}N$ values (b, d, & f) with days at liberty between captures for blacktip reef sharks (a & b), sicklefin lemon sharks (c & d), and bull sharks (e & f). Vectors represent plasma stable isotope data at day of initial capture (tail) and recapture (head) for each shark. Vectors of the same color in concurrent panels (i.e. a & b, c & d, e & f) are data from the same individual for each species.

0.02); Fig. 1e & f, Table 1). Mean slopes for plasma isotope values with days between captures were also significantly different from zero (mean $\delta^{13}C$/days at liberty = −0.03‰/day ± 0.04 (t = 5.18, $p < 0.01$), mean $\delta^{15}N$/days at liberty = −0.01‰/day ± 0.01 (t = 4.80, $p < 0.01$); Fig. 2e & f, Table 1). Power analysis revealed analytical power was between 0.6 and 0.9.

Among bull sharks in which plasma was collected upon capture and recapture, and muscle tissue was collected upon recapture (n = 5), most individuals exhibited similarities in the differences between plasma-plasma and plasma-muscle for $\delta^{13}C$ and $\delta^{15}N$ values ($z = 0.21$, $p = 0.83$; $z = 0.42$, $p = 0.67$ for $\delta^{13}C$ and $\delta^{15}N$, respectively; Table 2). Yet, there were notable differences in the magnitude of change across individuals ranging from 1.38 to 4.00‰ for $\delta^{13}C$ and 0.02 to 1.42‰ for $\delta^{15}N$.

3.3. Geographic/interspecific differences

When plasma isotope data were converted to absolute proportional values to account for geographic differences in food web structure (Table 3), no significant differences were found in the slopes of isotope vectors for the three species when quantified across shark total length using Kruskal-Wallis ANOVA ($\delta^{13}C_{prop}$/TL: $H = 0.34$, $p = 0.84$; $\delta^{15}N_{prop}$/TL: $H = 2.68$, $p = 0.26$). There was a significant difference in ranks of vector slopes, however, when absolute $\delta^{13}C_{prop}$ values were quantified across days at liberty ($H = 6.03$, $p < 0.05$). Post hoc Mann-Whitney tests revealed blacktip reef sharks ($z = 1.98$, $p < 0.05$) and sicklefin lemon sharks ($z = 2.44$, $p = 0.01$) exhibited faster rates of change in $\delta^{13}C_{prop}$ values (mean change in $\delta^{13}C_{prop}$ /days at liberty = 0.01/day for each species, i.e. 1% change in $\delta^{13}C$ per day) compared to bull sharks in Florida (mean = 0.005/day; Table 3).

4. Discussion

Newborn sharks, like many other vertebrates, undergo considerable changes in their trophic interactions during their first few months to years of life, transitioning from relying on maternal energy sources to being self-sufficient, and some undergoing ontogenetic shifts in their diets thereafter (e.g. Grubbs, 2010; Hussey et al., 2010b; Matich et al., 2010). After birth, placentatrophic sharks initially rely on energy reserves in their livers to sustain energetic needs while developing foraging skills (Belicka et al., 2012; Hussey et al., 2010b; Olin et al., 2011), and for some species, nursery habitats (e.g. coastal estuaries, lagoons) provide protection from predators during this critical period when young sharks refine hunting tactics and begin allocating energy to growth, development, and energy storage (Heithaus, 2007; Heupel et al., 2007). Stable isotope analysis offers multiple ways to quantify changes in trophic interactions during this period of development – e.g. collecting fast turnover tissues during multiple sampling events and collecting multiple tissues with different turnover tissues during one sampling event (Bearhop et al., 2004) – which provides insight into the timing of trophic shifts in juvenile sharks and interpretation of stable isotope values that may be influenced by maternal provisioning (McMeans et al., 2009; Olin et al., 2011). Our study employed successive samplings through time in order to evaluate shifts in energy pathways and trophic interactions of juvenile sharks.

Table 2
Difference in plasma and adjusted muscle $\delta^{13}C$ and $\delta^{15}N$ values (in ‰), and differences in plasma values upon capture and recapture (in ‰) of bull sharks (*C. leucas*).

ID	Plasma–muscle $\delta^{13}C$	Plasma–plasma $\delta^{13}C$	Plasma–muscle $\delta^{15}N$	Plasma–plasma $\delta^{15}N$
Cl2	−4.38	−3.00	−0.33	−0.31
Cl3	−5.87	−1.87	−0.60	−0.34
Cl4	−4.05	−6.02	−0.33	−0.25
Cl5	−2.35	−5.80	0.15	−1.27
Cl6	−2.71	0.75	−0.54	−0.85

Using stable isotope values from blood plasma, our study suggests shifts in trophic interactions are relatively fast among young-of-the-year carcharhinid sharks, at least for the species sampled in Florida and Moorea — the rate of change in plasma stable isotopes was 0.35‰ $\delta^{13}C$/cm total length (TL) and −0.16‰ $\delta^{15}N$/cm TL for blacktip reef sharks, 0.49‰ $\delta^{13}C$/cm TL and 0.19‰ $\delta^{15}N$/cm TL for sicklefin lemon sharks, and −0.82‰ $\delta^{13}C$/cm TL and −0.24‰ $\delta^{15}N$/cm TL for bull sharks, respectively, and up to 0.14‰/day. These changes in plasma isotope values suggest rapid changes in basal carbon sources among sharks during their first year, and the rates of change are considerably faster than previously calculated for young-of-the-year placentatrophic sharks using fast turnover tissue (e.g. calculated as −0.18‰/cm TL and −0.09‰/cm TL in liver $\delta^{13}C$ and $\delta^{15}N$ in *Rhizoprionodon terraenovae*, and −0.18‰/cm TL and −0.36‰/cm TL in liver $\delta^{13}C$ and $\delta^{15}N$ in *C. leucas*, based on data presented by Olin et al., 2011). From our results, we estimate that plasma isotopes likely reflect the diets of young sharks within 6–8 months after birth (blacktip reef sharks: 45–65 cm TL; bull sharks: 70–90 cm TL; sicklefin lemon sharks: 55–75 cm TL) based on the end points of maternal and neonate diets (Matich et al., 2010) and the growth rates of juvenile bull sharks in the Gulf of Mexico region (Neer et al., 2005) and juvenile blacktip reef sharks and juvenile sicklefin lemon sharks in the Pacific (Ebert et al., 2013; Mourier et al., 2013b). Recently, Shiffman et al. (2014) predicted that stable isotope values from muscle tissue (muscle $\delta^{15}N$ half-life = ~122 days; MacNeil et al., 2006; Kim et al., 2012; Malpica-Cruz et al., 2012) are indicative of dietary changes within two months of a diet switch, even for changes as small as 2‰ ($\delta^{13}C$) and 5‰ ($\delta^{15}N$) based on controlled feeding studies (Logan and Lutcavage, 2010). Our findings from sequentially sampled individuals, however, suggest a longer time period is needed to detect such changes, and that stable isotope data from a tissue with faster turnover rates (e.g. plasma $\delta^{15}N$ half-life = ~33 days, liver $\delta^{15}N$ half-life = ~39 days, whole blood $\delta^{15}N$ half-life = ~62 days; Caut et al., 2013; Kim et al., 2012; MacNeil et al., 2006; Malpica-Cruz et al., 2012), and/or using modeling approaches to test a priori hypotheses (e.g. Araujo et al., 2007; Lee et al., 2012; Matich and Heithaus, 2014) is more appropriate for detecting short-term shifts in elasmobranch trophic interactions.

The speed at which plasma $\delta^{13}C$ values changed in sharks varied across sampling sites and/or species, suggesting changes in energy pathways and trophic interactions may be context-specific among juvenile sharks. Indeed, blacktip reef sharks and sicklefin lemon sharks in Moorea both appear to undergo shifts in $\delta^{13}C$ twice as fast (~1%/day) as bull sharks in the Shark River Estuary (~0.5%/day) as reflected in their isotope values after being corrected for differences in dietary endpoints. Differences in predation pressure, life histories, environmental conditions, food availability, metabolisms, and species-specific discrimination values may all be responsible for the observed geographic differences in $\delta^{13}C$ shifts (reviewed by Kelly, 2000; Vanderklift and Ponsard, 2003; Martinez del Rio et al., 2009).

Large predatory sharks are extremely rare in the upstream portions of the Shark River Estuary (Heithaus et al., 2009; Matich and Heithaus, 2015; Wiley and Simpfendorfer, 2007), while sub-adult sicklefin lemon and adult blacktip reef sharks are often seen in close proximity to the shark nurseries of Moorea where we sampled (Mourier and Planes, 2013; Mourier et al., 2013a). These geographical differences in encounter rates suggest neonates in Moorea are likely to face higher risk than in the Shark River Estuary, and variation in predation regimes may contribute to behavioral differences among sharks that lead to variation in the speed at which they switch to self-feeding (Abrams, 2001; Brown and Kotler, 2004; McNamara, 1987).

Species specificity in stable isotope shifts may have also be shaped by interspecific differences in life history — bull sharks are born at larger sizes (60–70 cm TL; Curtis et al., 2011; Heithaus et al., 2009; Neer et al., 2005) than blacktip reef sharks (33–52 cm TL; Mourier et al., 2013b) and sicklefin lemon sharks (55–66 cm TL; Ebert et al., 2013). Thus, juvenile bull sharks may have larger livers and more energy stores to

Table 3
Slopes of length-isotope proportional value vectors and day-isotope proportional value vectors of recaptured blacktip reef sharks (*C. melanopterus*), sicklefin lemon sharks (*N. acutidens*), and bull sharks (*C. leucas*); p-values based on Newsome et al. (2007).

	Shark ID	$\Delta\ \delta^{13}C_{prop}$/TL	$\Delta\ \delta^{15}N_{prop}$/TL	$\Delta\ \delta^{13}C_{prop}$/days	$\Delta\ \delta^{15}N_{prop}$/days
C. melanopterus	Cm1	0.028	0.188	0.001	0.005
	Cm2	−0.004	−0.071	<0.001	−0.004
	Cm3	0.095	−0.094	0.007	−0.007
	Cm4	0.026	−0.019	0.005	−0.006
	Cm5	0.033	−0.027	0.008	−0.006
	Cm6	0.134	−0.053	0.010	−0.004
	Cm7	0.069	−0.031	0.004	−0.002
	Cm8	0.341	0.041	0.023	0.003
	Cm9	0.079	−0.023	0.007	−0.002
	Cm10	0.090	<0.001	0.010	<0.001
	Cm11	0.073	−0.121	0.003	−0.005
	Cm12	−0.151	−0.095	−0.011	−0.007
	Cm13	−0.191	0.003	−0.014	<0.001
	Cm14	0.320	0.032	0.032	0.003
N. acutidens	Na1	0.493	0.184	0.010	0.004
	Na2	0.080	−0.040	0.011	−0.006
	Na3	0.098	0.025	0.010	−0.003
	Na4	−0.060	0.027	−0.007	0.003
C. leucas	Cl1	−0.051	−0.303	−0.003	−0.018
	Cl2	−0.170	−0.079	−0.003	−0.001
	Cl3	−0.106	−0.088	−0.003	−0.003
	Cl4	−0.170	−0.033	−0.008	−0.001
	Cl5	−0.131	−0.131	−0.007	−0.007
	Cl6	0.043	−0.218	0.002	−0.009
	Cl7	−0.064	−0.022	−0.007	−0.002

rely on weeks or even months after birth (Hussey et al., 2010b), leading to slower shifts in energy pathways of young-of-the-year bull sharks as indicated in their plasma tissues. However, despite similarities in stable isotope shifts, sicklefin lemon sharks are much larger at birth than blacktip reef sharks yet exhibited similar rates of change in $\delta^{13}C$ and $\delta^{15}N$, suggesting mean size at birth may not have lead to the observed differences.

Seasonal variation in biotic and abiotic factors may also contribute to the geographic differences in stable isotope shifts observed. Environmental conditions and food availability vary seasonally in the Florida Everglades, with considerable shifts in freshwater flow, salinities, water temperatures, and allochthonous resources (Boucek and Rehage, 2013; Rosenblatt and Heithaus, 2011; Ruetz et al., 2005), while Moorea experiences more uniform conditions year-round (Hoegh-Guldberg and Salvat, 1995; Leichter et al., 2012). Therefore, seasonal variability in environmental conditions and food availability is likely more important in shaping trophic interactions and foraging development in bull sharks than blacktip reef sharks and sicklefin lemon sharks in our study (e.g. Ben-David et al., 1997; Cherel et al., 2009; Hall-Aspland et al., 2005). Seasonal variation in temperatures in Florida may also lead to seasonal variation in metabolic and growth rates and the assimilation of materials into tissues, and therefore affect estimates of shifts in energy pathways (Crawford et al., 2008; McIntyre and Flecker, 2006; Vanderklift and Ponsard, 2003).

Other factors, like prey preferences, species-specific isotopic discrimination factors, and isotopic differences of energy pathways can also lead to interspecific differences in behavior, and populations can be relatively specialized in their trophic interactions, which may also have contributed to interspecific variation in stable isotope shifts (reviewed in Bolnick et al., 2003; Holbrook and Schmitt, 1992; Post et al., 2000). The relatively low sample sizes for each species, however, limit our ability to draw conclusions on which extrinsic and intrinsic factors may be more important in shaping shark foraging development, especially considering the inherent variability in stable isotope values among individuals in each population.

In addition to the array of extrinsic and intrinsic factors that may lead to species- and/or site-specific differences in stable isotope shift, shifts in energy pathways were not uniform within species. Among bull sharks, the direction of change in $\delta^{13}C$ and $\delta^{15}N$ values was similar across most individuals, but the rate of change varied among individuals (range = 0.38‰/cm to −1.50‰/cm and −0.04‰/cm to −0.59‰/cm for $\delta^{13}C$ and $\delta^{15}N$, respectively), suggesting some individuals may develop foraging skills faster than others and/or individual differences in diets (e.g. specialists or generalists) may lead to differences in the speed at which stable isotopes change in juvenile bull sharks (Matich et al., 2011). Other factors, such as individual differences in growth rates and size at birth may also contribute to individual differences in plasma $\delta^{13}C$ and $\delta^{15}N$ shifts, as well as seasonal variation in prey availability, metabolic rates, and foraging behavior (e.g. Burrows and Hughes, 1991; Thiemann et al., 2011; Weise et al., 2010). Among blacktip reef sharks, individuals exhibited differences in the direction of change in plasma $\delta^{13}C$ and $\delta^{15}N$ – 79% of individuals (n = 11) became more enriched in $\delta^{13}C$ and more depleted in $\delta^{15}N$ as they grew, and 21% of sharks became more depleted in $\delta^{13}C$ and more enriched in $\delta^{15}N$ over time – suggesting individual differences in prey items blacktip reef sharks fed upon during the study. Temporal variability in environmental conditions and food availability likely did not lead to the observed differences in blacktip reef sharks considering all individuals were sampled within a three month period, however individual differences in size at birth, growth rates, and prey preferences may all lead to differences in energy pathway shifts in Moorea shark nurseries.

Individual differences were also exhibited among bull sharks when changes in plasma stable isotope values were compared to differences in plasma and muscle isotope values — some sharks exhibited similarities when the two methods were compared and other individuals showed differences, suggesting these two methods may provide different information. The limited number of sharks sampled and/or variation in time of capture and recapture may be responsible for the observed differences, but our previous work suggests that individual differences are prevalent early in the lives of at least some shark species, including bull sharks in the Shark River Estuary, and that changes in trophic interactions may not be uniform within shark nurseries (Matich and Heithaus, 2012, 2015; Matich et al., 2011). Yet, it is unclear if individual differences in intrinsic factors (e.g. size at birth, growth rates, body condition, prey preferences) may shape such individual differences and/or particular extrinsic factors (e.g. food availability, risk) are responsible for driving the observed differences (Bolnick et al., 2010; Sih et al., 2011). Continued work on the drivers of intraspecific variability among individuals early in their life-histories, and more studies

incorporating data from multiple ecosystems, including replicate nurseries of specific species in different contexts, will improve our understanding of foraging development in sharks and the development and persistence of individual differences in trophic interactions.

5. Conclusion

Sampling individuals multiple times provides the opportunity to investigate shifts in the sources of energy animals rely on for metabolic processes (e.g. Drago et al., 2010; Huckstadt et al., 2012). The results from our study using recaptured individuals support previous research suggesting shifts in energy pathways are both context- and species-specific (reviewed in Crawford et al., 2008; McIntyre and Flecker, 2006; Vanderklift and Ponsard, 2003). However, recapturing individuals, especially among highly mobile species like sharks, can be very difficult (recapture rates of sharks are often <10%; Skomal and Bernal, 2010), and therefore other approaches should also be considered when recapture rates are low. Serial sampling tissues that are metabolically inert, sampling large numbers of individuals from different life history classes, and comparing the isotopic values of multiple tissues with different turnover rates provide promising tools to overcome low recapture rates (e.g. Arthur et al., 2008; Estrada et al., 2006; Newsome et al., 2009; Wolf et al., 2009), and may improve our understanding of foraging development in juvenile animals, especially for cryptic or aquatic species that are not easily observed over long time periods. Our data suggest, however, that different approaches may provide different pieces of information, especially considering that differences in turnover rates among different tissues in elasmobranchs (e.g. plasma $\delta^{13}C$ half-life = ~22 days and muscle $\delta^{13}C$ half-life = ~100 days; Kim et al., 2012) could lead to differences in the time periods over which trophic comparison are made. Thus, we encourage the continued development of these methods to increase our abilities to study temporal variability in trophic interactions, especially in taxa that likely play important roles in their respective ecosystems.

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