Oecologia (2019) 189:621–636
https://doi.org/10.1007/s00442-019-04357-5

BEHAVIORAL ECOLOGY – ORIGINAL RESEARCH

Inter-individual differences in ontogenetic trophic shifts among three marine predators

Philip Matich[1,2] · Jeremy J. Kiszka[1] · Michael R. Heithaus[1] · Baptiste Le Bourg[3,4] · Johann Mourier[5,6,7]

Received: 30 May 2018 / Accepted: 11 February 2019 / Published online: 22 February 2019

Abstract

Ontogenetic niche shifts are widespread. However, individual differences in size at birth, morphology, sex, and personalities can cause variability in behavior. As such, inherent inter-individual differences within populations may lead to context-dependent changes in behavior with animal body size, which is of concern for understanding population dynamics and optimizing ecological monitoring. Using stable carbon and nitrogen isotope values from concurrently sampled tissues, we quantified the direction and magnitude of intraspecific variation in trophic shifts among three shark species, and how these changed with body size: spurdogs (*Squalus* spp.) in deep-sea habitats off La Réunion, bull sharks (*Carcharhinus leucas*) in estuarine habitats of the Florida Everglades, and blacktip reef sharks (*Carcharhinus melanopterus*) in coral reef ecosystems of Moorea, French Polynesia. Intraspecific variation in trophic shifts was limited among spurdogs, and decreased with body size, while bull sharks exhibited greater individual differences in trophic shifts, but also decreased in variability through ontogeny. In contrast, blacktip reef sharks exhibited increased intraspecific variation in trophic interactions with body size. Variability in trophic interactions and ontogenetic shifts are known to be associated with changes in energetic requirements, but can vary with ecological context. Our results suggest that environmental stability may affect variability within populations, and ecosystems with greater spatial and/or temporal variability in environmental conditions, and those with more diverse food webs may facilitate greater individual differences in trophic interactions, and thus ontogenetic trophic shifts. In light of concerns over environmental disturbance, elucidating the contexts that promote or dampen phenotypic variability is invaluable for predicting population- and community-level responses to environmental changes.

Keywords Dietary shifts · Elasmobranchs · Foraging development · Juveniles · Nursery

Introduction

Temporal variation in trophic interactions is widespread among animals, including many predators that undergo dietary shifts attributed to changes in energy requirements, morphology, and habitat use patterns through ontogeny (e.g., McClellan and Read 2007; Snover 2008; Newsome et al.

Communicated by Jeremy Long.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00442-019-04357-5) contains supplementary material, which is available to authorized users.

✉ Philip Matich
pmati001@fiu.edu

[1] Marine Sciences Program, Florida International University, 3000 NE 151st, North Miami, FL 33181, USA

[2] Texas Research Institute for Environmental Studies, Sam Houston State University, 2424 Sam Houston Avenue, Huntsville, TX 77341, USA

[3] LIttoral ENvironnement et Sociétés (LIENSs), UMR 7266 CNRS-Université de la Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France

[4] Laboratory of Oceanology, MARE Centre, Université de Liège, 4000 Liège, Belgium

[5] PSL Research University: EPHE-UPVD-CNRS, USR 3278 CRIOBE, 66860 Perpignan, France

[6] Laboratoire d'excellence 'CORAIL', EPHE, PSL Research University, UPVD, CNRS, USR 3278 CRIOBE, Papetoai, Moorea, French Polynesia

[7] UMR MARBEC (IRD, Ifremer, Univ. Montpellier, CNRS), 34203 Sète, France

2009a). Consequently, as individuals grow, shifts in trophic interactions can lead to changes in species ecological importance within food webs, especially among wide-ranging species that undertake movements across ecosystem boundaries (e.g., Woodward and Hildrew 2002; Field et al. 2007; Subalusky et al. 2009; McCauley et al. 2012). As such, quantifying ontogenetic variation in trophic interactions is important not only for understanding life-history traits, but also for elucidating how inherent intraspecific variation in the ecological roles of species may be shaped through ontogeny.

When species undergo ontogenetic niche shifts, some models predict that most or all individuals within a population follow a similar trajectory through their life history. However, an increasing number of studies highlight the prevalence of individual variation within populations, necessitating studies to account for such individual differences (reviewed by Skulason and Smith 1995; Bolnick et al. 2003; Sih et al. 2004; Mittelbach et al. 2014). Given the wide array of species that undergo shifts in trophic interactions through ontogeny, and the diversity of species that exhibit intraspecific variation within size classes, it is likely that there are size (and age)-based changes in intraspecific variability in many taxa (i.e., changes in how similar or dissimilar individuals are in their feeding ecology). For example, species that undergo ontogenetic habitat shifts from coastal to pelagic ecosystems may experience decreases in intraspecific variation in trophic interactions due to fewer $\delta^{13}C$ sources in pelagic food webs (Hyndes et al. 2014; Bird et al. 2018). Comparatively, species that undergo ontogenetic niche shifts from coastal to estuarine habitats may exhibit greater trophic variability through ontogeny, because of the inherent fluctuations in environmental conditions and food resources (Pritchard 1967; Elliot and Quintino 2007; Fig. 1). An understanding of how behaviour and trophic interactions vary among individuals with ontogeny is important for elucidating intraspecific variations of the ecological roles of species, as well as their susceptibility to disturbance and environmental change based on resource use (e.g., Colles et al. 2009; Clutton-Brock and Sheldon 2010; Salisbury et al. 2012; de Roos and Persson 2013).

Stable isotope analysis is commonly used to investigate ontogenetic shifts in trophic interactions, because it presents time-integrated views of trophic interactions over relatively predictable time periods (e.g., Post 2002; Martinez del Rio et al. 2009; Wolf et al. 2009). However, approaches using stable isotope analysis can provide a limited understanding of species feeding ecologies, since this method traditionally averages the diet of a given individual

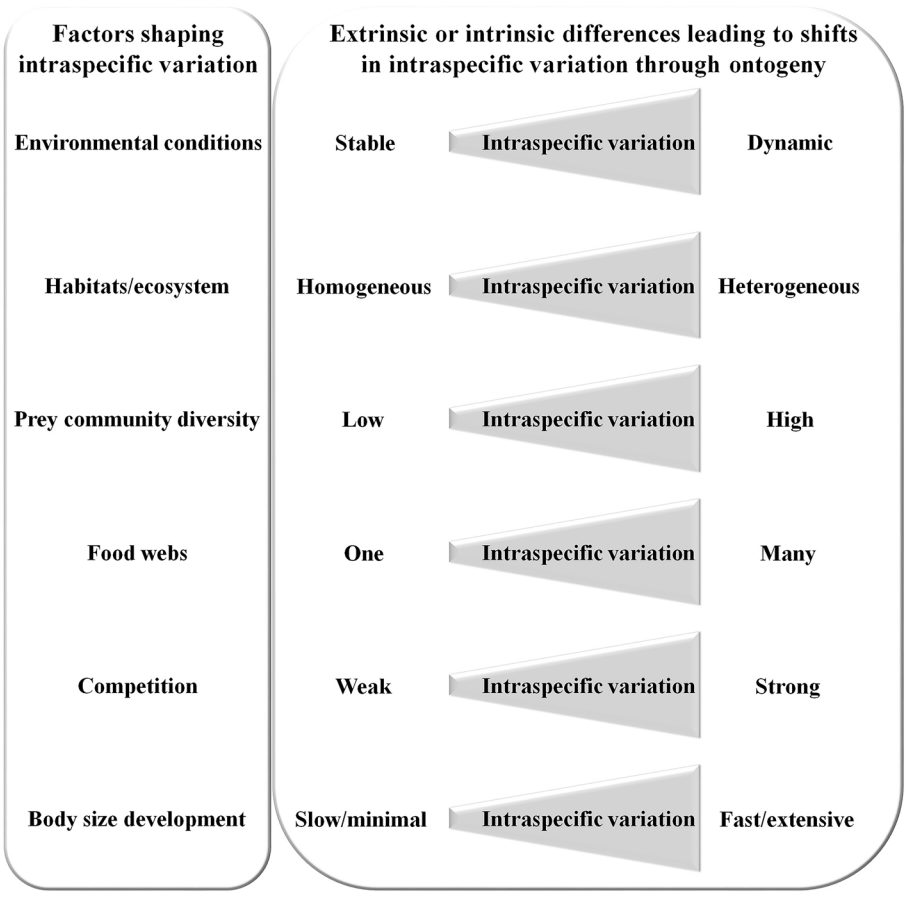

Fig. 1 Potential factors that may lead to differences in intraspecific variation within populations. Ontogenetic shifts in behavior that lead to greater use of habitats with more dynamic environmental conditions, more heterogeneous structure, more diverse prey communities, multiple food webs, and stronger competition may lead to greater variability in behavior among individuals. Intrinsic factors, including the speed at which individual grow and/or develop may also play a role in phenotypic diversity through ontogeny

over a time period dependent on the turnover rate of the tissue(s) considered (Layman et al. 2012). Yet, several approaches can be used to investigate both ontogenetic niche shifts and variability in niche shifts across individuals using stable isotopes (e.g., Estrada et al. 2006; Knoff et al. 2008; Matich et al. 2015):

1. Using traditional methods with one tissue type analyzed—the residuals of regression models are quantified to elucidate if variability around the mean is similar or different across animal sizes.
2. Using metabolically inert tissues, such as bone, cartilaginous tissues or vibrissae—serial sampling provides a continuous temporal record (i.e., history) of trophic interactions, which can be used to identify sizes that exhibit greater or lesser intraspecific variability than others; this method often requires access to dead individuals (e.g., Estrada et al. 2006; Knoff et al. 2008; Newsome et al. 2009b).
3. Sampling a single tissue type from individuals upon multiple captures—a longitudinal record provides insight into trophic variability through ontogeny; but low recapture rates can limit the use of this approach (but see Drago et al. 2010; Hückstadt et al. 2012; Matich et al. 2015).
4. The collection of multiple tissues with different turnover rates—differences among tissues can provide estimates of the direction and magnitude of shifts of average trophic interactions, and intraspecific variation in these trophic shifts across a size range (Bearhop et al. 2004; Matich et al. 2011; Rosenblatt et al. 2015).

While all four approaches provide rigorous methods to investigate ontogenetic trophic shifts and variability in such trophic shifts among individuals, the first and fourth approaches feature fewer logistical constraints (animals must only be sampled once and do not need to be dead/euthanized). Key to comparing multiple tissues (approach 4) is selecting tissues that are metabolically active and have considerably different turnover/incorporation rates, to ensure differences in tissues are indicative of shifts in trophic interactions.

Here, we use both single-tissue and two-tissue approaches to quantify variability in ontogenetic trophic shifts using stable carbon and nitrogen isotope analysis ($\delta^{13}C$, $\delta^{15}N$) from concurrently collected tissues of three shark species—spurdogs (*Squalus* spp.), bull sharks (*Carcharhinus leucas*), and blacktip reef sharks (*Carcharhinus melanopterus*). Each species was sampled in a different ecosystem type including deep-sea (spurdog), estuarine (bull shark), and coral reef-dominated lagoon (blacktip reef shark) habitats.

Methods

Study sites and field methods

La Réunion is a tropical volcanic island located in the south-western Indian Ocean (21°07′S, 55°32′E), ca. 800 km east of Madagascar and 60 km west of Mauritius. The island is characterized by a steep topography, and deep oceanic waters occur in close proximity to coastal waters. Bottom-set longline fishing surveys were undertaken in slope waters around the island to sample spurdogs between November and December 2011 in depths between 100 and 400 m. Upon capture, shark total length was measured to the nearest 0.5 cm, weight was measured to the nearest 0.1 kg, and sex was determined. Muscle and liver tissues were collected from each individual, then were dried and ground into a fine powder. Liver has a much faster isotopic turnover rate (14- to 39-day half-life) than muscle tissue (98- to 216-day half-life) in elasmobranchs, with an average difference of ca. 131 days (MacNeil et al. 2006; Kim et al. 2012). Lipids were removed from muscle and liver samples by three successive extractions prior to stable isotope analysis (1-h shaking in 4 cm^3 of cyclohexane at room temperature and subsequent centrifugation; Chouvelon et al. 2011). After drying, lipid-free sub-samples were analyzed at the isotope facility of the University of La Rochelle. Replicate measurements of a laboratory standard (acetanilide) indicated that analytical errors were < 0.1‰ SD for $\delta^{13}C$ and $\delta^{15}N$. Percent C and N elemental composition of tissues were obtained using the elemental analyzer and used to calculate the sample C:N ratio (mean C:N ± SD: 2.77 ± 0.16 for muscle and 3.28 ± 0.24 for liver).

The Shark River Estuary, Florida, USA (25°25′N, 80°59′W) serves as a nursery for juvenile bull sharks year-round until sharks disperse to coastal marine habitats after 2–5 years of residence (Matich and Heithaus 2015). Bull sharks were sampled during long-term monitoring from 2008 to 2013 using bottom-set longlines fished between 1- and 7-m depth (Heithaus et al. 2009). Sharks were externally tagged using a numbered roto tag affixed through the first dorsal fin to identify recaptured individuals, shark total length was measured to the nearest 0.5 cm, weight was measured to the nearest 1 kg, and sex was determined. An 18-gauge needle was used to collect 3 mL of blood from the caudal vein of each individual, and 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 1 min at 3000 rpm. Muscle samples (0.5 cm^3 of tissue) were collected from each bull shark using a biopsy punch ca. 5 cm lateral to the first dorsal

fin. Blood plasma has a much faster isotopic turnover rate (16–63-day half-life) than muscle tissue (98- to 216-day half-life) in elasmobranchs, with an average difference of ca. 118 days (MacNeil et al. 2006; Kim et al. 2012; Caut et al. 2013). Plasma and muscle samples were put on ice and frozen before laboratory preparations. All tissue samples were dried and homogenized prior to stable isotopic analysis at Florida International University's Stable Isotope Laboratory, during which variation among standards was 0.12‰ and 0.10‰ ± SD for $\delta^{13}C$ and $\delta^{15}N$, respectively. Mean C:N values ± SD were 1.99 ± 0.17 for plasma, and 3.06 ± 0.20 for muscle.

Blacktip reef sharks were sampled in the waters of Moorea, French Polynesia (17°30′S, 149°51′W), a high island surrounded by shallow sand-bottom lagoons that serve as nurseries for juvenile sharks. Fringing coral reefs in deeper waters, as well as the outer slope, serve as habitat for sub-adults and adults (Mourier et al. 2013). Using small gillnets and baited rod and tackle, blacktip reef sharks were captured during sampling efforts from 2008 to 2012 in depths between 1 and 20 m. Juvenile blacktip reef sharks were externally tagged using a numbered identification tag implanted next to the first dorsal fin, while a photograph of each side of the first dorsal fin was taken of adult sharks for identification (Mourier et al. 2012). Sharks were processed in the same manner as bull sharks, except for the collection of muscle, which was not sampled. Instead, scissors were used to collect a 0.5 cm^3 tissue clip from the first dorsal fin of each individual, which has an isotopic half-life of ca. 134 days in elasmobranchs, with an average difference in the isotopic half-life of plasma and fin tissue of ca. 95 days (MacNeil et al. 2006). Fin and plasma samples were processed in the same manner as those for bull sharks, with variation among standards = 0.06‰ and 0.08‰ ± SD for $\delta^{13}C$ and $\delta^{15}N$, respectively. Mean C:N values ± SD were 1.91 ± 0.19 for plasma, and 2.80 ± 0.12 for fin.

Quantitative analysis

Single-tissue analysis

All statistical analyses were conducted in IBM SPSS 22. We quantified size-based changes in $\delta^{13}C$ and $\delta^{15}N$ values of spurdogs (liver and muscle), bull sharks (plasma and muscle), and blacktip reef sharks (plasma and fin) to compare ontogenetic niche shifts of each population across short-term (liver and plasma) and long-term (muscle and fin) trophic averaging. While sampled tissues exhibit different turnover rates, fast turnover tissues (liver and plasma) and slow turnover tissues (muscle and fin) provide trophic information on comparable timeframes (weeks to months, and months to years, respectively; Table 1) as other studies comparing different predator species have shown (e.g., Saporiti et al. 2016; Matich et al. 2017; Valls et al. 2017; Yurkowski et al. 2017). Raw, uncorrected $\delta^{13}C$ and $\delta^{15}N$ values were plotted against shark total length for each species, respectively, and least squares regression was used to quantify linear and polynomial trends. Polynomial selection was based on significant improvements of R^2 and F values. Post hoc Z tests were used to compare slopes of best-fit lines within species when both tissue types (e.g., plasma and muscle of bull sharks) exhibited significant ontogenetic shifts for one or both stable isotopes.

Table 1 Isotopic half-lives and tissue discrimination factors (in ‰) of elasmobranch blood plasma, liver, muscle, and fin tissues from MacNeil et al. (2006)[a] (*Potamotrygon motoro*), Kim et al. (2012)[b] (*Triakis semifasciata*), and Malpica-Cruz et al. (2012)[c] (*Triakis semifasciata*)

Tissue	$\delta^{13}C$		$\delta^{15}N$	
	Half-life	Discrimination	Half-life	Discrimination
Plasma	22 days[b]	ca. 3.3[b]	33 days[b]	ca. 3.2[b]
Liver	NA	ca. 2.4[c]	39 days[a]	ca. 1.4[c]
Muscle	100 days[b,c]	ca. 2.9[b,c]	120 days[a,b,c]	ca. 3.9[b,c]
Fin	NA	ca. 4.2[c]	134 days[a]	ca. 1.9[c]

The residuals of $\delta^{13}C$ or $\delta^{15}N$ vs. length regression plots were then analyzed to quantify if and how variability in trophic shifts changed with animal size. Among populations with relatively uniform/predictable shifts in trophic interactions, intraspecific variation in $\delta^{13}C/\delta^{15}N$–TL relationships should be minimal (i.e., individuals are similar in the direction and magnitude of diet changes), and residuals of regression models should be small, with no trend with size. However, if individuals are not uniform in diet shifts, then residuals may vary in magnitude and/or exhibit a directional relationship with size, providing insight into the variability and predictability of ontogenetic shifts.

Least squares regression requires residuals to be normally distributed to avoid violating assumptions. However, trends in residuals from untransformed data provide information on how variation in trophic shifts changes with size or other dependent variables (e.g., time if such an approach is used to quantify shifts in trophic interactions across seasons or years)—as variability in trophic shifts increases or decreases with animal size, the range of residuals from the regression model will increase or decrease with size, respectively. Thus, we plotted the absolute values of $\delta^{13}C$ and $\delta^{15}N$ residuals against shark total length for each species. Least squares regression was used to quantify linear and polynomial trends. Polynomial selection was based on significant improvements of R^2 and F values. Interpretations of best-fit lines were made under the following assumptions:

1. Small residuals suggest limited intraspecific variation in trophic shifts, and large residuals suggest relatively high intraspecific variation in trophic shifts.
2. A non-significant slope (i.e., no relationship) indicates no change in intraspecific variation in trophic shifts with size.
3. A negative slope indicates that intraspecific variation in trophic shifts decreases through ontogeny, and individuals become more similar as they grow larger.
4. A positive slope indicates that intraspecific variation in trophic shifts increases through ontogeny, and individuals become less similar as they grow larger.

Post hoc t-tests were also used to quantify the effects of sex and body condition on ontogenetic shifts. Body condition was calculated using residuals of body length vs. body mass (Online Appendix I).

Two-tissue analysis

To compare the utility of individually using $\delta^{13}C$ and $\delta^{15}N$ values from one tissue with the efficacy of concurrently using $\delta^{13}C$ and $\delta^{15}N$ values from two tissues, we plotted the paired differences of $\delta^{13}C$ and $\delta^{15}N$ values (isotope value of a slow turnover tissue subtracted from a discrimination-corrected isotope value of a fast turnover tissue; Matich et al. 2010) against total length for each species. Muscle $\delta^{13}C$ and $\delta^{15}N$ values were subtracted from discrimination-corrected liver $\delta^{13}C$ and $\delta^{15}N$ values for spurdogs; muscle $\delta^{13}C$ and $\delta^{15}N$ values were subtracted from discrimination-corrected plasma $\delta^{13}C$ and $\delta^{15}N$ values for bull sharks; and fin $\delta^{13}C$ and $\delta^{15}N$ values were subtracted from discrimination-corrected plasma $\delta^{13}C$ and $\delta^{15}N$ values for blacktip reef sharks (Hussey et al. 2012; Table 1). Least squares regression was used to quantify linear and polynomial trends. Polynomial selection was based on significant improvements of R^2 and F values. Interpretations of best-fit lines were made under the following assumptions (Matich et al. 2010):

1. A non-significant slope (i.e., no relationship) indicates a consistent magnitude and direction in $\delta^{13}C$ or $\delta^{15}N$ shifts, or no shift in trophic interactions.
2. A positive slope indicates a faster enrichment in ^{13}C or ^{15}N of the fast turnover tissue compared to the slow turnover tissue with shark size, which would be indicative of increased consumption of ^{13}C- or ^{15}N-enriched food with ontogeny.
3. A negative slope indicates a faster depletion in ^{13}C or ^{15}N of the fast turnover tissue compared to the slow turnover tissue with shark size, which would be indicative of increased consumption of ^{13}C- or ^{15}N-depleted food with ontogeny.
4. A positive y-value indicates an enrichment in ^{13}C or ^{15}N with shark size, a negative y-value indicates a depletion in ^{13}C or ^{15}N with shark size, and a y-value of zero indicates equilibrium of fast turnover and slow turnover $\delta^{13}C$ or $\delta^{15}N$ values.

The absolute values of $\delta^{13}C$ and $\delta^{15}N$ paired difference residuals (i.e., liver–muscle, plasma–muscle, and plasma–fin) were plotted against shark total length for each species, respectively, to compare findings from analyzing a single tissue and analyzing two tissues concurrently. Least squares regression was used to quantify linear and polynomial trends. Polynomial selection was based on significant improvements of R^2 and F values. Interpretations of best-fit lines were made under the same assumptions as single-tissue analysis. Post hoc Z tests were used to compare slopes of best-fit lines within species for single-tissue analysis and two-tissue analysis when both exhibited significant ontogenetic shifts, to evaluate each method. Post hoc t tests were used to quantify the effects of sex and body condition on ontogenetic shifts.

Results

Spurdogs

Liver and muscle samples were collected from 53 individuals ranging from 29.5 to 77 cm TL. Raw $\delta^{13}C$ and $\delta^{15}N$ values showed that spurdogs exhibited small but significant enrichments in ^{13}C and ^{15}N with total length (Fig. 2a, d), with liver exhibiting a faster enrichment in ^{13}C than muscle ($Z = 2.88$, $p < 0.01$). This species did not exhibit an ontogenetic shift in liver $\delta^{15}N$. Sex did not affect ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ (Table 2). Body condition did not affect ontogenetic shifts in $\delta^{13}C$, and did not affect liver $\delta^{15}N$ (Table 2). Body condition affected ontogenetic shift in muscle $\delta^{15}N$ ($t = 2.40$, $p = 0.02$; Table 2), with a greater enrichment of $\delta^{15}N$ with total length among sharks with negative body conditions (i.e., negative residuals) than sharks with positive body conditions (Online Appendix II).

Liver–muscle paired differences suggested that spurdogs exhibited a relatively consistent ontogenetic shift in $\delta^{13}C$ (Fig. 3a), and a decreasing enrichment in ^{15}N through ontogeny (Fig. 3d). Spurdogs approached equilibrium (liver–muscle = 0‰) at ca. 60–70 cm TL. Neither sex nor body condition affected ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ paired differences (Table 2).

Residuals of both muscle and liver least squares regression exhibited no trend in $\delta^{13}C$ shifts with spurdog size (Fig. 4a), with no effect of sex or body condition (Table 2). Residuals of muscle least squares regression exhibited no trend in $\delta^{15}N$ with spurdog size (Fig. 4d), with no effect

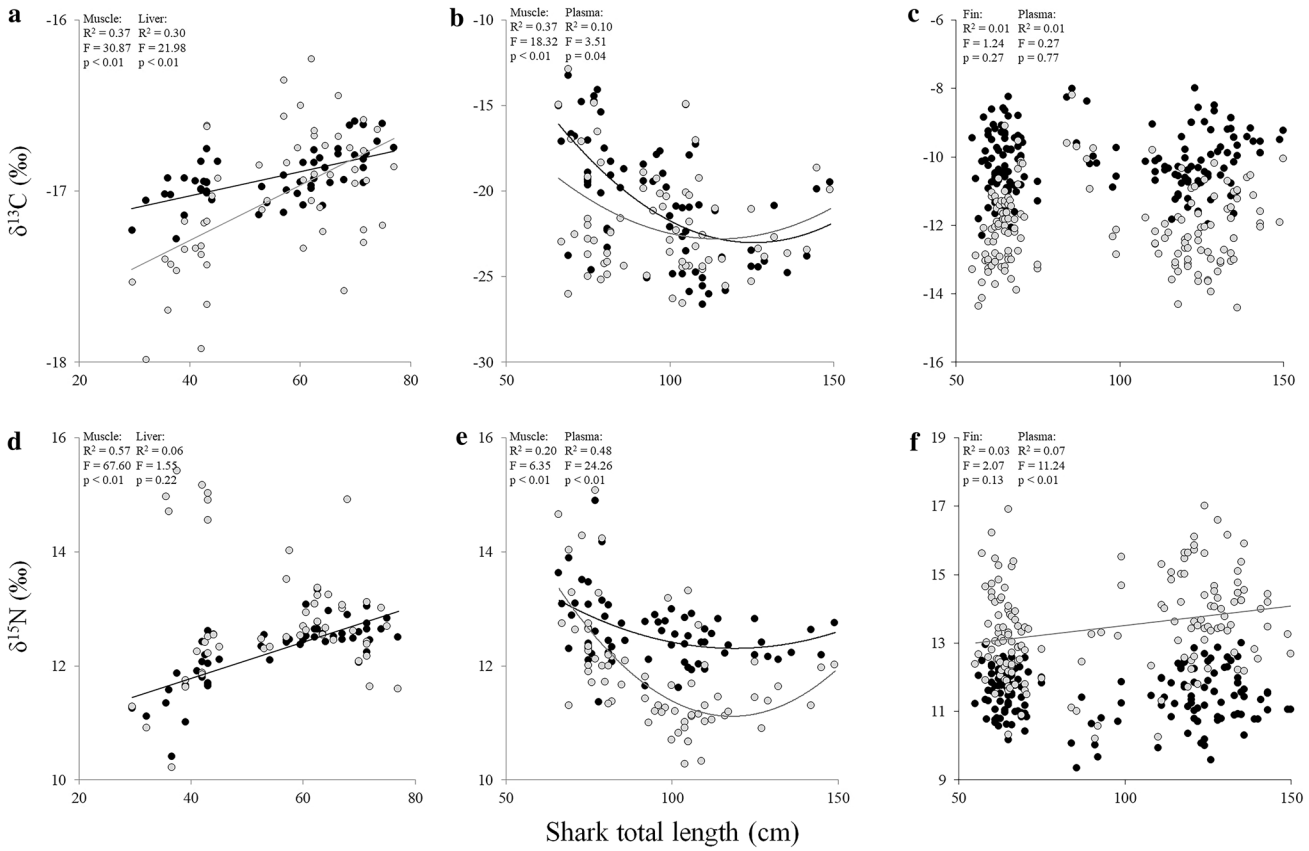

Fig. 2 Relationships between shark total length and $\delta^{13}C$ (**a**–**c**) and $\delta^{15}N$ values (**d**–**f**) among spurdogs (**a**, **d**; $N=53$), bull sharks (**b**, **e**; $N=66$), and blacktip reef sharks (**c**, **f**; $N=158$). Muscle stable isotope values for spurdogs are black data points, and liver stable isotope values are gray data points (**a**, **d**). Muscle stable isotope values for bull sharks are black data points, and plasma stable isotope values are gray data points (**b**, **e**). Fin stable isotope values for blacktip reef sharks are black data points, and plasma stable isotope values are gray data points (**c**, **f**). The color of best-fit lines corresponds to the color of data points. Only significant relationships are displayed

of sex or body condition (Table 2). Spurdogs exhibited a significant decrease in liver $\delta^{15}N$ residuals with shark size (Fig. 4d), with no effect of sex or body condition (Table 2).

Residuals of liver–muscle paired differences showed no change, and limited intraspecific variability in $\delta^{13}C$ shifts with spurdog size (Fig. 5a). Spurdogs exhibited a significant decrease in liver–muscle $\delta^{15}N$ shifts with shark total length (− 0.04‰/cm TL), with sharks approaching equivalence at ca. 75 cm TL (Fig. 5d). Neither sex nor body condition affected the residuals of $\delta^{13}C$ or $\delta^{15}N$ paired differences (Table 2). Residuals of $\delta^{13}C$ exhibited no significant shift with shark total length, and therefore, differences in single-tissue and two-tissue data were not tested. The slope of liver $\delta^{15}N$ residuals was not significantly different from the slope of liver–muscle $\delta^{15}N$ residuals ($Z=0.22$, $p=0.83$)

Bull sharks

Plasma and muscle samples were collected from 66 immature bull sharks in the Shark River Estuary, FL from November 2008 to September 2013, ranging from 66 to 149 cm TL (0–5 years old; males mature at 176–220 cm TL, females mature at 189–225 cm TL; Branstetter and Stiles 1987; Natanson et al. 2014). Raw $\delta^{13}C$ and $\delta^{15}N$ values showed that bull sharks exhibited significant depletions then enrichments in ^{13}C and ^{15}N with total length (Fig. 2b and e), with no differences in muscle and plasma for $\delta^{13}C$ ($Z=1.78$, $p=0.08$) or $\delta^{15}N$ ($Z=0.13$, $p=0.90$). Neither sex nor body condition affected ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ (Table 2).

Plasma–muscle paired differences showed a slower depletion and a faster enrichment in plasma ^{13}C than muscle ^{13}C through ontogeny (Fig. 3b), and relatively consistent shifts in $\delta^{15}N$ across tissues with total length (Fig. 3e). Bull sharks approached equilibrium in $\delta^{13}C$ at ca. 120 cm TL, and equilibrium in $\delta^{15}N$ at ca. 160 cm TL. Sex did not affect ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ paired differences (Table 2). Body condition did not affect ontogenetic shifts in $\delta^{15}N$ paired differences (Table 2). Body condition did affect ontogenetic shifts in $\delta^{13}C$ paired differences ($t=2.23$,

Table 2 Results of post hoc *t*-tests used to quantify the effects of sex and body condition on stable isotope values, paired differences, and residuals of regression plots

Squalus spp	Muscle	Liver	Muscle–liver	Residual (muscle)	Residual (liver)	Residual (muscle–liver)
Sex						
$\delta^{13}C$	1.00, 0.32	0.05, 0.96	0.40, 0.69	1.05, 0.30	0.07, 0.95	0.42, 0.68
$\delta^{15}N$	1.19, 0.24	0.70, 0.49	0.36, 0.72	0.23, 0.82	0.65, 0.52	0.57, 0.57
Body condition						
$\delta^{13}C$	1.52, 0.14	0.56, 0.58	0.02, 0.98	1.74, 0.09	0.18, 0.86	0.12, 0.90
$\delta^{15}N$	**2.40, 0.02**	0.21, 0.84	0.12, 0.90	0.58, 0.57	0.99, 0.33	0.29, 0.77
C. leucas	Muscle	Plasma	Muscle–plasma	Residual (muscle)	Residual (plasma)	Residual (muscle–plasma)
Sex						
$\delta^{13}C$	1.11, 0.27	0.79, 0.43	1.60, 0.12	1.25, 0.22	0.39, 0.70	0.90, 0.37
$\delta^{15}N$	0.11, 0.91	0.06, 0.95	0.19, 0.85	0.56, 0.58	0.05, 0.96	1.06, 0.29
Body condition						
$\delta^{13}C$	1.65, 0.11	0.80, 0.43	**2.23, 0.03**	0.20, 0.84	0.05, 0.96	0.53, 0.60
$\delta^{15}N$	0.18, 0.86	0.89, 0.38	0.22, 0.83	1.22, 0.23	0.90, 0.37	0.14, 0.89
C. melanopterus	Fin	Plasma	Fin–plasma	Residual (fin)	Residual (plasma)	Residual (fin–plasma)
Sex						
$\delta^{13}C$	0.80, 0.43	0.79, 0.43	0.35, 0.73	1.10, 0.28	1.13, 0.26	1.21, 0.23
$\delta^{15}N$	1.27, 0.21	1.04, 0.30	0.01, 0.99	0.44, 0.66	1.17, 0.25	0.07, 0.94
Body condition						
$\delta^{13}C$	0.02, 0.99	0.25, 0.81	1.19, 0.24	NA	NA	0.93, 0.36
$\delta^{15}N$	0.34, 0.74	0.10, 0.92	0.92, 0.36	NA	NA	1.87, 0.07

Values presented are *t* values, *p* values. Values in bold are significant at $\alpha = 0.05$. Body condition values for the residuals of fin and plasma $\delta^{13}C$ and $\delta^{15}N$ values of *Carcharhinus melanopterus* unavailable due to low sample size

$p = 0.03$; Table 2), with a slower depletion and a faster enrichment in plasma ^{13}C than muscle ^{13}C among sharks with negative body conditions compared to sharks with positive body conditions (Online Appendix II).

Residuals of muscle least squares regression exhibited no trend in $\delta^{13}C$ with bull shark size (Fig. 4b), with no effect of sex or body condition (Table 2). Bull sharks exhibited a significant decrease in plasma $\delta^{13}C$ residuals with shark size (Fig. 4b), with no effect of sex or body condition (Table 2). Residuals of both muscle and plasma $\delta^{15}N$ residuals exhibited significant, negative trends with bull shark size (Fig. 4e), with no effect of sex or body condition (Table 2). The best-fit lines for muscle and plasma $\delta^{15}N$ residuals were not significantly different ($Z = 0.28$, $p = 0.78$).

Residuals of plasma–muscle paired differences showed no change, and limited intraspecific variability in $\delta^{15}N$ shifts with bull shark size (Fig. 5e). Bull sharks exhibited a moderately significant decrease and increase in residuals of plasma–muscle paired differences of $\delta^{13}C$ shifts with shark total length, with greatest similarity among individuals at ca. 110 cm TL (Fig. 5b). Neither sex nor body condition affected the residuals of ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ paired differences (Table 2). Residuals of plasma $\delta^{13}C$ exhibited a negative linear relationship with shark size, while residuals of plasma–muscle $\delta^{13}C$ paired differences exhibited a polynomial relationship with shark size, suggesting differences in interpretation based on methodology. There was no relationship between plasma–muscle $\delta^{15}N$ residuals and shark total length, and therefore, differences in single-tissue and two-tissue data were not tested.

Blacktip reef sharks

Plasma and fin samples were collected from 158 juvenile and adult blacktip reef sharks in Moorea from May 2008 to May 2012, ranging from 55 to 150 cm TL (0–15 years old; males mature at 110-114 cm TL, females mature at ca. 120 cm TL; Mourier et al. 2013; Mourier *unpublished data*). Raw $\delta^{13}C$ and $\delta^{15}N$ values showed that blacktip reef sharks exhibited no significant change in ^{13}C with total length (Fig. 2c), but a significant enrichment in plasma ^{15}N with total length (Fig. 2f). Neither sex nor body condition affected ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ (Table 2).

Plasma–fin paired differences suggested that blacktip reef sharks exhibited minimal change in ^{13}C depletion through ontogeny (Fig. 3c), but a significant increase in

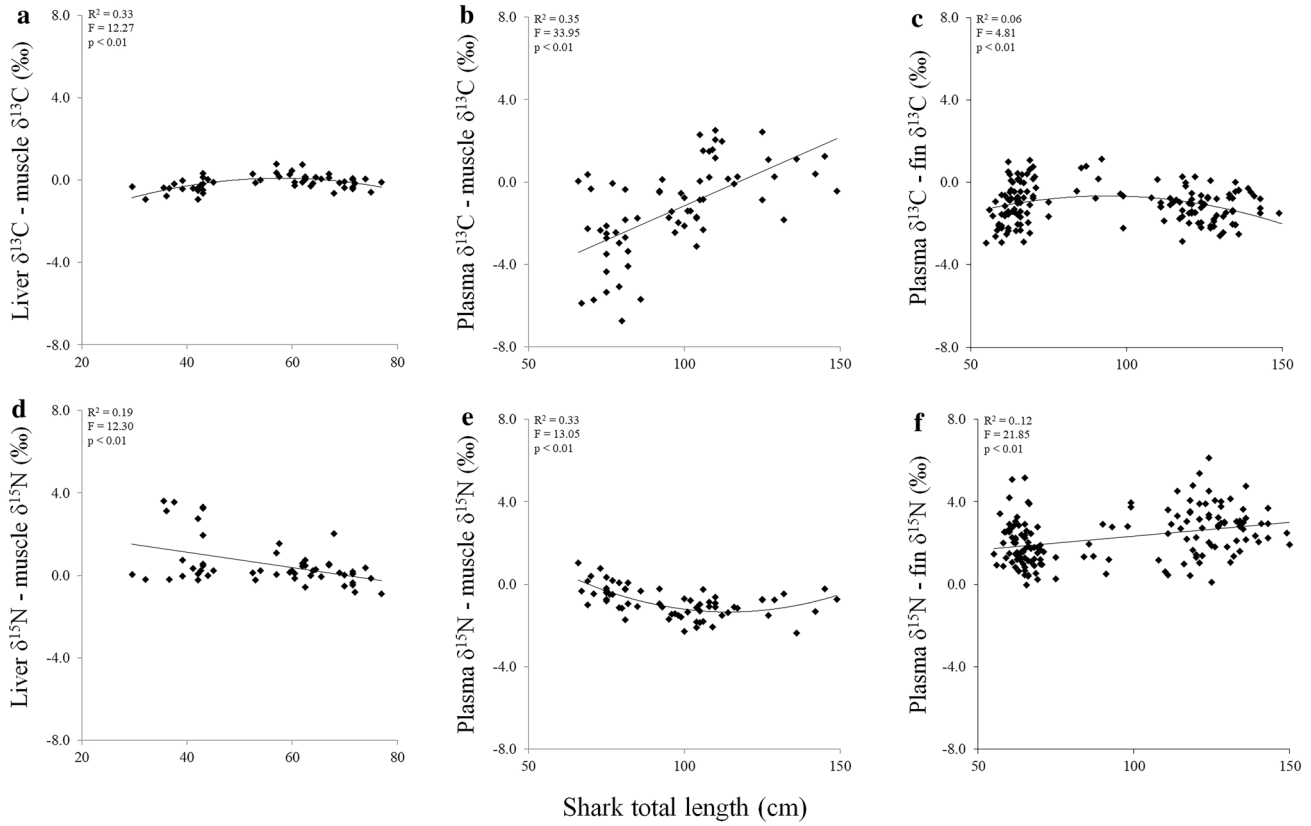

Fig. 3 Relationships between shark total length and $\delta^{13}C$ (**a**–**c**) and $\delta^{15}N$ (**d**–**f**) paired differences (value of long turnover tissue subtracted from discrimination-corrected value of short turnover tissue) among spurdogs (**a**, **d**; $N=53$), bull sharks (**b**, **e**; $N=66$), and blacktip reef sharks (**c**, **f**; $N=158$). Data points and sizes at which best-fit lines equal zero represent the size at which sharks reach isotopic equilibrium when short-term trophic interactions are isotopically equivalent to long-term trophic interactions

plasma ^{15}N enrichment with total length (Fig. 3f). Neither sex nor body condition affected ontogenetic shifts in $\delta^{13}C$ or $\delta^{15}N$ paired differences (Table 2).

Residuals of fin and plasma least squares regression exhibited no trends in $\delta^{13}C$ with blacktip reef shark size (Fig. 4c), with no effect of sex (Table 2). Residuals of both fin and plasma $\delta^{15}N$ residuals exhibited no trends with blacktip reef shark size (Fig. 4f), with no effect of sex (Table 2). The best-fit lines for muscle and plasma $\delta^{15}N$ residuals were not significantly different ($Z=0.28$, $p=0.78$). Too few individuals had body condition metrics to test the effects of body condition on $\delta^{13}C$ and $\delta^{15}N$ residuals.

Residuals of plasma–muscle paired differences showed a small and marginally significant decrease in intraspecific variability of $\delta^{13}C$ shifts with shark total length (− 0.002‰/cm TL; Fig. 5c), and a small, significant increase in intraspecific variability of $\delta^{15}N$ shifts with shark total length (0.004‰/cm TL; Fig. 5f). Neither sex nor body condition affected the residuals of $\delta^{13}C$ or $\delta^{15}N$ paired differences (Table 2). There was no relationship between fin and plasma $\delta^{13}C$ and $\delta^{15}N$ residuals and shark total length, and therefore, differences in single-tissue and two-tissue data were not tested.

Discussion

Ontogenetic niche shifts are common among vertebrates, leading to variability in resource use, interspecific interactions, and exposure to predation risk as animals grow (Wilbur 1980; Werner and Gilliam 1984; Snover 2008). However, individuals may not be uniform in their feeding behavior during such shifts and/or the speed and direction of shifts, which can affect fitness, life history, and ecological role (Gross and Charnov 1980; Post 2003; Newsome et al. 2009a). We found that spurdogs, bull sharks, and blacktip reef sharks each underwent ontogenetic shifts in trophic interactions based on stable isotope analysis, and exhibited intraspecific variation in $\delta^{13}C$ and $\delta^{15}N$ shifts. Across all three shark species, total length was, as expected, a significant predictor of $\delta^{15}N$ values, with enrichment in ^{15}N with shark size. As sharks and other aquatic predators grow, swimming speeds, gape widths, and energetic needs

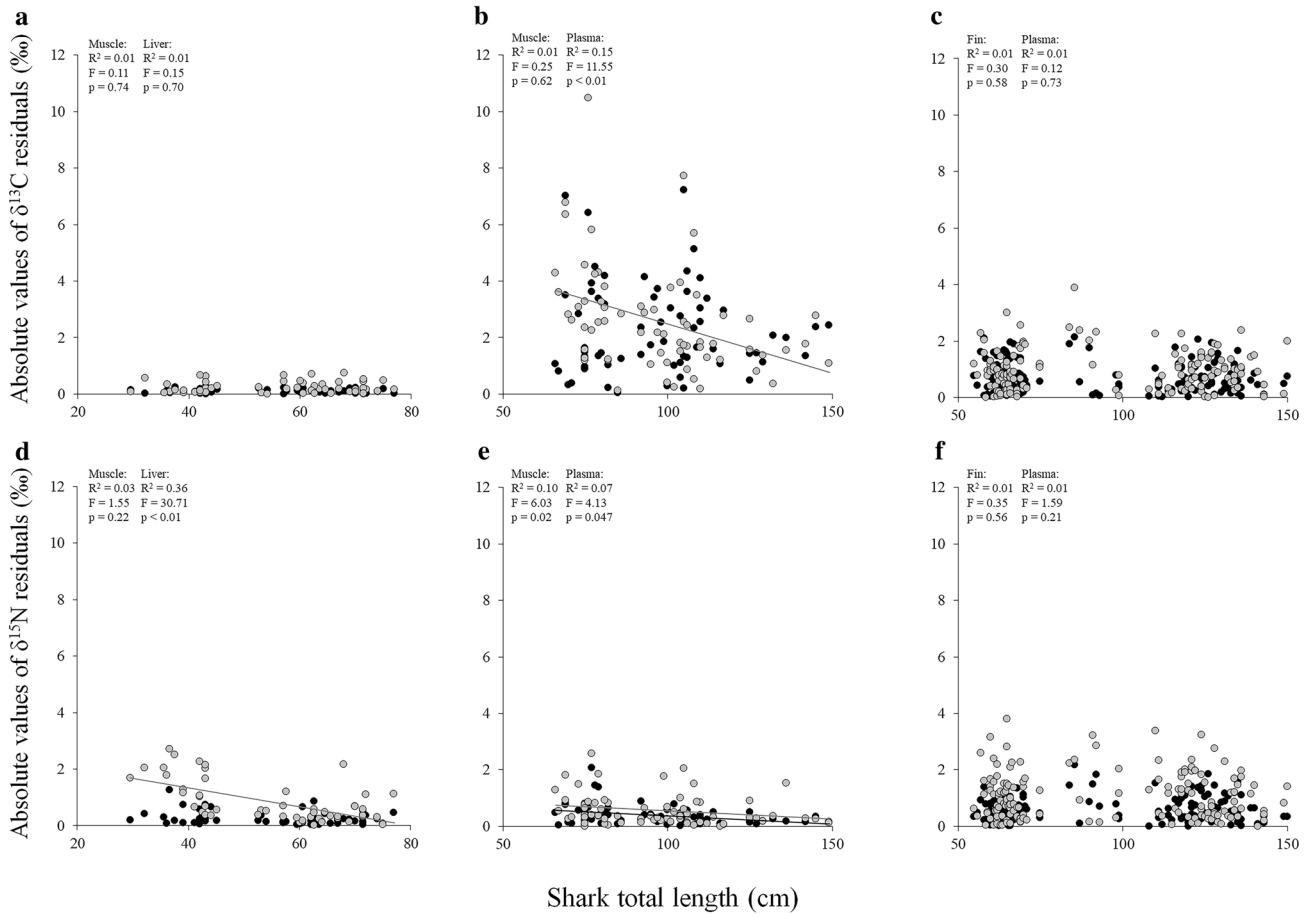

Fig. 4 Relationships between shark total length and the absolute value of $\delta^{13}C$ (**a**–**c**) and $\delta^{15}N$ (**d**–**f**) residuals indicative of intraspecific variation in trophic shifts among spurdogs (**a**, **d**; $N=53$), bull sharks (**b**, **e**; $N=66$), and blacktip reef sharks (**c**, **f**; $N=158$) using single tissues. Muscle and liver stable isotope values for spurdogs are black and gray data points, respectively (**a**, **d**). Muscle and plasma stable isotope values for bull sharks are black and gray data points, respectively (**b**, **e**). Fin and plasma stable isotope values for blacktip reef sharks are black and gray data points, respectively (**c**, **f**). The color of best-fit lines corresponds to the color of data points. Only significant relationships are displayed. Values are correlated with variability in the predicted speed of trophic shifts—greater values indicate faster trophic shifts than predicted. Negative slopes of best-fit lines indicate decreasing variability in predicted trophic shifts with shark size

typically increase, often leading to increases in trophic level within respective food webs (e.g., Estrada et al. 2006; Newsome et al. 2009a). Intraspecific variability in ontogenetic trophic shifts among spurdogs and bull sharks was also correlated with body size, with a decrease in variability as total length increased. Other shark species also exhibit greater variability among smaller individuals (e.g., *Hemigaleus australiensis*, Taylor and Bennett 2008; *Negaprion brevirostris*, Newman et al. 2010; *Sphyrna lewini*, Torres-Rojas et al. 2010), which may be attributed to intraspecific variability in size at birth, growth rates, learning and social behavior, and maternal provisioning.

Yet, sampled sharks exhibited differences in the direction and magnitude of ontogenetic shifts, and variability in these shifts, which were likely influenced by inherent species-specific differences in life histories, growth rates, and physiologies (Grubbs 2010; de Roos and Persson 2013), as well as other factors, including environmental conditions and food web structure (e.g., Zhao et al. 2014; Sánchez-Hernández et al. 2017). Spurdogs exhibited small, but clear enrichments of ^{13}C and ^{15}N with body length, and reached equilibrium at ca. 60–70 cm TL, within the range of maturity for other *Squalus* species (Ebert et al. 2013). Spurdogs exhibited minimal variability in trophic shifts, with increasing similarities among larger individuals. Bull sharks exhibited much greater changes in $\delta^{13}C$ and $\delta^{15}N$ compared to spurdogs, influenced partially by maternal meddling (McMeans et al. 2009; Olin et al. 2011; Belicka et al. 2012). Bull sharks reached equilibrium in $\delta^{13}C$ and $\delta^{15}N$ tissue values at body lengths ca. 125–160 cm TL, and exhibited considerable variability in $\delta^{13}C$ shifts. Yet, similar to spurdogs, bull sharks also exhibited greater similarities

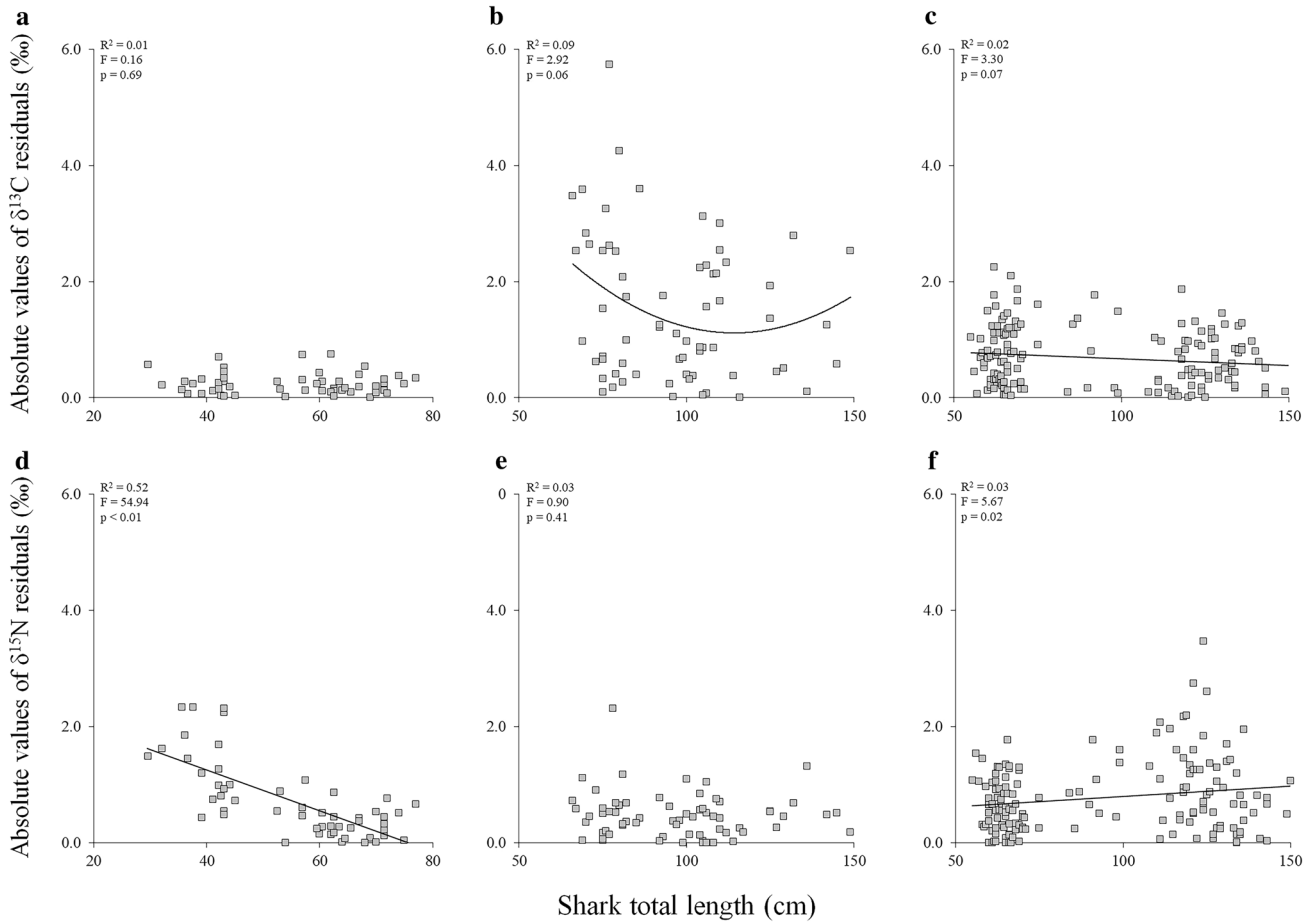

Fig. 5 Relationships between shark total length and the absolute value of $\delta^{13}C$ (**a**–**c**) and $\delta^{15}N$ (**d**–**f**) paired difference residuals indicative of intraspecific variation in trophic shifts among spurdogs (**a**, **d**; $N=53$), bull sharks (**b**, **e**; $N=66$), and blacktip reef sharks (**c**, **f**; $N=158$) using paired tissues. Values are correlated with variability in the predicted speed of trophic shifts—greater values indicate faster trophic shifts than predicted. Positive slopes of best-fit lines indicate increasing variability in predicted trophic shifts with shark size, and negative slopes of best-fit lines indicate decreasing variability in predicted trophic shifts with shark size

in trophic shifts among larger individuals. Comparatively, blacktip reef sharks exhibited considerable variability in trophic interactions among individuals, and did not reach a state of isotopic equilibrium among the tissues sampled. Blacktip reef sharks showed limited patterns in trophic interactions through ontogeny, although intraspecific variation in $\delta^{15}N$ shifts exhibited a small, but significant increase with body length.

Differences in environmental conditions could play an important role in shaping variability in trophic shifts among the studied species (Moran 1992; Donohue et al. 2000; Niemelä et al. 2013). In many deep-sea habitats (e.g., off La Réunion), conditions are typically stable over daily, monthly, and annual time-scales (e.g., Tyler 2003), limiting changes in energetic requirements or microhabitat preferences that would cause populations to exhibit variation in trophic interactions (reviewed by Rigby and Simpfendorfer 2015). Within subtropical estuaries, such as the Florida Coastal Everglades, tidal cycles and diurnal variability in available light lead to daily shifts in salinity, water temperature, and dissolved oxygen, with seasonal fluctuations and inter-annual variation in temperature, precipitation, freshwater flow, and salinity (e.g., Davis and Ogden 1994), which lead to temporal variability in species assemblages and foraging behaviors (e.g., Rosenblatt et al. 2013; Matich et al. 2017). As such, greater environmental stability should lead to increasing similarities in trophic shifts among individuals, as exhibited by the lower intraspecific variability exhibited by spurdogs compared to bull sharks. Contrary to this hypothesis, blacktip reef sharks in Moorea exhibited considerable variability in trophic interactions despite relatively stable environmental conditions within this tropical ecosystem (e.g., Edmunds et al. 2010; Leichter et al. 2012; Rivest and Gouhier 2015). However, spatial variability in resources attributed to habitat complexity (e.g., fringing reefs, lagoons, and outer reef slopes differ in potential prey biomass and distribution), and human

impacts may play an important role in shaping variability in trophic interactions among blacktip reef sharks (reviewed by Leu et al. 2008; Saporiti et al. 2014; Brena et al. 2015; Wright and Kyhn 2015).

Food web structure also likely played a key role in shaping ontogenetic niche shifts and variability in these shifts. Deep-sea habitats (e.g., off La Réunion) support relatively simple food webs, whereas estuaries (e.g., Florida Coastal Everglades) and coral reefs (e.g., Moorea) support diverse species assemblages across numerous microhabitats, and spatially discrete food webs available to highly mobile species like sharks (reviewed by Sala and Knowlton 2006). The diverse food webs of the Everglades and Moorea are also temporally variable (e.g., Boucek and Rehage 2013; Lamy et al. 2015), which may lead to greater variability in the foraging behavior of predator species, like bull sharks and blacktip reef sharks. Less diverse prey assemblages can lead to fewer trophic connections and simplified food webs, with more predictable interspecific interactions and ontogenetic niche shifts among predators (Chapin et al. 1997; Riede et al. 2010). In contrast, generalist predators that feed on a wide array of species in complex food webs are more likely to exhibit individual differences in foraging behaviors, and potentially ontogenetic trophic shifts (e.g. Bolnick et al. 2002, 2003). Similarly, food webs with narrower $\delta^{13}C$ and $\delta^{15}N$ ranges (e.g., deep-sea habitats off La Réunion) also likely lead to less variability in trophic interactions among individuals compared to those feeding in more isotopically diverse food webs (e.g., Moorea and the Shark River Estuary; e.g., Arias-Gonzalez et al. 1997; Matich et al. 2017). Thus, while bull sharks and spurdogs both exhibited decreased intraspecific variability through ontogeny, environmental conditions, food web structure, and other intrinsic factors likely led to greater variability among bull sharks than spurdogs.

Inherent variation within populations is also an important consideration. Spurdogs off La Réunion likely use similar habitats throughout their life history, with limited diversity in available resources, leading to predictable trophic shifts (Kyne and Simpfendorfer 2010). Comparatively, bull sharks in the Everglades use a variety of microhabitats within estuaries and coastal waters (Wiley and Simpfendorfer 2007; Matich and Heithaus 2015), and exhibit a diversity of behavioral patterns during ontogenetic niche shifts, including specialized and plastic foraging tactics, condition-dependent movement patterns, and risk-averse habitat use (Matich et al. 2011; Matich and Heithaus 2015). Indeed, bull sharks in poor body condition exhibited a slower depletion and a faster enrichment in plasma ^{13}C than muscle ^{13}C compared to sharks with positive body conditions, suggesting body condition affects behavior and the speed of ontogenetic trophic shifts of bull sharks (Matich and Heithaus 2015). Blacktip reef sharks also undergo ontogenetic shifts in habitat use, transitioning from exclusive use of nearshore lagoons by juveniles, to more reef-associated habitat use patterns, although these habitats are relatively proximate and characterized by high connectivity (Mourier et al. 2013). However, sexual dimorphism in adult habitat use, with females predominantly remaining in lagoons and males using forereefs (Mourier et al. 2012, 2013), likely leads to increased intraspecific variability in $\delta^{15}N$ shifts with blacktip reef shark size. Provisioning among some adult blacktip reef sharks may also lead to greater divergence in trophic interactions (Brena et al. 2015). Although sex was not a significant factor in analyses, similarities among male and female juvenile blacktip reef sharks may have been responsible for insignificant results when all individuals were pooled for analyses. Important for consideration, the full size range of bull sharks was not sampled (only juveniles and subadults; Branstetter and Stiles 1987; Natanson et al. 2014); thus, patterns of variation in trophic interactions and ontogenetic shifts may differ if adults from southwest Florida were considered.

Consequently, consideration of inter- and intraspecific variation in ontogenetic niche shifts is important as we move forward for research, management, and conservation across an array of taxonomic groups. Our results show that the speed of ontogenetic trophic shifts and variability in such shifts is significantly different among spurdogs, bull sharks, and blacktip reef sharks. Such dissimilarities are likely driven by inherent differences in species life histories and trophic ecologies, as well as their ecological contexts. As food webs become more complex, and discrete food webs spatially overlap and/or are proximately located, intraspecific variability in ontogenetic niche shifts is likely to be greater (e.g., bull sharks in the Everglades and blacktip reef sharks in Moorea) compared to species in more homogeneous habitats with less complex food webs (e.g., spurdogs in deep-sea), bolstering food web complexity or simplicity (Beckerman et al. 2006; Riede et al. 2010; Moya-Laraño 2011). Intraspecific variation is also likely to be greater among species that reside in more spatially and temporally dynamic environments, like estuaries that afford individuals the opportunity to access and use resources differently from conspecifics (Moran 1992; Elliot and Quintino 2007; Niemelä et al. 2013). Testing these hypotheses among populations of the same species in different context will be valuable for behavioral and trophic ecology as these fields aim to predict the consequences of environmental change in the future. Identifying suitable populations and ecosystems to test these hypotheses is of importance moving forward.

Single-tissue and two-tissue approaches

Our results suggest that intraspecific variation in ontogenetic niche shifts is likely context- and species specific, and adds to the applications of stable isotopes to understand not

only how megafauna populations and individuals vary in trophic interactions across space (e.g., Bird et al. 2018), but also in time. Our interpretations did, however, differ among single-tissue analysis and two-tissue analysis for some species, suggesting the utility of these methods may vary based on species, context(s), and question(s) of interest. Among spurdogs, results were very similar using stable isotope data from one tissue and two tissues. Increased spurdog size led to an enrichment in ^{13}C and ^{15}N, with limited variability in $\delta^{13}C$ shifts, and decreasing variability in $\delta^{15}N$ shifts. In deep-sea habitats, slow, predictable growth rates and isotopic incorporation rates (Kyne and Simpfendorfer 2010; Rigby and Simpfendorfer 2015; Shipley et al. 2017a), coupled with stable environments (Tyler 2003) likely promote a high degree of similarity between single-tissue analysis that quantifies trophic averaging, and two-tissue analysis that quantifies trophic shifts due to slow, predictable changes in spurdog $\delta^{13}C$ and $\delta^{15}N$ values.

Bull sharks and blacktip reef sharks also exhibited similar patterns in ontogenetic trophic shifts when interpreted with one tissue or two tissues; however, apparent differences in model output suggest that the two approaches can provide different, but complementary information. As placentatrophic sharks, bull shark embryos can exhibit diversity in maternal enrichment, leading to variability in size at birth and neonatal $\delta^{15}N$ values within a litter (McMeans et al. 2009; Olin et al. 2011). After birth, maternal investments and catabolic processes lead to elevated $\delta^{15}N$ values among neonates and young-of-the-year, which persist until anabolic metabolic processes balance catabolism, and stable isotopes from diets are incorporated into tissue(s) of interest (McMeans et al. 2009; Olin et al. 2011; Belicka et al. 2012). Because paired differences quantify variability/shifts in trophic interactions rather than absolute trophic values, inherent variability in ^{15}N among individuals attributed to maternal investment may be masked in paired differences, leading to greater intraspecific variation in bull shark $\delta^{15}N$ shifts when interpreting results from a single tissue, as observed in our study. In contrast, intraspecific variation in bull shark $\delta^{13}C$ shifts was greater when interpreting results from two tissues, which could result from temporally dynamic prey sources. Local prey pulses lead to seasonal trophic shifts among juvenile bull sharks in the Florida Everglades (Boucek and Rehage 2013; Matich and Heithaus 2014), and deviations in long-term dietary patterns may be more apparent when using paired differences that quantify shifts in trophic interactions compared to trophic averaging presented by one tissue.

Among blacktip reef sharks, single-tissue analysis indicated no trends in intraspecific variability with shark size, whereas two-tissue analysis indicated a decrease in intraspecific variability of $\delta^{13}C$ shifts with shark total length, and an increase in intraspecific variability of $\delta^{15}N$ shifts with shark total length. Differences in model output may be a product of limited ($\delta^{15}N$) or a lack of ($\delta^{13}C$) ontogenetic trophic shifts in blacktip reef sharks based on single-tissue analysis, limited sample size of intermediate-length individuals, the inherent complexity of coral reef food webs (Arias-Gonzalez et al. 1997; Bascompte et al. 2005), and/or sexually dimorphic habitat use patterns of adults that were not accounted for in analyses (Mourier et al. 2012, 2013). As such, the utility of evaluating paired differences in stable isotope values that provide insight into individual trophic shifts, compared to trophic averaging presented by a single tissue, should be further investigated to identify the contexts in which one approach may be more suitable than the other.

Several considerations should be made concerning the use of a single tissue or multiple tissues for stable isotope analysis. The ability to detect variability in trophic shifts using stable isotopes will vary among species and tissue type(s) collected. Our study compared three species using different tissue types with different turnover rates and discrimination factors (Table 1). For any study employing stable isotope analysis, species- and tissue-specific differences in stable isotope turnover rates and discrimination factors should be considered when comparing the values of multiple tissues, and transforming isotopic data (e.g., Newsome et al. 2007) may improve the quality of inferences. While confounded by tissue-specific differences, corrections were made based on discrimination factors, and slow turnover tissues (muscle and fin) and fast turnover tissues (liver and plasma) were selected based on similarities in turnover rates across species, with comparable turnover rate differences for each set of paired differences (ca. 131 days for spurdogs, ca. 118 days for bull sharks, ca. 95 days for blacktip reef sharks; Table 1; Saporiti et al. 2016; Matich et al. 2017; Valls et al. 2017; Yurkowski et al. 2017).

If employing two tissues, species with very slow and very fast metabolisms may be problematic if isotopic turnover rates for each tissue provide information on trophic interactions over the same time frame, potentially hindering the ability to draw conclusions about dietary shifts. Based on Vander Zanden et al.'s (2015) review of published stable isotope turnover rates, blood plasma and liver are likely to be the best fast turnover tissues [mean half-life = 17.2 days ± 18.5 SD (plasma) and 18.6 days ± 26.3 (liver) of birds, elasmobranchs, mammals, reptiles, and teleosts] to use in studies of dietary shifts. Red blood cells and muscle tissue are appropriate slower turnover tissues [mean half-life = 51.7 days ± 54.3 SD (red blood cells) and 57.8 days ± 55.7 (muscle) of birds, elasmobranchs, mammals, reptiles, and teleosts]. However, there remain challenges in selecting appropriate tissues for studies. For example, in birds, all tissues have relatively fast isotopic turnover rates (half-life range = 0.5–29.8 days). In amphibians and invertebrates, more studies are needed to identify suitable

tissues, and liver and muscle tissues likely have lipids that may affect stable isotope values in many species (Post et al. 2007; Vander Zanden et al. 2015).

Minimizing the effects of materials stored within tissues (e.g., lipids, urea) is also an important consideration (e.g., Hussey et al. 2012). Lipids were removed from tissues known to have high lipid content (liver and muscle of spurdogs), and C:N ratios suggested minimal effects of lipids on $\delta^{13}C$ values of treated and non-treated samples (Post et al. 2007). Urea can also affect $\delta^{13}C$ and $\delta^{15}N$ values, especially among elasmobranchs (e.g., Kim and Koch 2012; Churchill et al. 2015), and may lead to small depletions in ^{15}N within tissues (ca. 1‰), especially those with faster turnover rates (i.e., liver and plasma). Future studies are encouraged to evaluate appropriate removal methods for lipids, urea, and other compounds that may affect isotopic discrimination (Hussey et al. 2012; Shipley et al. 2017b).

Finally, gaining insight about trophic shifts from stable isotope values of different tissues may be limited when $\delta^{13}C$ pools are not distinct, primary producers vary widely in $\delta^{15}N$ values, and stable isotope values of food items vary seasonally in response to environmental change. As with all studies employing stable isotope analysis, an understanding of food web dynamics, or at least basal carbon sources and baseline $\delta^{15}N$ levels is necessary for interpretation beyond species-level inferences, including elucidating factors that may lead to temporal shifts in intraspecific variation.

Conclusion

Food web structure, environmental conditions, and other behavioral patterns likely contribute to the magnitude of intraspecific variation in ontogenetic niche shifts within predator populations (Moran 1992; Bolnick et al. 2003; Niemelä et al. 2013). Of particular interest are the consequences of changing environments and community compositions attributed to climate change, human disturbance, and associated food web perturbations (Leu et al. 2008; Snover 2008; Yang and Rudolf 2010). Our results suggest that in more stable ecosystems with limited variability in food availability and lower potential prey diversity, individuals may exhibit greater similarities in ontogenetic niche shifts than in populations found in more complex ecosystems. The latter systems may feature temporally dynamic prey pulses and/or micro-habitat variation in prey abundance and diversity, which can lead to phenotypic divergence and potentially greater resilience (Duffy 2009; Naeem et al. 2009; Hooper et al. 2012).

However, it is still uncertain if greater variability within populations promotes resilience, or increases vulnerability in particular contexts (reviewed in Nilsson et al. 2018). In situ sampling provides an important mean by which to test hypotheses concerning species resilience, relying on both long-term and opportunistic sampling (Hooper et al. 2005; Lindenmayer et al. 2012; Rosenblatt et al. 2013). Pairing data collection with expanding modeling methods will broaden our understanding of the contexts under which intraspecific variation through ontogeny is more prevalent, as well as its consequences for individuals and populations responding to disturbance (de Roos and Persson 2013; Persson and de Roos 2013). Our results suggest that more dynamic ecosystems promote more widespread variability among individuals compared to stable environments, supporting the hypothesis that phenotypic diversity may improve resilience (Tilman et al. 2006; Duffy 2009; Hooper et al. 2012; Baskett et al. 2014; Oliver et al. 2015). Yet, it is apparent that intraspecific variability increases through ontogeny in some species and/or some contexts (i.e., blacktip reef sharks in Moorea coral reefs), and decreases in others (i.e. bull sharks in the Everglades). As such, our study illustrates the need to further investigate intraspecific variability through ontogeny and how to categorize individuals in predictive models (Fig. 1), in order to identify species and/or phenotypes that may be at elevated risk in the face of disturbance (Barton 2010; Yang and Rudolf 2010; Bolnick et al. 2011).

Acknowledgements Data collection and sample analysis were funded by the National Science Foundation through the Florida Coastal Everglades LTER Program (DEB1237517, DBI0620409, DEB9910514), Florida International University's Marine Sciences Program, the PADI Foundation, and National Geographic. J Mourier was supported by funding from the Ministère de l'Environnement, du Développement Durable et de l'Energie (MEDDE) together with Agence Nationale des Aires Marines Protégées (AAMP). We thank the many volunteers who assisted with shark fishing in both Moorea and the Florida Everglades, and sample preparation and processing. We thank IFREMER La Réunion and ARVAM (especially JP Quod) for providing sharks in La Réunion. We thank the Texas Research Institute for Environmental Studies for providing logistical support in preparation for the manuscript. Research was approved by and conducted under the protocols of Florida International University's Institutional Animal Care and Use Committee, and in accordance to sampling permits EVER-2013-SCI-0020, EVER-2011-SCI-0031, EVER-2009-SCI-0024, and EVER-2007-SCI-0025 granted by Everglades National Park. This is the sixth publication for the Coastal Marine Ecology Program, and contribution #121 from the Center for Coastal Oceans Research within the Institute of Water and Environment at Florida International University. Data are made available through the Florida Coastal Everglades LTER program (http://fce.lternet.edu).

Author contribution statement PM, JJK, MRH, BLB, and JM conceived and designed the sampling protocols. PM, JJK, and JM conducted the fieldwork and collected the data. PM analyzed the data. PM, JJK, MRH, BLB, and JM developed the questions investigated within the manuscript. PM, JJK, MRH, BLB, and JM wrote the manuscript.

References

Arias-Gonzalez JE, Delesalle B, Salvat B et al (1997) Trophic functioning of the Tiahura reef sector, Moorea Island, French Polynesia. Coral Reefs 16:231–246

Barton BT (2010) Climate warming and predation risk during herbivore ontogeny. Ecology 91:2811–2818

Bascompte J, Melian C, Sala E (2005) Interaction strength combinations and the voerfishing of a marine food web. PNAS 102:5443–5447

Baskett ML, Fabina NS, Gross K (2014) Response diversity can increase ecological resilience to disturbance in coral reefs. Am Nat 184:E16–E31

Bearhop S, Adams CE, Waldron S et al (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J An Ecol 73:1007–1012

Beckerman AP, Petchey OL, Warren PH (2006) Foraging biology predicts food web complexity. PNAS 103:13745–13749

Belicka LL, Matich P, Jaffé R et al (2012) Fatty acids and stable isotopes as indicators of early-life feeding and potential maternal resource dependency in the bull shark *Carcharhinus leucas*. Mar Ecol Prog Ser 455:245–256

Bird CS, Veríssimo A, Magozzi S et al (2018) A global perspective on the trophic geography of sharks. Nature Ecol Evol 2:305–399

Bolnick DI, Yang LH, Fordyce JA et al (2002) Measuring individual-level resource specialization. Ecology 83:2936–2941

Bolnick DI, Svanbäck R, Fordyce JA et al (2003) The ecology of individuals: incidence and implications of individual specialization. Am Nat 161:1–28

Bolnick DI, Amarasekare P, Araújo MS et al (2011) Why intraspecific trait variation matters in community ecology. Trend Ecol Evol 26:183–192

Boucek RE, Rehage JS (2013) No free lunch: displaced marsh consumers regulate a prey subsidy to an estuarine consumer. Oikos 122:1453–1464

Branstetter S, Stiles R (1987) Age and growth estimates of the bull shark, *Carcharhinus leucas*, from the northern Gulf of Mexico. Environ Biol Fish 20:169–181

Brena PF, Mourier J, Planes S et al (2015) Shark and ray provisioning: functional insights into behavioral, ecological and physiological responses across multiple scales. Mar Ecol Prog Ser 538:273–283

Caut S, Jowers MJ, Michel L et al (2013) Diet- and tissue-specific incorporation of isotopes in the shark *Scyliorhinus stellaris*, a North Sea mesopredator. Mar Ecol Prog Ser 492:185–198

Chapin FS III, Walker BH, Hobbs RJ et al (1997) Biotic control over the functioning of ecosystems. Science 277:500–504

Chouvelon T, Spitz J, Cherel Y et al (2011) Inter-specific and ontogenic differences in $\delta^{13}C$ and $\delta^{15}N$ values and Hg and Cd concentrations of cephalopods. Mar Ecol Prog Ser 433:107–120

Churchill DA, Heithaus MR, Grubbs RD (2015) Effects of lipid and urea extraction on $\delta^{15}N$ values of deep-sea sharks and hagfish: Can mathematical correction factors be generated? Deep Sea Res Part II 115:103–108

Clutton-Brock T, Sheldon BC (2010) Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. Trend Ecol Evol 25:562–573

Colles A, Liow LH, Prinzing A (2009) Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. Ecol Lett 12:849–863

Davis S, Ogden JC (1994) Everglades: the ecosystem and its restoration. CRC Press, London

de Roos AM, Persson (2013) Population and community ecology of ontogentic development. Princeton Univ Press, Princeton

Donohue K, Messiqua D, Pyle EH et al (2000) Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade-avoidance responses in *Impatiens capensis*. Evolution 54:1956–1968

Drago M, Cardona L, Aguilar A et al (2010) Diet of lactating South American sea lions, as inferred from stable isotopes, influences pup growth. Mar Mamm Sci 26:309–323

Duffy JE (2009) Why biodiversity is important to the functioning of real-world ecosystems. Front Ecol Environ 7:437–444

Ebert DA, Fowler S, Compagno L (2013) Sharks of the world. Wild Nature Press, Plymouth

Edmunds PJ, Leichter JJ, Adjeroud M (2010) Landscape-scale variation in coral recruitment in Moorea, French Polynesia. Mar Ecol Prog Ser 414:75–89

Elliot M, Quintino V (2007) The Estuarine Quality Paradox, Environmental Homeostastis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Mar Pollut Bull 54:640–645

Estrada JA, Rice AN, Natanson LJ et al (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology 87:829–834

Field IC, Bradshaw CJA, van der Hoff J et al (2007) Age-related shifts in the diet composition of southern elephant seals expand overall foraging niche. Mar Biol 150:1441–1452

Gross MR, Charnov EL (1980) Alternative male life histories in bluegill sunfish. Proc Nat Acad Sci 77:6937–6940

Grubbs RD (2010) Ontogenetic shifts in movement and habitat use. In: Carrier JC, Musick JA, Heithaus MR (eds) Sharks and their relatives II. Biodiversity, adaptive physiology, and conservation. CRC Press, London, pp 319–350

Heithaus MR, Delius BK, Wirsing AJ et al (2009) Physical factors influencing the distribution of a top predator in a subtropical oligotrophic estuary. Limnol Oceanogr 54:472–482

Hooper DU, Chapin FS, Ewel JJ et al (2005) Effects of biodiversity on ecosystems functioning: a consensus of current knowledge. Ecol Mono 75:3–35

Hooper DU, Adair EC, Cardinale BJ et al (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105–108

Hückstadt LA, Koch PL, McDonald BI et al (2012) Stable isotope analyses reveal individual variability in the trophic ecology of a top predator, the southern elephant seal. Oecologia 169:395–406

Hussey NE, MacNeil MA, Olin JA et al (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol 80:1449–1484

Hyndes GA, Nagelkerken I, McLeod RJ et al (2014) Mechanisms and ecological roles of carbon transfer within coastal seascapes. Biol Rev 89:232–254

Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fish 95:53–63

Kim SL, del Rio CM, Casper D et al (2012) Isotopic incorporation rates for shark tissues from a long-term captive feeding study. J Exper Biol 215:2495–2500

Knoff A, Hohn A, Macko S (2008) Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. Mar Mamm Sci 24:128–137

Kyne PM, Simpfendorfer CA (2010) Deepwater chondrichthyans. In: Carrier JC et al (eds) Sharks and their relatives II: biodiversity, adaptive physiology, and conservation. CRC Press, London, pp 37–113

Lamy T, Legendre P, Chancerelle Y et al (2015) Understanding the spatio-temporal response of coral reef fish communities to natural disturbances: insights from beta-diversity decomposition. PLoS One 10:e0138696

Layman CA, Araujo MS, Boucek R et al (2012) Applying stable isotopes to examine food web structure: an overview of analytical tools. Biol Rev 87:542–562
Leichter JJ, Stokes MD, Hench JL et al (2012) The island-scale internal wave climate of Moorea, French Polynesia. J Geophys Res Oceans 117:C06008
Leu M, Hanser SE, Knick ST (2008) The human footprint in the west: a large-scale analysis of anthropogenic impacts. Ecol App 18:1119–1139
Lindenmayer DB, Likens GE, Andersen A et al (2012) Value of long-term ecological studies. Austral Ecol 37:745–757
MacNeil MA, Drouillard KG, Fisk AT (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can J Fish Aquat Sci 63:345–353
Martinez del Rio C, Wolf N, Carelton SA et al (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84:91–111
Matich P, Heithaus MR (2014) Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. J An Ecol 83:199–213
Matich P, Heithaus MR (2015) Individual variation in ontogenetic niche shifts in habitat use and movement patterns of a large estuarine predator (*Carcharhinus leucas*). Oecologia 178:347–359
Matich P, Heithaus MR, Layman CA (2010) Size-based variation in inter-tissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). Can J Fish Aquat Sci 67:877–885
Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J An Ecol 80:295–304
Matich P, Kiszka JJ, Heithaus MR et al (2015) Short-term shifts of stable isotope ($\delta^{13}C$, $\delta^{15}N$) values in juvenile sharks within nursery areas suggest rapid shifts in trophic interactions. J Exper Mar Biol Ecol 465:83–91
Matich P, Ault JS, Boucek RE et al (2017) Ecological niche partitioning within a large predator guild in a nutrient-limited estuary. Limnol Oceanog 62:934–953
McCauley DJ, Young HS, Dunbar RB et al (2012) Assessing the effects of large mobile predators on ecosystem connectivity. Ecol App 22:1711–1717
McClellan CM, Read AJ (2007) Complexity and variation in loggerhead sea turtle life history. Biol Lett 3:592–594
McMeans BC, Olin JA, Benz GW (2009) Stable-isotope comparisons between embryos and mothers of a placentatrophic shark species. J Fish Biol 75:2464–2474
Mittelbach GG, Ballew NG, Kjevik MK (2014) Fish behavioral types and their ecological consequences. Can J Fish Aquat Sci 71:1–18
Moran NA (1992) The evolutionary maintenance of alternative phenotypes. Am Nat 139:971–989
Mourier J, Vercelloni J, Planes S (2012) Evidence of social communities in a spatially structured network of a free-ranging shark species. Annu Behav 83:389–401
Mourier J, Mills SC, Planes S (2013) Population structure, spatial distribution and life-history traits of blacktip reef sharks Carcharhinus melanopterus. J Fish Biol 82:979–993
Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure. Philos Trans R Soc Lond B 366:1425–1437
Naeem S, Bunker DE, Hector A et al (2009) Biodiversity, ecosystem-functioning, and human wellbeing. Oxford University Press, New York
Natanson LJ, Adams DH, Winton MV et al (2014) Age and growth of the bull shark in the western North Atlantic Ocean. Trans Am Fish Soc 143:732–743
Newman SP, Handy RD, Gruber SH (2010) Diet and prey preference of juvenile lemon sharks *Negaprion brevirostris*. Mar Ecol Prog Ser 398:221–234
Newsome SD, Martinze del Rio C, Bearhop S et al (2007) A niche for isotopic ecology. Front Ecol Environ 5:429–436
Newsome SD, Etnier MA, Monson DH et al (2009a) Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Mar Ecol Prog Ser 374:229–242
Newsome SD, Tinker MT, Monson DH et al (2009b) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90:961–974
Niemelä PT, Vainikka A, Forsman JT et al (2013) How does variation in the environment and individual cognition explain the existence of consistent behavioral differences? Ecol Evol 3:457–464
Nilsson KA, McCann KS, Caskenette AL (2018) Interaction strength and stability in stage-structured food web modules. Oikos 5:5. https://doi.org/10.1111/oik.05029
Olin JA, Hussey NE, Fritts M et al (2011) Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. Rapid Comm Mass Spect 25:1008–1016
Oliver TH, Heard MS, Isaac NJB et al (2015) Biodiversity and resilience of ecosystem functions. Trends Ecolo Evol 30:673–684
Persson L, de Roos AM (2013) Symmetry breaking in ecological systems through different energy efficiencies of juveniles and adults. Ecology 94:1487–1498
Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718
Post DM (2003) Individual variation in the timing of ontogenetic niche shifts in largemouth bass. Ecology 84:1298–1310
Post DM, Layman CA, Arrington DA et al (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189
Pritchard DW (1967) What is an estuary: physical standpoint. In: Estuaries (ed) Lauf GH, pp 3–5. Washington, DC, USA: American Association for the Advancement of Science, Publication 83
Riede JO, Rall BC, Banasek-Richter C et al (2010) Scaling of food-web properties with diversity and complexity across ecosystems. Adv Ecol Res 42:139–170
Rigby C, Simpfendorfer CA (2015) Patterns in life history traits of deep-water chondrichthyans. Deep-Sea Res II 115:30–40
Rivest EB, Gouhier TC (2015) Complex environmental forcing across the biogeographical range of coral populations. PLoS One 10:e0121742
Rosenblatt AE, Heithaus MR, Mather ME et al (2013) The roles of large top predators in coastal ecosystems: new insights from Long-Term Ecological Research. Oceanography 26:156–167
Rosenblatt AE, Nifong JC, Heithaus MR et al (2015) Factors affecting individual foraging specialization and temporal diet stability across the range of a large "generalist" apex predator. Oecologia 178:5–16
Sala E, Knowlton N (2006) Global marine biodiversity trends. Annu Rev Environ Res 31:93–122
Salisbury CL, Seddon N, Cooney CR et al (2012) The latitudinal gradient in dispersal constraints: ecological specialisation drives diversification in tropical birds. Ecol Lett 15:847–855
Sánchez-Hernández J, Eloranta AP, Finstad AG et al (2017) Community structure affects trophic ontogeny in a predatory fish. Ecol Evol 7:358–367
Saporiti F, Bearhop S, Silva L et al (2014) Longer and less overlapping food webs in anthropogenically disturbed marine ecosystems: confirmations from the past. PLoS One 9:e103132
Saporiti F, Bearhop S, Vales DG et al (2016) Resource partitioning among air-breathing marine predators: are body size and mouth diameter the major determinants? Mar Ecol 37:957–969

Shipley ON, Brooks EJ, Madigan DJ et al (2017a) Stable isotope analysis in deep-sea chondrichthyans: recent challenges, ecological insights, and future directions. Rev Fish Biol Fish 27:481–497

Shipley ON, Murchie KJ, Frisk MG et al (2017b) Low lipid and urea effects and inter-tissue comparisons of stable isotope signatures in three nearshore elasmobranchs. Mar Ecol Prog Ser 579:233–238

Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. Trend Ecol Evol 19:372–378

Skulason S, Smith TB (1995) Resource polymorphisms in vertebrates. Trend Ecol Evol 10:366–370

Snover ML (2008) Ontogenetic habitat shifts in marine organisms: Influencing factors and the impact of climate variability. Bull Mar Sci 83:53–67

Subalusky AL, Fitzgerald LA, Smith LL (2009) Ontogenetic niche shifts in the American Alligator establish functional connectivity between aquatic systems. Biol Conserv 142:1507–1514

Taylor SM, Bennett MB (2008) Cephalopod dietary specialization and ontogenetic partitioning of the Australian weasel shark *Hemigaleus australiensis* White, Last & Compagno. J Fish Biol 72:917–936

Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629–632

Torres-Rojas YE, Hernandez-Herrera A, Galván-Magaña F et al (2010) Stomach content analysis of juvenile, scalloped hammerhead shark *Sphyrna lewini* captured off the coast of Mazatlan, Mexico. Aquat Ecol 44:301–308

Tyler PA (2003) Ecosystems of the deep oceans. Elsevier, USA

Valls M, Rueda L, Quetglas A (2017) Feeding strategies and resource partitioning among elasmobranchs and cephalopods in Mediterranean deep-sea ecosystems. Deep-Sea Res Part I: 28-41

Vander Zanden JM, Clayton MK, Moody EK et al (2015) Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLoS One 10:e0116182

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. Annu Rev Ecol System 15:393–425

Wilbur HM (1980) Complex life cycles. Annu Rev Ecol Syst 11:67–93

Wiley TR, Simpfendorfer CA (2007) The ecology of elasmobranches occurring in the Everglades National Park, Florida: implications for conservation and management. Bull Mar Sci 80:171–189

Wolf N, Carleton SA, Martínez del Rio C (2009) Ten years of experimental animal isotopic ecology. Func Ecol 23:17–26

Woodward G, Hildrew AG (2002) Body-size determinants of niche overlap and intraguild predation within a complex food web. J Annu Ecol 71:1063–1074

Wright AJ, Kyhn LA (2015) Practical management of cumulative anthropogenic impacts with working marine examples. Conserv Biol 29:333–340

Yang LH, Rudolf VHW (2010) Phenology, ontogeny and the effects of climate change on the timing of species interactions. Ecol Lett 13:1–10

Yurkowski DJ, Hussey NE, Fisk AT et al (2017) Temporal shifts in intraguild predation pressure between beluga whales and Greenland halibut in a changing Arctic. Biol Lett 13:20170433

Zhao T, Villéger S, Lek S et al (2014) High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. Ecol Evol 4:4649–4657