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Article *in* Proceedings of the Royal Society B: Biological Sciences · April 2017

DOI: 10.1098/rspb.2017.0166

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Cite this article: Hussey NE *et al*. 2017 Risky business for a juvenile marine predator? Testing the influence of foraging strategies on size and growth rate under natural conditions. *Proc. R. Soc. B* **284**: 20170166. http://dx.doi.org/10.1098/rspb.2017.0166

Received: 25 January 2017
Accepted: 9 March 2017

Subject Category:
Ecology

Subject Areas:
behaviour, ecology, evolution

Keywords:
food web, lemon shark, life history, natural selection, predation risk, stable isotopes

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3723955.

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Risky business for a juvenile marine predator? Testing the influence of foraging strategies on size and growth rate under natural conditions

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Mechanisms driving selection of body size and growth rate in wild marine vertebrates are poorly understood, thus limiting knowledge of their fitness costs at ecological, physiological and genetic scales. Here, we indirectly tested whether selection for size-related traits of juvenile sharks that inhabit a nursery hosting two dichotomous habitats, protected mangroves (low predation risk) and exposed seagrass beds (high predation risk), is influenced by their foraging behaviour. Juvenile sharks displayed a continuum of foraging strategies between mangrove and seagrass areas, with some individuals preferentially feeding in one habitat over another. Foraging habitat was correlated with growth rate, whereby slower growing, smaller individuals fed predominantly in sheltered mangroves, whereas larger, faster growing animals fed over exposed seagrass. Concomitantly, tracked juveniles undertook variable movement behaviours across both the low and high predation risk habitat. These data provide supporting evidence for the hypothesis that directional selection favouring smaller size and slower growth rate, both heritable traits in this shark population, may be driven by variability in foraging behaviour and predation risk. Such evolutionary pathways may be critical to adaptation within predator-driven marine ecosystems.

1. Background

Spatial plasticity in selection is known to occur as a consequence of the biotic and abiotic conditions that shape the sea or landscape [1]. This dynamicity naturally leads to population divergence through localized adaptation, whereby traits in one population are favoured based on local conditions, irrespective of the fitness consequences or adaptive value of those traits among other populations [2], or even at later life stages (e.g. [3]). Nonetheless, selection for large size and fast growth has long been considered a central force that dictates population fitness and evolutionary success [4,5]. Larger size allows organisms to mature earlier [6], consume a wider size spectrum of prey to maximize growth and fitness [7], improves manoeuvrability to locate prey and evade predators [8], increases survival potential during extreme conditions [9] and when exposed to disease [10], as well as improves reproductive output in terms of both the number and size of young [11]. Indeed, larger individuals within a cohort have reduced predation risk relative to conspecifics within that same age class (the 'bigger is better' hypothesis [12]). Faster growth is similarly thought to increase survival, because organisms require less time to transit through juvenile phases when they are most vulnerable to predators (the 'stage-duration' hypothesis [13]).

More recent data, however, are questioning the benefits of larger size and faster growth. Instead, negative fitness consequences have been shown as a direct result of increased risk behaviours to facilitate larger size and faster growth [14,15]. For example, heightened locomotory performance associated with larger size can drive foraging activity over larger spatial scales, which in turn requires a threshold of consumed prey to balance the energetic cost of those movements. Expansion of home ranges also increases encounter probabilities with predators, heightening the potential for reduced fitness through both unprofitable foraging excursions and stress related non-consumptive predator interactions [16]. This is further exacerbated at early life stages when individuals are still naive and developing foraging skills [17].

The direction of selection for body size and growth rate, i.e. larger versus smaller body size and faster versus slower growth rate, is likely more plastic than originally thought and an important component of adaptation within any predator-driven marine ecosystem. Yet our understanding of the mechanisms that drive selection for size remains limited. Typically, to examine the mechanisms of selection, one of four approaches is used: experimental studies (e.g. size-selective predation), selection or other genetic experiments under laboratory conditions, quantitative selection analysis and correlational approaches using field data [5]. Most adopt experimental and laboratory-based studies where parameters can easily be controlled and manipulated, but how these results transfer to natural conditions is unclear. For aquatic organisms, specifically large marine vertebrates, field studies provide the only viable option to determine the mechanisms of selection but these are typically hindered by logistical challenges.

An exhaustively sampled population of juvenile lemon sharks, *Negaprion brevirostris* (Poey, 1868) at Bimini, Bahamas presents a unique model to overcome these limitations and allow examination of the mechanisms underpinning selection and adaptation in a large bodied marine vertebrate. Lemon sharks are born and remain within the Bimini nursery for a minimum of 3 years [18], with at least some mature females known to be philopatric to natal sites for parturition [19]. The nursery habitat consists of seagrass beds fringed by dense mangroves along the shoreline that provide abundant prey resources for developing juveniles during their residency phase (figure 1). Using quantitative selection analysis, DiBattista *et al*. [3] examined approximately 700 individuals over a 4-year period, encompassing more than 99% of the total juveniles born in the Bimini nursery. The authors demonstrated that smaller, slower growing lemon sharks were selected for, facilitating an evolutionary trajectory that may be offset by selection for faster growth and larger size during later life stages or at other nursery sites [20,21]. A subsequent genetic analysis and pedigree reconstruction of this population confirmed heritability of these size-related traits [22]. The mechanism proposed for driving selection of slower growth and smaller size in these sharks was preferential foraging within protected fringing mangroves, where predator encounter rates are much lower compared with risky foraging behaviour over exposed seagrass beds [3]. Indeed, sub-adult and adult lemon sharks are the main predators of juveniles on the exposed seagrass beds in the nursery [23–25].

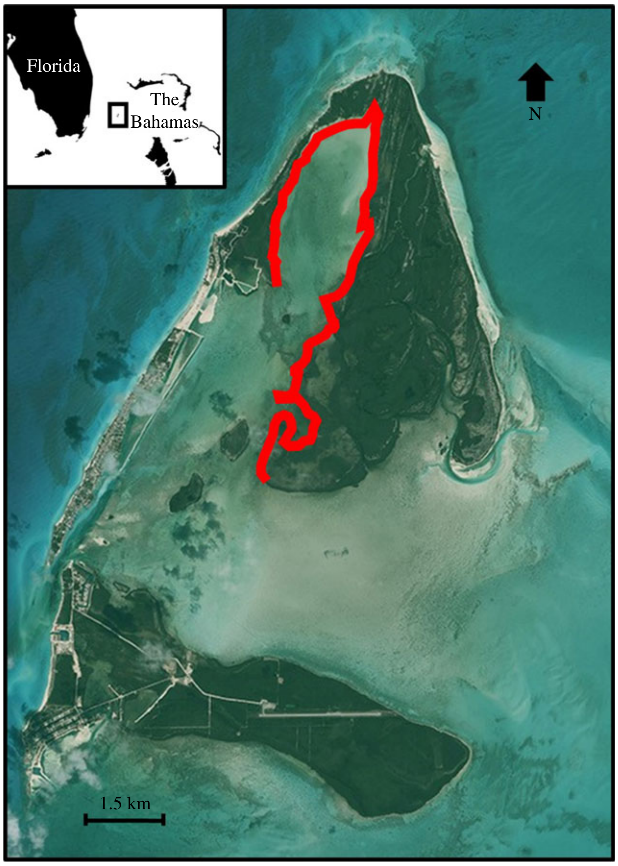

Figure 1. The interconnected nursery habitat at Bimini Islands in the Bahamas where juvenile lemon sharks (*Negaprion brevirostris*) remain resident for up to 3 years. The red line indicates the mangrove fringed shoreline of the nursery region. The aerial photograph depicts seagrass beds in the lagoon areas. (Online version in colour.)

Stable isotopes provide a method to systematically track energy flow within marine ecosystems, allowing differentiation of the proportional importance of distinct baseline producers (or habitats) to consumer diets [26]. For example, when an animal consumes prey, preferential loss of the lighter carbon isotope, ^{12}C, compared to the heavier isotope, ^{13}C occurs at each hierarchical level of consumption in a food web [27]. Consequently, if carbon isotope values ($\delta^{13}C$) of prey that reside in distinct habitats within an ecosystem can be readily distinguished, isotope values of predators that consume that prey can then be used to retrospectively track their foraging locations [28]. Serial sampling of individual predators, through capture and recapture, can then be used to examine inter-and intra-individual variation in foraging behaviours over time. An additional isotopic tracer, sulfur

(δ^{34}S), which exhibits minimal fractionation between consumer and prey, can also provide a novel proxy to identify individual variation in consumer foraging locations, thus complementing the more traditional δ^{13}C approach [29].

A combined stable isotope (δ^{13}C and δ^{34}S), field sampling and telemetry approach was adopted to test the proposed hypothesis of localized selection within the juvenile lemon shark population at Bimini. Carbon stable isotope values of both juvenile lemon sharks and their most common teleost prey group (family Gerreidae) were measured. This prey group was targeted in both mangrove and seagrass habitats given their dominance in the diet of juvenile lemon sharks [30], the known distinct δ^{13}C values of these two habitats [31] and previous work discriminating δ^{13}C values of Gerreidae fishes sampled in mangrove and seagrass [32]. Carbon isotope data for predator and prey were then incorporated into an individual-level hierarchical Bayesian mixing model to quantify inter-individual variation in foraging locations. A subset of juvenile sharks were; (i) analysed for δ^{34}S to provide a second (complementary) tracer and (ii) sampled at two time points 1 year apart to examine uniformity in δ^{13}C isotope values as a measure of consistent foraging behaviour. Growth rates derived from field morphometric data on recaptured individuals were used to test if slow and fast growth were correlated with foraging in mangrove and seagrass habitats, respectively. Finally, acoustic telemetry tracking of juvenile sharks was used to examine variation in habitat use relative to the predation risk associated with the occurrence of sub-adult sharks. Specifically, we tested whether juvenile lemon sharks show variable growth rates and foraging strategies along the mangrove–seagrass isotopic continuum, which would be consistent with the hypothesis that the trade-off between foraging behaviour and predation risk drives known selection for small juvenile size in this population.

2. Material and methods

(a) Study site

The subtropical Bimini Islands are located on the western edge of the Great Bahama Bank (25°44 N, 79°16 W; figure 1). The two main islands are separated by a shallow central lagoon and are predominantly fringed by red mangroves (*Rhizophora mangle*). This study focused on the north island's inner shoreline, the North Sound and Shark Land, both previously identified as an interconnected lemon shark nursery [19] (figure 1). This area is characterized by two distinct habitats, the mangrove fringed shoreline and shallow seagrass beds dominated by turtle grass (*Thalassia testudinum*) and to a lesser extent (*Halodule wrightii*), interspersed with areas of exposed sand substrate.

(b) Sample collection and preparation

Juvenile lemon sharks were captured using monofilament gillnets and biological data were recorded (electronic supplementary material, S1). During processing, a fin clip was taken from the trailing edge of the anal fin and stored in 20% DMSO. Sharks were then placed in a holding pen for recovery and released back into the nursery. This annual population census of juvenile lemon sharks has been ongoing for the past 21 years, but for the purposes of this study, capture and morphometric data (size, sex and umbilical scar) were used from individuals sampled between 2005 and 2010. For the stable isotope component of the analysis, we used fin clips taken from individuals captured in 2009 and 2010, including multiple animals that were recaptured following periods at liberty. The healing of the umbilical connection ranked from open wound (newborn–neonate) to healed (more than or equal to 1 year old juvenile) was used to assign age class.

To define the carbon/sulfur source endpoints, seagrass blades ($n = 3$) and mangrove leaves ($n = 3$) from exposed seagrass beds in the central area of the nursery and from fringing habitats, respectively, were sampled. To sample the principle prey species of juvenile lemon sharks, mojarra (*Eucinostomus* spp.) [30], a hand-pulled seine net (75 m length, 2 m depth and 1 cm stretched mesh size) was closed in a parallel direction to the shoreline and pursed. Captured fish were retained, tissue sampled and biological data recorded (electronic supplementary material, S2). All samples were processed and then analysed for carbon (δ^{13}C) and a subset of samples for sulfur (δ^{34}S) using an elemental analyser (Costech 4010) interfaced to a Thermo Finnigan Delta$^{\text{PLUS}}$ mass spectrometer (electronic supplementary material, S3). Nitrogen isotope data (δ^{15}N) were also measured for all samples to allow presentation of sharks and primary prey in isotopic space (figure 2).

(c) Telemetry tracking

To track the movements of newborn/juvenile (less than 100 cm precaudal length (PCL)) and sub-adult (more than 100 cm PCL) lemon sharks, individuals were captured using either monofilament gill nets (as described above) or rod and line. On capture, an acoustic transmitter (Sonotronics—individually coded continuous signal tag; 68–78 KHz) was surgically implanted into each shark or externally attached and standard morphometric measurements recorded. Following release, sharks were actively tracked using a hydrophone (Sonotronics, DH4) and receiver (Sonotronics, USR-96) mounted on a small flat-bottomed skiff for periods up to 48 h. Locations were recorded every 5–15 min with a hand-held GPS (Garmin 72H) along with a compass bearing and the distance to the shark estimated (to nearest 5 m based on audible signal intensity; see [25] for details).

(d) Analyses

Previous work has shown distinct δ^{13}C and δ^{34}S isotopic differences between mangrove versus seagrass food webs [31,33] and distinct δ^{13}C isotope profiles for individual *Eucinostomus* spp. sampled within those respective habitats [32]. We first examine if δ^{13}C and δ^{34}S values of mangrove and seagrass in Bimini are distinct and if juvenile sharks show a large range in δ^{13}C and δ^{34}S values that are highly correlated, as would be predicted. We then assume that the δ^{13}C values of *Eucinostomus* spp. sampled at Bimini are representative of the distinct carbon source habitats (mangrove versus seagrass) where they occur. ANCOVA was used to test for differences in δ^{13}C values of *Eucinostoimus* spp. between habitats while accounting for individual size using *lme* in R (R Development Core Team, 2014).

A hierarchical Bayesian mixing model was constructed to examine individual variation in the foraging locations of 1–3 year old sharks within the nursery based on consumption of *Eucinostomus* spp. Specifically, the model quantified the percentage contributions of mangrove- and seagrass-origin *Eucinostomus* spp. compared to the δ^{13}C values of individual sharks (i.e. sharks feeding exclusively on prey in seagrass, mangrove or some combination of the two habitats). Bayesian mixing models allow for propagation of uncertainty into estimates of posterior probability distributions of contributions to isotopic mixtures ([34]; for further model details, see the electronic supplementary material, S4). All priors were set to be flat or diffuse with source proportions assigned a Dirichlet prior ($\alpha = 1$), which is a multivariate extension of the beta distribution (uniform on the compositional simplex). Posterior probability distributions of parameters were quantified using Markov

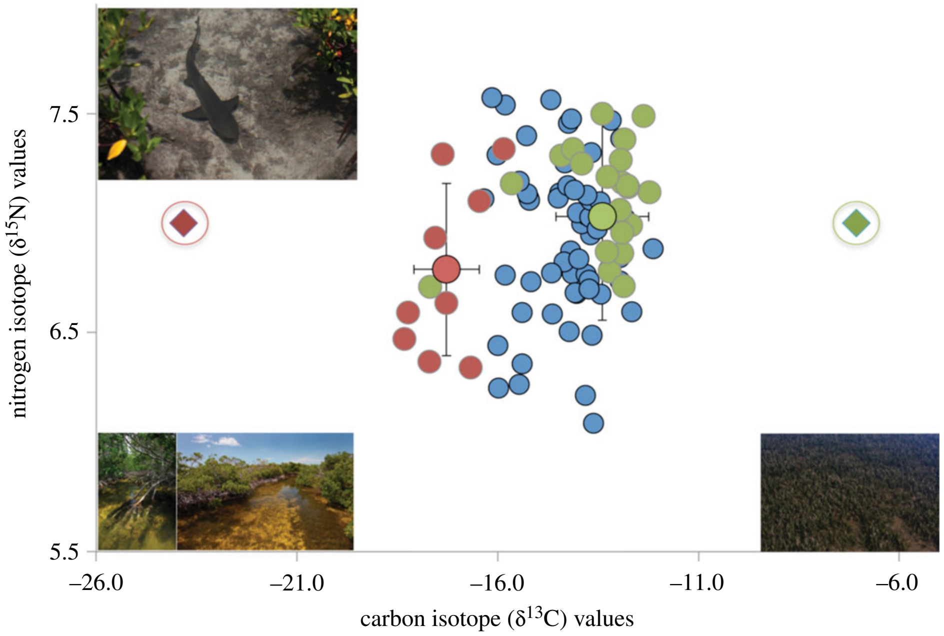

Figure 2. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic biplot of the principal ecosystem components in the nursery ground: (i) the average baseline carbon sources, mangrove leaves (red diamond in circle) versus seagrass blades (green diamond in circle), (ii) the principal prey group in the diet of juvenile lemon sharks, mojarra spp. (*Eucinostomus* spp.) sampled in both fringing sheltered mangroves (red dots) versus exposed seagrass beds (green dots) and 1–3 year old juvenile lemon sharks (blue dots) corrected for isotopic discrimination (see Material and methods). Isotope data for individual prey and sharks and mean (±1 s.d.) are presented. The δ^{15}N value of mangrove and seagrass is set to 7‰ for ease of interpretation. Actual mean δ^{15}N values for mangrove and seagrass are −8.9‰ and −2.7‰, respectively. The δ^{34}S values for mangrove and seagrass are not shown but are −16.9‰ and 12.0‰, respectively. (Online version in colour.)

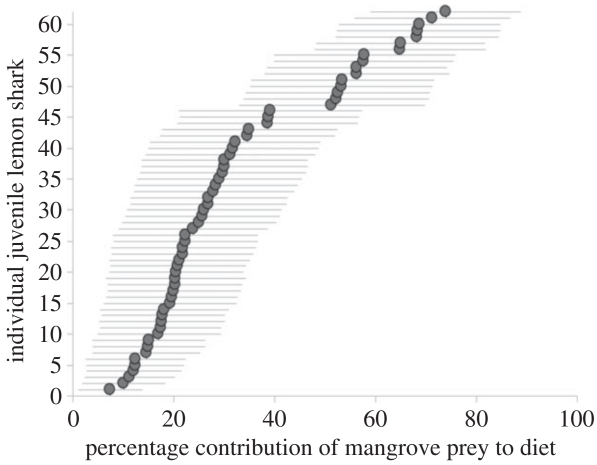

Figure 3. Individual variation in foraging locations of juvenile lemon sharks (*Negaprion brevirostris*) within the nursery habitat, quantified through a Bayesian hierarchical mixing model incorporating carbon stable isotopes (δ^{13}C) of prey (*Eucinostomus* spp.) sampled in fringing sheltered mangroves and on exposed seagrass beds and predators sampled throughout the nursery. Each point shows the estimated proportional contribution of mangrove-derived prey to each of the 62 individual sharks, with lines representing ±1 s.d. Individual sharks were stacked based on ranked carbon stable isotope proportions.

chain Monte Carlo (MCMC) sampling implemented in JAGS in R [35]. Three parallel chains, each with 150 000 iterations and a burn-in phase of 100 000 iterations were used, retaining every other sample. All MCMC chains showed visual evidence of convergence, exhibited low levels of auto-correlation and Gelman and Rubin diagnostics were all less than 1.05.

To determine whether 1–2 year old lemon sharks consistently foraged in the same habitat over time, δ^{13}C values of sharks captured in the nursery ground in 2009 and recaptured in 2010 following a year at liberty ($n = 19$) were determined. To measure individual foraging uniformity over the 1 year period, the difference of the mean ($\mu_1 - \mu_2$) and standard deviation ($\sigma_1 - \sigma_2$) parameters between years and the normality of the data within groups (υ) were simultaneously estimated using a Bayesian *t*-test approach, BEST in R [36]. A non-committal prior was used that has minimal impact on the posterior distribution and the shape of the data in each group described by a *t*-distribution [36]. The posterior distribution of differences (mean and standard deviation) and effect size were estimated, and the 95% high density intervals (HDIs) were used to describe the credibility interval for each. If the 95% HDI is significantly above zero, the parameter estimates are credibly different.

Growth rates of 1 and 2 year old recaptured sharks were then calculated from the difference in length (PCL, cm) and mass (kg) between capture and recapture, and multiplied by the total number of days at liberty, where 365 days = 1. We used one year old sharks that were born in 2008 (sampling time points 2008 and 2009; isotopic data from 2009; $n = 9$) and 2009 (sampling time points 2009 and 2010; isotopic data from 2010; $n = 43$) and 2 year old sharks born in 2007 (sampling time periods 2008 and 2009, isotopic data 2009; $n = 6$) and 2008 (sampling time periods 2009 and 2010, isotopic data 2010; $n = 10$). This included nine individuals with growth data for both years 1 and 2 in the nursery ground. To examine whether the isotopic values of these sharks were related to growth rate, the relationship between δ^{13}C values for each individual and the covariates of growth rate, age and sex were examined using a mixed effects model fit with maximum likelihood in the *lme* package in R. Growth rate, sex and age were included as fixed effects and birth year as a random effect. An ANOVA was used to compare models with progressively simplified fixed effects. Linear regression was used to determine the relationship between δ^{13}C values of the sharks (i.e. depleted ^{13}C values, foraging in mangroves or enriched ^{13}C values, foraging in seagrass) and growth rate (i.e. slow versus fast). Only sharks that were greater than or equal to 1 year and less than 3 years old were included in these analyses given (i) maternal effects on isotopic signatures of less than 1 year old sharks and

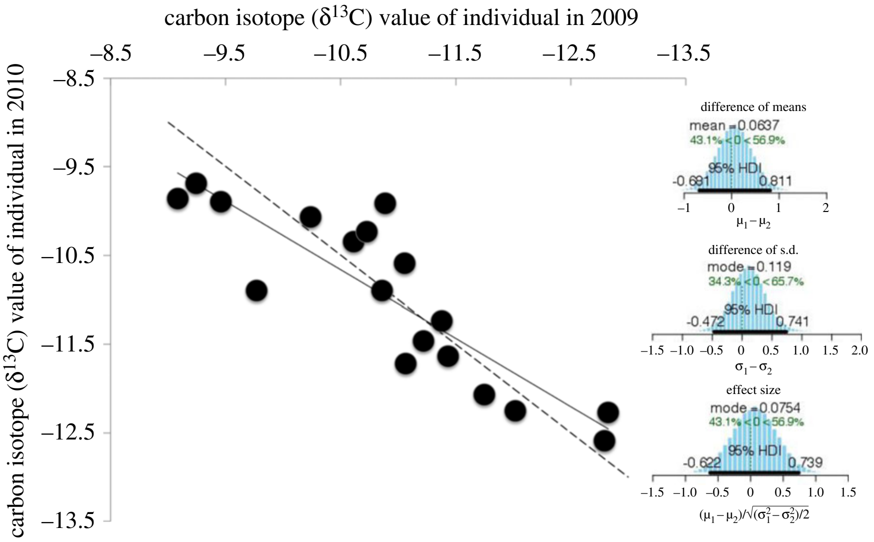

Figure 4. Uniformity in foraging location by lemon sharks (*Negaprion brevirostris*) within the nursery over a 1-year period. Carbon isotope data (δ^{13}C) are for two sampling points per individual shark (June 2009 and 2010) and location is inferred based on significant differences in carbon isotope values of the main prey sampled in mangrove versus seagrass beds. The continuous black line depicts the linear regression between isotopic sample points per individual for each sampling time point and the dashed line represents the one to one relationship for these data. Histograms show posterior distribution of differences and effects size; 'HDI' denotes highest density interval. (Online version in colour.)

(ii) 3 year old animals start to make broader scale movements within the nursery and lagoon areas. We focus solely on δ^{13}C for the latter analyses given the correlation between δ^{13}C and δ^{34}S values.

To examine whether habitat use of juvenile lemon sharks was biased towards mangrove or exposed seagrass habitat as a measure of predation risk, the mean (±s.d.) distance from shore was calculated for each shark from all GPS locations recorded per month. For individuals tracked at more than 1 month intervals, size was re-estimated based on growth rate (or from recaptures) and a new mean distance-to-shoreline value was calculated for that individual. Shark–shoreline distances were calculated using the Animal Movement extension in ArcGIS. For sub-adult sharks, location data were divided by tidal cycle (low versus high; data from the National Oceanic and Atmospheric Administration) and data for all individuals presented to show their overall distribution around the Bimini Islands.

3. Results

(a) Ecosystem isotopic variation

The δ^{13}C and δ^{34}S values for mangrove leaves and seagrass blades, the source endpoints in the nursery ground, were markedly different ($\delta^{13}\text{C} = -23.8 \pm 0.3$‰ and -7.1 ± 0.6‰ and $\delta^{34}\text{S} = -16.9$ and 12.0; mangrove and seagrass, respectively) in agreement with previous findings [31]. *Eucinostomus* spp. sampled in mangrove (mean ± s.d.: -17.3‰ $\pm$ 0.8) and seagrass habitats (-13.4‰ $\pm$ 1.2) had significantly different δ^{13}C values that scaled accordingly between the carbon source endpoints ($F_{2,29} = 42.77$, $p < 0.0001$; figure 2). There was no effect of size on δ^{13}C values of *Eucinostomus* spp. For juvenile lemon sharks sampled within the nursery in 2010 ($n = 62$), δ^{13}C were highly variable with a minimum and maximum value of -12.6‰ and -8.4‰ (range 4.2‰; figure 2). Equally, large variation in δ^{34}S values was observed for the subset of sharks analysed ($n = 15$), ranging from a minimum of -0.4‰ to a maximum of 5.7‰ (range = 6.1‰). As predicted, the δ^{13}C and δ^{34}S values of sharks were highly correlated further supporting foraging across the mangrove–seagrass continuum (electronic supplementary material, figure S1). Juvenile sharks ranged in size from 49.5 to 74.5 cm PCL (mean ± s.d. = 55.7 ± 5.5 cm) and 1.1 to 5.5 kg (1.9 ± 0.8 kg).

(b) Individual foraging behaviour

Our hierarchical Bayesian stable isotope-mixing model found that individual sharks adopted variable foraging strategies. The median proportional contribution of mangrove-derived *Eucinostomus* spp. to the 62 individual sharks ranged from 73.9 to 7.4% (figure 4). The median shark was estimated to feed on approximately 26.9% of mangrove-derived *Eucinostomus* spp. As expected, the estimation of posterior probabilities for individual sharks had some level of uncertainty (figure 3). This uncertainty could be reduced by treating individuals as random effects (rather than fixed effects as implemented here), but we were primarily interested in estimating where individuals fell on the gradient between mangrove and seagrass habitats. These data identify that some sharks forage predominantly on *Eucinostomus* spp. in mangrove habitat, others mostly on *Eucinostomus* spp. in seagrass habitat, with the remaining shark foraging locations scaling between the two habitats (figure 3).

(c) Temporal uniformity in foraging behaviour

For 1 and 2 year old sharks that were sampled both in 2009 and 2010 ($n = 18$), δ^{13}C values were consistent between the two sampling periods (mean central tendency (CT) = 0.06; 95% HDI −0.69, 0.80 $t_{17} = 0.55$; $p = 0.59$), with only minor variation (s.d. CT = 0.11; 95% HDI −0.48, 0.73: 0.03–0.80) and effect size (effect CT = 0.01; 95% HDI −0.64, 0.72; figure 4). This suggests that juvenile lemon sharks undertake systematic individual-level foraging behaviour in mangrove, seagrass or combined mangrove–seagrass habitats over an annual cycle during their first 3 years in the nursery habitat.

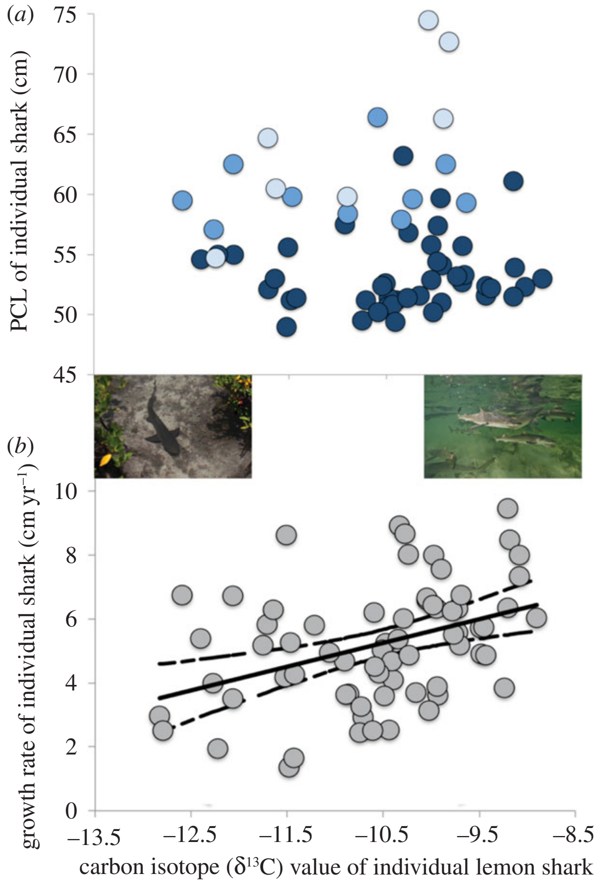

Figure 5. (*a*) Body size distribution versus carbon stable isotope values of individual sharks born in 2007, 2008 and 2009 and sampled in 2010 (1–3 years old; dark to light blue circles, respectively) and; (*b*) the relationship between growth rate of individual lemon sharks (*Negaprion brevirostris*; 1 and 2 year olds) calculated from field derived measurements and the mean carbon isotope values (δ^{13}C) of fin tissue sampled from each shark at two time points approximately 1 year apart. The δ^{13}C values range over a continuum from sharks feeding predominantly on prey in fringing sheltered mangroves to those feeding on prey over exposed seagrass beds. The continuous line depicts the fitted regression line for growth versus δ^{13}C values of sharks and the dashed line the 95% CIs. (Online version in colour.)

(d) Foraging versus body size traits

One, 2 and 3 year old sharks had variable δ^{13}C values that reflected the range of the two distinct foraging habitats. For 1 year olds, a large proportion of individuals had δ^{13}C values that were indicative of foraging in both seagrass and mangrove habitats but predominantly on seagrass beds (figure 5). For 2 and 3 year old individuals, the spread of δ^{13}C values was more even. Three years old sharks, with enriched ^{13}C values indicative of foraging over seagrass beds, were also larger (mean δ^{13}C values of -9.9 ± 0.1 and PCL of 71.2 ± 4.3 for three most enriched ^{13}C sharks versus mean δ^{13}C values of -11.9 ± 0.3 and PCL of 60.0 ± 5.0 cm for the three most depleted ^{13}C sharks; figure 5*a*). Growth rates for 1 and 2 year old sharks ($n = 52$ and $n = 16$, respectively) ranged from 1.4 to 9.5 cm yr^{-1} (PCL) and -0.4 to 1.7 kg yr^{-1} (mass). Only PCL growth rate data were modelled due to known error in body mass field measurements and the confounding effect of recent feeding events, contributing up to 5% of stomach mass to total body weight. The progressively simplified mixed effect model found δ^{13}C values were significantly affected by growth rate, but sex, age and birth year had no effect. While growth variability was observed, as would be expected under natural conditions, there was a significant positive linear relationship between growth rate and δ^{13}C values ($F_{1,66} = 11.97$, $p < 0.001$; $r^2 = 0.2$; figure 5*b*). Juvenile lemon sharks foraging predominantly in mangrove habitats had significantly slower growth rates than those foraging over seagrass beds, with growth rate increasing between the two habitat endpoints.

(e) Variability in juvenile movements and predation risk

Tracked juvenile lemon sharks ($n = 19$) displayed highly variable movements with some individuals remaining predominantly close to the mangroves versus others that occupied exposed seagrass habitat (figure 6*a*). There was no effect of animal size on the observed movement patterns (figure 6*a*). A track of a newborn shark, measuring 44.3 cm PCL, ended when it appeared to remain stationary for more than 10 min over exposed seagrass beds. Upon entering the water, remains of the dead shark were found documenting an active predation event (figure 6*c*). Tracking data for sub-adult sharks ($n = 67$) showed that they occupied both inshore and offshore habitats throughout the Bimini Islands that was dependent on tidal state, and thus suitable water depth to manoeuvre and forage (figure 6*b*).

4. Discussion

Resolving the underlying mechanisms driving selection processes in the wild, particularly for large, long-lived and highly mobile marine organisms is complex and consequently is often assumed rather than empirically tested [37]. Given known cannibalism and predation on juvenile lemon sharks over exposed seagrass habitat by larger conspecifics ([23,24]; figure 6*c*), we used the distinct isotopic baselines of mangrove versus seagrass to assess the foraging locations of individual sharks. This facilitated an indirect test of the mechanism for negative directional selection acting on size-related traits proposed by DiBattista *et al*. [3]. Carbon and sulfur isotope values identified variable foraging strategies among juvenile sharks, and hierarchical Bayesian mixing models revealed that some individuals fed predominantly on prey from mangrove habitat while others fed predominantly on prey from seagrass habitat. This was supported by variable movement among juveniles recorded in the field via acoustic tracking. Foraging strategies were also correlated with growth rates, whereby sharks that fed in sheltered mangrove habitats had slower growth rates than those that fed over exposed seagrass beds. These cumulative findings support a continuum of foraging strategies by juvenile lemon sharks between mangrove and seagrass habitat during their first years of residency in the Bimini nursery. Concurrently, there was a notable shift from 1 year old sharks feeding predominantly over seagrass to more evenly distributed foraging over both habitats by age three. This provides evidence for a size range of juveniles within the nursery where selection is free to act, favouring smaller size and slower growth [3].

Our data provide compelling evidence that larger, faster growing individuals do occur in the Bimini nursery habitat, but where do these individuals originate from to allow for continuing selection for small size given the much larger individuals at age and faster juvenile growth observed at other nurseries [21,22]? We suggest that male-mediated gene flow

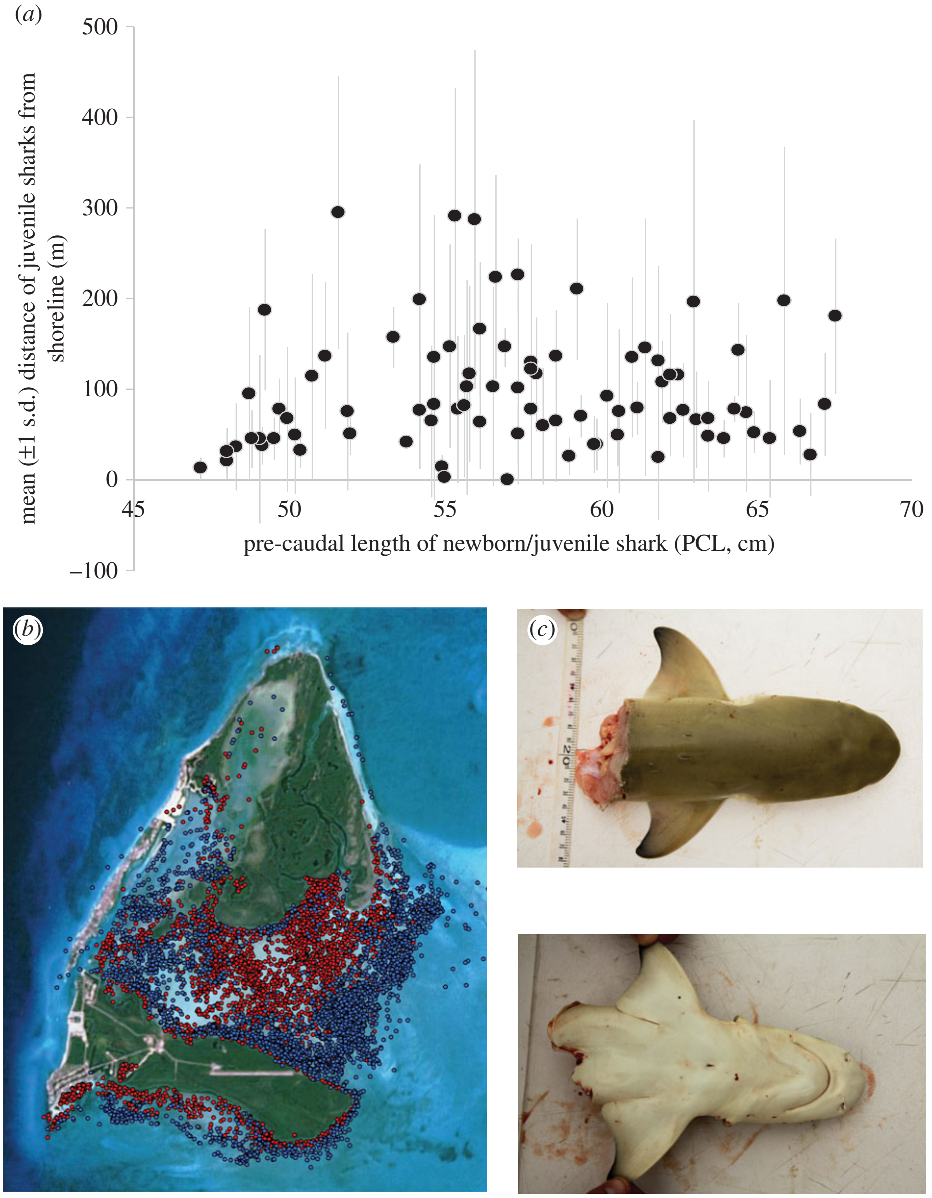

Figure 6. (*a*) Variation in proximity of juvenile lemon sharks (*Negaprion brevirostris*) to the shoreline of the nursery habitat (distance in metres ± 1 s.d.) as a measure of low (near mangrove) versus high (over exposed seagrass bed) predation risk. Data are presented for 19 sharks, with multiple data points per individual if tracked over consecutive months (see Material and methods); (*b*) tracks of sub-adult lemon sharks ($n = 67$) detected throughout the Bimini Islands with each dot representing derived location estimates. Red and blue dots depict high and low tide, respectively; and (*c*) remains of a newborn lemon shark that was consumed during an active acoustic track over exposed seagrass beds within the nursery. (Online version in colour.)

and occasional stray pregnant females [38] at the adult stage maintains selection for smaller size at Bimini through constrained local adaptation. Under this scenario, maladaptive genes are continually introduced to Bimini, thus maintaining a body size above the optimum and consequently promoting selection. The larger juvenile lemon sharks seen at Marquesas Key, Florida, for example, are genetically similar to the Bimini sharks, suggesting gene flow between these two populations [38]. Indeed, it would appear that selection for slow growth and smaller size may be stronger at Bimini than other neighbouring nursery grounds leading to a smaller equilibrium body size.

The appearance of distinct behavioural foraging strategies (over exposed seagrass versus in sheltered mangroves) among these juvenile sharks may be attributable to underlying differences in personality. Juvenile lemon sharks (age 1–2 years) have been shown to display persistent individual differences in the rate of movement within a novel open field test at the Bimini nursery [39]. Importantly, the sharks showed habituation in movement pattern over repeated trials, indicating that this was a reaction to novelty and not representative of general activity. Recent research also suggests that personality variation is determined by an individual's environment and age-related experience as well as a heritable component [40] that may be under strong selection [41]. The mechanism driving the proposed continual selection for smaller size and slower growth at Bimini may therefore be an effect of introduced maladaptive genes from other populations that not only include individuals of larger size and faster growth, but also personality variation. Alternatively, this dichotomy could relate to differences in habitat type among geographically separate nursery habitat. For example, juvenile lemon sharks at Cape Canaveral, Florida reside in an exposed coastal nursery habitat and undertake seasonal migrations of up to 190 km, which contrasts with the fidelity and small home ranges of juveniles at Bimini [42]. Selection for larger size and bolder personality traits may support this exploratory behaviour at Cape Canaveral, despite the potential for increased predation risk because of a lack of available refuging habitat (i.e. sheltered mangrove). Moreover, evidence for gene flow between these two geographically isolated populations (Cape Canaveral and Bimini) has been shown [38].

Both carbon and sulfur isotopes and growth data unequivocally show that juvenile sharks at Bimini adopt variable foraging strategies with effects on growth rate and size, but data are not available to directly quantify intra-specific predation rates. Substantial evidence within this exhaustively studied system, however, supports predation on juvenile lemon sharks within the nursery [23,24]. For example, telemetry tracking data in this study, and that reported previously, found that the sub-adult lemon shark population at Bimini have larger home ranges and predominantly use exposed seagrass and sand flats within the lagoon [43], a likely result of habitat structure (mangrove roots) and shallow water depths restricting the size of sharks that can manoeuvre through the fringing mangroves. Recently, Guttridge *et al*. [25] demonstrated that the movements of juvenile lemon sharks in an adjacent nursery area at Bimini (Bonefish Hole) were influenced by the presence of sub-adult sharks in the area as it related to tidal changes in water depth. These data suggested that juveniles make fine-scale habitat selection decisions in response to intra-specific predation risk. Moreover, observations of juveniles feeding on prey in the field were rare, which may also suggest a trade-off between foraging and refuging [25], with impacts for both body size and growth rate. Refuging and anti-predator avoidance has been reported for several shark species and is considered one of the fundamental behaviours associated with coastal or estuarine nursery habitats [44]. The predominant use of sheltered, shallow habitats by juvenile lemon sharks is also documented at two more nurseries in the Atlantic Ocean (Cape Eleuthera in the Bahamas and Atol das Rocas in Brazil) that have similar habitat structure to Bimini [45,46]. As a result, the likelihood of predation in shallow waters areas (particularly mangroves) is minimized both by habitat type, water depth and known movements of the sub-adult lemon shark population. By contrast, several shark predation events have been reported while tracking juvenile lemon sharks along the exposed nursery shoreline [24], including one of the individuals tracked in this study (figure 6*c*). This includes transmitted juveniles suddenly changing behaviour or being detected in unexpected habitats, rapidly increasing swimming speeds to unrealistic levels or as we report here, an acoustic signal abruptly ending and the discovery of the remains of the predated juvenile (figure 6*c*). Importantly, our data and previous juvenile tracking data reveal a range of behaviours, with some juvenile sharks predominantly moving in the mangroves or remaining very close to the mangrove edge, while similar-sized individuals favour exposed seagrass habitats hundreds of metres from shore ([24,46]; figure 6*a*), where large sharks may co-occur (figure 6*b*; TL Guttridge 2017, unpublished data).

5. Conclusion

Through a combination of isotopically distinct habitats and prey that reside in those habitats as well as careful consideration of pragmatic assumptions (electronic supplementary material, S5), fine-scale tracking of the foraging behaviour of juvenile sharks within their nursery ground was possible. Although several studies have tracked spatial movements of animals using stable isotopes [47], this study represents one of few that were able to elucidate foraging patterns over a fine spatial scale. When combined with field sampling and telemetry tracking, this allowed novel insights into body size selection in a large marine vertebrate. Identifying the mechanism driving selection for slower growth and smaller size supports a growing body of work showing that bigger is not always better and that size selection is more plastic than originally thought. While an alternate explanation for the observed variable growth rates could result from competition among conspecifics, the nursery ground is not resource limited [48], aggression between juveniles has not previously been reported over extensive monitoring periods [49] and population density does not appear to be correlated with the strength of selection acting on these size-related traits [3]. Equally, while faster or slower growth rates may represent a form of adaptive phenotypic plasticity, whereby individuals, for example, have lower growth rates in response to poorer quality mangrove habitat, we favour evolutionary change given the evidence of selection for smaller body size and heritability of these traits in this population [3,22]. It is likely that predator-dominated systems may influence size selection and that the interplay of predator abundance, personality and sociality, as well as available habitat types (exposed versus refuges) at localized scales influenced by broader scale variance in adaptation of mobile marine species, shape the direction of selection.

Ethics. All handling of juvenile lemon sharks and sampling of prey items was undertaken under a permit issued from the Department of Fisheries of the Commonwealth of the Bahamas.

Data accessibility. Data are available in the electronic supplementary material.

Authors' contributions. N.E.H., D.D.C. and J.D.D. conceived of the study, designed the study and coordinated the study; N.E.H., A.T.F. and O.C. undertook laboratory work; N.E.H., J.W.M. and E.J.W. carried out the statistical analyses; S.T.K., K.A.F., T.L.G., J.D.D. and S.H.G. collected field data; N.E.H. wrote the manuscript with support from J.D.D. and input from all authors. All authors gave final approval for publication.

Competing interests. We have no competing interests.

Funding. This study was financially supported by the Bimini Biological Field Station and grants from the Canadian Research Chair Program to A.T.F. N.E.H. was supported by the Ocean Tracking Network and the Government of Nunavut.

Acknowledgements. The authors would like to thank the numerous volunteers at the Bimini Biological Field Station who have participated in the annual sampling of lemon sharks as part of this long-term population census. Photographic credits in all figures to Mathew D Potenski. We thank Anna J. Hussey for stable isotope sample preparation and analysis.

References

1. Williams GC. 1996 *Adaptation and natural selection: a critique of some current evolutionary thought*. Princeton, NJ: Princeton University Press.
2. Kawecki TJ, Ebert D. 2004 Conceptual issues in local adaptation. *Ecol. Lett.* **7**, 1225–1241. (doi:10.1111/j.1461-0248.2004.00684.x)
3. Dibattista JD, Feldheim KA, Gruber SH, Hendry AP. 2007 When bigger is not better: selection against large size, high condition and fast growth in

juvenile lemon sharks. *J. Evol. Biol.* **20**, 201–212. (doi:10.1111/j.1420-9101.2006.01210.x)

4. Roff DA. 1992 *Evolution of life histories: theory and analysis*. Berlin, Germany: Springer Science & Business Media.
5. Blanckenhorn WU. 2000 The evolution of body size: what keeps organisms small? *Q. Rev. Biol.* **75**, 385–407. (doi:10.1086/393620)
6. Rowe D, Thorpe J. 1990 Differences in growth between maturing and non-maturing male Atlantic salmon, *Salmo salar* L., parr. *J. Fish Biol.* **36**, 643–658. (doi:10.1111/j.1095-8649.1990.tb04319.x)
7. Juanes F, Conover D. 1994 Piscivory and prey size selection in young-of-the-year bluefish-predator preference or size-dependent capture success. *Mar. Ecol. Prog. Ser.* **114**, 59–69. (doi:10.3354/meps114059)
8. Parker RR. 1971 Size selective predation among juvenile salmonid fishes in a British Columbia inlet. *J. Fish. Res. Board Can.* **28**, 1503–1510. (doi:10.1139/f71-231)
9. Henderson P, Holmes R, Bamber RN. 1988 Size-selective overwintering mortality in the sand smelt, *Atherina boyeri* Risso, and its role in population regulation. *J. Fish Biol.* **33**, 221–233. (doi:10.1111/j.1095-8649.1988.tb05465.x)
10. West CJ, Larkin P. 1987 Evidence for size-selective mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia. *Can. J. Fish. Aquat. Sci.* **44**, 712–721. (doi:10.1139/f87-086)
11. Hendry AP, Day T, Cooper AB. 2001 Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *Am. Nat.* **157**, 387–407. (doi:10.1086/319316)
12. Miller TJ, Crowder LB, Rice JA, Marschall EA. 1988 Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Can. J. Fish. Aquat. Sci.* **45**, 1657–1670. (doi:10.1139/f88-197)
13. Houde ED. 1989 Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fish. Bull.* **87**, 471–495.
14. Biro PA, Abrahams MV, Post JR, Parkinson EA. 2004 Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proc. R. Soc. Lond. B* **271**, 2233–2237. (doi:10.1098/rspb.2004.2861)
15. Sundström LF, Löhmus M, Devlin RH. 2005 Selection on increased intrinsic growth rates in coho salmon, *Oncorhynchus kisutch*. *Evolution* **59**, 1560–1569. (doi:10.1111/j.0014-3820.2005.tb01805.x)
16. Sogard SM. 1997 Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull. Mar. Sci.* **60**, 1129–1157.
17. Warburton K. 2003 Learning of foraging skills by fish. *Fish Fish.* **4**, 203–215. (doi:10.1046/j.1467-2979.2003.00125.x)
18. Chapman DD *et al.* 2009 Long-term natal site-fidelity by immature lemon sharks (*Negaprion brevirostris*) at a subtropical island. *Mol. Ecol.* **18**, 3500–3507. (doi:10.1111/j.1365-294X.2009.04289.x)
19. Feldheim KA, Gruber SH, DiBattista JD, Babcock EA, Kessel ST, Hendry AP, Pikitch EK, Ashley MV, Chapman DD. 2014 Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. *Mol. Ecol.* **23**, 110–117. (doi:10.1111/mec.12583)
20. Barker MJ, Gruber SH, Newman SP, Schluessel V. 2005 Spatial and ontogenetic variation in growth of nursery-bound juvenile lemon sharks, *Negaprion brevirostris*: a comparison of two age-assigning techniques. *Environ. Biol. Fishes* **72**, 343–355. (doi:10.1007/s10641-004-2584-3)
21. Freitas RHA, Rosa RS, Gruber SH, Wetherbee BM. 2006 Early growth and juvenile population structure of lemon sharks (*Negarpion brevisrostris*) in the Atol das Rocas Biological Reserve, off northeast Brazil. *J. Fish. Biol.* **68**, 1319–1332. (doi:10.1111/j.0022-1112.2006.00999.x)
22. DiBattista JD, Feldheim KA, Garant D, Gruber SH, Hendry AP. 2009 Evolutionary potential of a large marine vertebrate: quantitative genetic parameters in a wild population. *Evolution* **63**, 1051–1067. (doi:10.1111/j.1558-5646.2008.00605.x)
23. Vorenberg MM. 1962 Cannibalistic tendencies of lemon and bull sharks. *Copeia* **59**, 455–456. (doi:10.2307/1440934)
24. Morrissey JF, Gruber SH. 1993 Habitat selection of juvenile lemon sharks, *Negaprion brevirostris*. *Environ. Biol. Fish.* **38**, 311–319. (doi:10.1007/BF00007524)
25. Guttridge TL, Gruber SH, Franks BR, Kessel ST, Gledhill KS, Uphill J, Krause J, Sims DW. 2012 Deep danger: intra-specific predation risk influences habitat use and aggregation formation of juvenile lemon sharks *Negaprion brevirostris*. *Mar. Ecol. Prog. Ser.* **445**, 279–291. (doi:10.3354/meps09423)
26. Peterson BJ, Fry B. 1987 Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **18**, 293–320. (doi:10.1146/annurev.es.18.110187.001453)
27. DeNiro MJ, Epstein S. 1978 Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 495–506. (doi:10.1016/0016-7037(78)90199-0)
28. Rasmussen JB, Trudeau V, Morinville G. 2009 Estimating the scale of fish feeding movements in rivers using δ^{13}C signature gradients. *J. Anim. Ecol.* **78**, 674–685. (doi:10.1111/j.1365-2656.2008.01511.x)
29. Hesslein RH, Capel MJ, Fox DE, Hallard KA 1991 Stable isotopes of sulfur, carbon and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River Basin, Canada. *Can. J. Fish. Aquat. Sci.* **48**, 2258–2265. (doi:10.1139/f91-265)
30. Newman SP, Handy RD, Gruber SH. 2010 Diet and prey preference of juvenile lemon sharks *Negaprion brevirostris*. *Mar. Ecol. Prog. Ser.* **398**, 221–234. (doi:10.3354/meps08334)
31. Loneragan N, Bunn S, Kellaway D. 1997 Are mangroves and seagrasses sources of organic carbon for penaeid prawns in a tropical Australian estuary? A multiple stable-isotope study. *Mar. Biol.* **130**, 289–300. (doi:10.1007/s002270050248)
32. Gning N, Le Loc'h F, Thiaw OT, Aliaume C, Vidy G. 2010 Estuarine resources use by juvenile flagfin mojarra (*Eucinostomus melanopterus*) in an inverse tropical estuary (Sine Saloum, Senegal). *Estuar. Coast. Shelf Sci.* **86**, 683–691. (doi:10.1016/j.ecss.2009.11.037)
33. Newell RIE, Marshall N, Sasekumar A, Chong VC 1995 Relative contributions of benthic macroalgae, phytoplankton and mangroves as sources of nutrition for panaeid prawns and other coastal invertebrates from Malaysia. *Mar. Biol.* **123**, 595–606. (doi:10.1007/BF00349238)
34. Ward EJ, Semmens BX, Schindler DE. 2010 Including source uncertainty and prior information in the analysis of stable isotope mixing models. *Environ. Sci. Technol.* **44**, 4645–4650. (doi:10.1021/es100053v)
35. Plummer M. 2003 JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. In *Proc. of the 3rd Int. workshop on distributed statistical computing*. Technische Universit at Wien, 125. See http://www.ci.tuwien.ac.at/Conferences/DSC-2003/.
36. Kruschke JK. 2013 Bayesian estimation supersedes the *t* test. *J. Exp. Psychol. Gen.* **142**, 573–603. (doi:10.1037/a0029146)
37. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. Oxford, UK: Oxford University Press.
38. Ashe JL, Feldheim KA, Fields AT, Reyier EA, Brooks EJ, O'Connell MT, Skomal G, Gruber SH, Chapman DD. 2015 Local population structure and context-dependent isolation by distance in a large coastal shark. *Mar. Ecol. Prog. Ser.* **520**, 203–216. (doi:10.3354/meps11069)
39. Finger JS, Dhellemmes F, Guttridge TL, Kurvers RHJM, Gruber SH, Krause J. 2016 Rate of movement of juvenile lemon sharks in a novel open field, are we measuring activity or reaction to novelty? *Anim. Behav.* **116**, 75–82. (doi:10.1016/j.anbehav.2016.03.032)
40. Sinn D, Apiolaza L, Moltschaniwskyj N. 2006 Heritability and fitness-related consequences of squid personality traits. *J. Evol. Biol.* **19**, 1437–1447. (doi:10.1111/j.1420-9101.2006.01136.x)
41. Brown C, Jones F, Braithwaite V. 2005 *In situ* examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Anim. Behav.* **70**, 1003–1009. (doi:10.1016/j.anbehav.2004.12.022)
42. Reyier EA, Franks BR, Chapman DD, Scheidt DM, Stolen ED, Gruber SH. 2014 Regional-scale migrations and habitat use of juvenile lemon sharks (*Negaprion brevirostris*) in the US South Atlantic. *PLoS ONE* **9**, e88470. (doi:10.1371/journal.pone.0088470)
43. Gruber SH, Nelson DR, Morrissey JF. 1988 Patterns of activity and space utilization of lemon sharks, *Negaprion brevirostris*, in a shallow Bahamian lagoon. *Bull. Mar. Sci.* **43**, 61–76.
44. Heithaus M. 2004 *Predator–prey interactions.* Biology of sharks and their relatives, pp. 487–521. Boca Raton, FL: CRC.
45. Wetherbee BM, Gruber SH, Rosa RS. 2007 Movement patterns of juvenile lemon sharks *Negaprion brevirostris* within Atol das Rocas, Brazil: a nursery characterized by tidal extremes. *Mar. Ecol. Prog. Ser.* **343**, 283–293. (doi:10.3354/meps06920)

46. Murchie K *et al.* 2010 Spatial ecology of juvenile lemon sharks (*Negaprion brevirostris*) in tidal creeks and coastal waters of Eleuthera, The Bahamas. *Environ. Biol. Fishes* **89**, 95–104. (doi:10.1007/s10641-010-9693-y)
47. Rubenstein DR, Hobson KA. 2004 From birds to butterflies: animal movement patterns and stable isotopes. *Trends. Ecol. Evol*. **19**, 256–263. (doi:10.1016/j.tree.2004.03.017)
48. Newman SP, Gruber SH. 2002 Comparison of mangrove and seagrass fish and macroinvertberates in Bimini, Bahamas. *Bahamas J. Sci*. **9**, 19–27.
49. Gruber SH. 1982 Role of the lemon shark as predator in the tropical marine environment: a multidisciplinary study. *Flo. Scient.* **45**, 46–75.