

Hunted hunters: an experimental test of the effects of predation risk on juvenile lemon shark habitat use

K. L. Stump^{1,2,*}, C. J. Crooks³, M. D. Fitchett⁴, S. H. Gruber^{1,2}, T. L. Guttridge²

¹Department of Marine Biology and Ecology, University of Miami Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA

²Bimini Biological Field Station Foundation, 9300 SW 99th Street, Miami, Florida 33176, USA

³University of Manchester, Oxford Road, Manchester M13 9PL, UK

⁴Department of Marine Ecosystems and Society, University of Miami Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA

ABSTRACT: Predation effects can occur either through consumption or as risk effects, which can include alterations in prey behavior to decrease encounters, detection and/or capture by predators. Perceived predation threats lead to behavioral responses which may manifest as changes in habitat use by the prey, and these effects can be significant and even greater in population-level consequences and across multiple trophic levels than consumptive effects. In Bimini, Bahamas, juvenile lemon sharks (*Negaprion brevirostris*, Poey 1868) use mangrove-fringed shorelines as nursery areas in part due to protection afforded from predators, namely, large conspecifics. We investigated small-scale use (on the order of meters) of artificial mangrove structure by juvenile *N. brevirostris* as an antipredatory response to perceived predation risk. Controlled experimental trials were conducted on semi-captive individuals to compare the degree to which sharks used artificial subtidal refugia when solitary, with a size-matched conspecific and with a potential predator (large conspecific). Test shark size and predator presence were significant drivers of artificial mangrove use. There was a negative relationship between body size and refuge use in the presence of a predator, indicating that size is an important factor influencing antipredatory behavior. Test sharks exhibited a high degree of social swimming behaviors with size-matched conspecifics, but the presence of a large conspecific predator elicited fright responses. This study provides the first experimental evidence of the influence of intraspecific predator–prey interactions on juvenile shark habitat use and demonstrates use of subtidal structure as a habitat-specific escape tactic.

KEY WORDS: Risk effects · Risk of predation · Habitat loss · Mangroves · Predator–prey interactions · Refuge

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Predators influence prey behavior, feeding rates, movement patterns, habitat use, morphology and population structure as a result of both direct predation and through risk effects (Lima & Dill 1990, Creel & Christianson 2008). Historically, studies investigating predator–prey interactions have focu-

sed on direct predation (e.g. Boutin 1995, Eberhardt et al. 2003). In recent years, however, the importance of risk effects, or behavioral changes in prey resulting from a perceived predation threat (Heithaus et al. 2008), has been demonstrated in both marine and terrestrial systems (e.g. Lima 1998, Creel & Christianson 2008, Heithaus et al. 2009, Vaudo & Heithaus 2013).

Antipredatory behavioral responses are major factors influencing habitat use in many marine vertebrates. For example, Indian Ocean dolphins *Tursiops aduncus*, green turtles *Chelonia mydas*, pied cormorants *Phalacrocorax variax*, and dugongs *Dugong dugong* shift from shallow, productive seagrass foraging areas to deeper and safer, but less productive, habitats when their predator the tiger shark *Galeocerdo cuvier* is present (Heithaus & Dill 2002, Heithaus et al. 2008). Similarly, Hammerschlag et al. (2012) found that tarpon *Megalops atlanticus* alter swimming speed and overall habitat use to avoid encounters with their bull shark *Carcharhinus leucas* predators. While there have been observational studies of risk effects focused on large marine vertebrates in the wild, to our knowledge, there have been few experimental manipulations investigating the effects of predation risk on such taxa (e.g. Rizzari et al. 2014). These types of studies may reveal important relationships between predator–prey interactions and their effects on habitat use, but experimental manipulations are particularly problematic due to the difficulty of maintaining large marine predators in captivity for experimental trials.

Many large shark species are apex predators in the marine environment, and individuals tend not to engage in antipredatory behavior as adults. However, small-bodied species and juveniles of larger species occupy a mesopredator trophic level (e.g. Heithaus 2007, Roff et al. 2016) and are therefore subject to the risk of predation, often by larger sharks (Springer 1967, Compagno 1984), including larger conspecifics (e.g. Snellson et al. 1984, Clarke 1971). To manage this risk, many species use nursery areas to decrease the probability of encountering larger sharks (e.g. Heithaus 2007, Grubbs 2010).

Mangroves, which commonly fringe low-energy, shallow shorelines at tropical and subtropical latitudes, provide important intertidal and subtidal nursery habitats (see Faunce & Serafy 2006 for a review). Laegdsgaard & Johnson (2001) suggested that one of the most important aspects of submerged mangrove habitats is structural complexity, which both maximizes prey resource availability and minimizes predation risks. Individuals of many marine species seek refuge below the waterline in the mangroves as a habitat-specific escape tactic when a predation threat is perceived (Cocheret de la Moriniere et al. 2004), though this habitat can actually be riskier at night (Hammerschlag et al. 2010).

It has been hypothesized that use of mangrove-fringed lagoons and creeks as nursery areas for juvenile lemon sharks in Bimini, The Bahamas (25° 45' N,

79° 15' W), is linked to both resource availability and predator avoidance (Morrissey & Gruber 1993a,b, Franks 2007, Hammerschlag et al. 2010, Newman et al. 2010), but the relative importance of each of these characteristics in driving movements within the nursery has rarely been investigated. Juveniles exhibit site fidelity to mangrove-fringed natal nurseries for several years before they are large enough to expand their home ranges into the wider lagoon (Morrissey & Gruber 1993a, Franks 2007, Chapman et al. 2009, Guttridge et al. 2012). A recent study in our insular system used acoustic tracking and observations on small, wild juvenile lemon sharks and larger conspecifics likely to be cannibalistic predators, finding an increase in small shark refuge use coinciding with an increase in predator presence in the surrounding areas (Guttridge et al. 2012). Previous studies have highlighted this separation of activity spaces between small and large juvenile lemon sharks within the insular Bimini system (Gruber et al. 1988, Chapman et al. 2009). Ontogenetic habitat shifts from mangrove-fringed natal nurseries to the wider lagoon are continuous and likely related to a perceived decrease in predation risk concurrent with an increase in body size (Grubbs 2010). Guttridge et al. (2012) found through observations in the wild that antipredator investment was in accordance with body size, as smaller juvenile lemon sharks used a specific mangrove refuge more often and for longer periods than larger juveniles. That study recorded 2 observations of larger lemon sharks pursuing smaller conspecifics, with the latter retreating to the back of the mangrove inlet, upon detection of the predator, via a shallow channel with mangrove root structure that precluded access to the larger predator. The difference in body size at which a juvenile lemon shark perceives reduced predation risk from a larger shark, including conspecifics, is unknown. This knowledge gap and the importance of mangrove structure to antipredatory behavior for juvenile sharks warrant further investigation (Yates et al. 2015).

While there have been several studies on teleosts using artificial mangroves to examine the use of structural refugia as an antipredator behavior (e.g. Nagelkerken & Faunce 2008), to our knowledge, no studies have employed a similar design to investigate elasmobranchs. Although mangrove shorelines are often major components of juvenile lemon shark nursery habitat, the hypothesis that these large marine vertebrates use submerged mangrove structure to avoid predators has never been empirically tested using experimental manipulations. Observing predator–prey interactions between 2 sharks in the

natural environment is difficult due to their relatively large-scale free-ranging movements. Using experimental manipulations, the present study assessed juvenile lemon sharks' use of subtidal structural complexity as a refuge when faced with a potential predation risk. We aimed to discover whether there is a relationship between body size and time spent near artificial mangrove structure in the presence of a potential predator. We hypothesized that within the experimental configuration, small juveniles would use artificial mangrove structure as a habitat-specific refuge more often in the presence of a potential predator (large conspecific) than when solitary or when paired with a size-matched conspecific. Based on previous studies (Guttridge et al. 2009, 2012), we predicted that size-matched juvenile lemon sharks would exhibit sociality with each other, while the presence of a large conspecific would elicit fright responses from small juveniles.

MATERIALS AND METHODS

Shark capture and experimental configuration

Juvenile lemon sharks used for this study were captured via gillnet following the methods described in Gruber et al. (2001). Upon capture, sharks were transported to a holding pen, where they were later measured (pre-caudal length, L_{PC}), weighed, and tagged with a passive integrated transponder tag (PIT, Destron Fearing®) for individual identification. Fifteen juveniles from 3 age classes (Age-0, Age-1 and Age-2) were used for the study. Age was determined from a database of first capture dates of each individual as a neonate (DiBattista et al. 2007) from concurrent mark-recapture studies. Of the 15 juveniles, 12 were test sharks (mean \pm SD; $\bar{L}_{PC} = 57.0 \pm 6.1$ cm; Age-0 $\bar{L}_{PC} = 48.6 \pm 3.4$ cm [$n = 3$]; Age-1 $\bar{L}_{PC} = 57.5 \pm 1.5$ cm [$n = 6$]; Age-2 $\bar{L}_{PC} = 64.0 \pm 3.3$ cm [$n = 3$]), and 3 served only as size-matched conspecifics ($L_{PC} = 56.7 \pm 1.2$ cm) in experimental treatments. For predator-presence trials, an Age-5 large juvenile lemon shark (116 cm L_{PC}) was captured within Bimini's central lagoon using the block rig technique described in Guttridge et al. (2012) and transported to the trial pen. The large juvenile was acclimated and fed for 2 d in the pen before trials began.

Small juvenile test sharks were held in circular 5-m-diameter plastic mesh (5×5 cm) pens on a shallow, sandy flat adjacent to the trial pen. While in one holding pen, the 12 test sharks were exposed to natural ambient conditions including tidal and lunar cycles,

and natural fluctuations in temperature and salinity. Pens were built 25 m apart and oriented to limit olfactory and visual cues between holding and test pens. The 3 additional small juveniles retained for size-matched conspecific treatments were held in one nearby, separate, but identical circular 5-m-diameter mesh pen to eliminate the possibility of the formation of any associative interactions between test sharks and size-matched conspecifics prior to trial runs (Guttridge et al. 2009). All individuals were fed to satiation every third day with fresh and/or frozen local fish. Feeding was intended to exclude confounding effects of foraging behavior within the trial pen.

A 10×10 m plastic mesh (5×5 cm) trial pen was constructed adjacent to the holding pens (Fig. 1). The trial pen was divided into 2 sectors demarcated by orange concrete rings: two-thirds of the pen were empty, while one-third of the pen contained artificial mangrove units (AMUs) similar to those described in Cocheret de la Moriniere et al. (2004), embedded along the length of the pen. AMUs ($80 \times 80 \times 150$ cm) were constructed of white PVC piping (diameter = 2 cm) at a density of 56 pipes m^{-2} , a value obtained by calculating the mean density of *Rhizophora mangle*

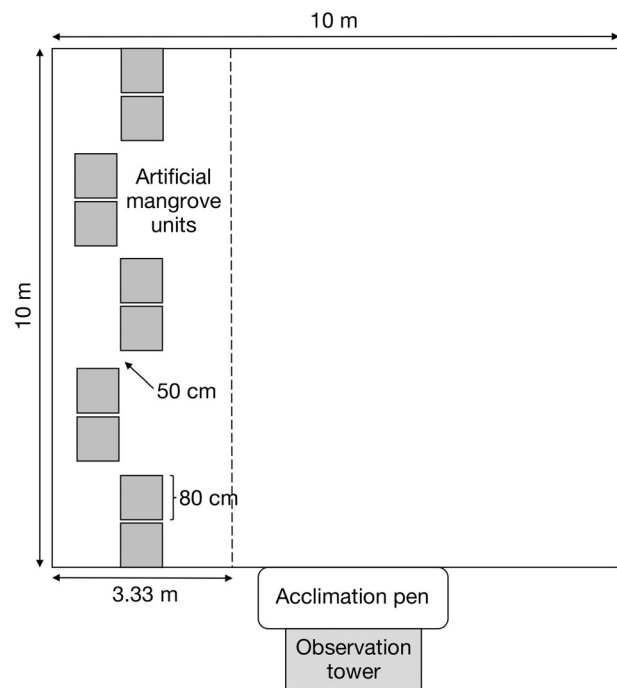


Fig. 1. Schematic of trial pen. One-third of the 10×10 m pen was visually demarcated as an artificial mangrove unit (AMU) sector (dashed line), two-thirds were empty. Staggered AMUs (gray squares) mimicked the natural mangrove shoreline of Bimini's lemon shark nurseries, allowing enough space between them for small sharks to pass through but prohibiting a large predator from maneuvering into the area behind them

prop roots in a representative area of the mangrove shoreline of Bimini's lemon shark nursery. The AMUs were spaced evenly in a repeating staggered pattern within one sector of the trial pen, mimicking the natural mangrove shoreline of the North Sound and allowing the smaller-bodied juvenile test sharks (but not the predator) to swim between and behind the artificial structures. AMUs were of a height such that they emerged from the water at all tidal levels. An adjacent 4-m-high tower allowed observers a complete view of the trial pen. At the midpoint of one side of the trial pen, a 2 m² acclimation pen was built between the trial pen and the tower, separated by a wooden trap door (1.5 × 1.5 m) that could be manually operated from the tower. The wooden door blocked a test shark's view of the trial pen during acclimation periods.

Treatments and trials

Test shark space use within the trial pen was observed in 3 treatments: (1) solitary, (2) in the presence of a size-matched conspecific and (3) in the presence of a large conspecific. Treatments 1 and 2 represented non-predation threat scenarios, while treatment 3 introduced a potential predator. Thirty-minute trials were conducted using a balanced design. There were 72 total trials conducted with 12 different test individuals whose order was selected randomly from the pool of test sharks. To account for any inherent bias for a particular side of the test pen, each shark underwent each treatment a total of 2 times: once with AMUs on one side of the pen, and once with AMUs on the opposite side. Therefore, each test shark underwent 6 total trials. At the completion of each trial, the test individual was returned to the holding pen until it was randomly selected for another trial. Treatments 1 and 2 were carried out first, and the order of both shark and treatment were randomized. However, all trials with the predator were condensed into a 1-wk period following treatments 1 and 2 due to the logistical difficulties of maintaining a large shark in semi-captive conditions on a shallow tidal flat. Therefore, all trials with the predator were first conducted with AMUs on one side of the pen. The units were then moved to the opposite side, and treatment 3 trials were conducted again. In both cases, the order of test sharks within treatment 3 was randomized.

At the start of each trial, a test shark was moved with minimal handling from its holding pen to the acclimation pen via a 100 l plastic transport box and allowed to recover from handling stress for a period

of 15 min (Guttridge et al. 2009). If the treatment involved a size-matched conspecific or predator, that individual was already within the test pen before the start of the trial. Following the test shark's recovery and acclimation period, the trap door was opened, and the test shark was free to move into the test pen. The trap door was raised slowly to minimize disturbance to both the test shark in the acclimation pen and any stimulus shark in the trial pen. The moment the test shark entered the trial pen marked the start of a 30 min trial. Water temperature ($\bar{x} \pm SD = 27.62 \pm 2.07^\circ\text{C}$), depth ($\bar{x} \pm SD = 63.91 \pm 23.74$ cm) and salinity ($\bar{x} \pm SD = 34.67 \pm 4.29$ ppt) were recorded at the start and end of each trial. Wind speeds during trials were <20 knots, and trials were conducted between 08:00 and 18:00 h (local time) to ensure complete visibility of the test pen.

Data treatment

Three behaviors were quantified during trial periods using the program JWatcher (JWatcher Video v1.0, Macquarie University and UCLA): pen sector use, sociality (i.e. behaviors such as following or paralleling) and fright responses (i.e. explosive glide) (Table 1). Nominal trial duration was 30 min ($\bar{x} SD = 29:47 \pm 0:49$ SD), as unforeseen circumstances such as sudden weather changes caused some trials to end before reaching 30 min. However, disparities in trial lengths varied at most by 2.7%. Therefore, it is assumed that behavioral responses are likely not biased by trial lengths.

Behavioral response data for AMU usage and sociality were treated as proportions (observed proba-

Table 1. Ethogram of behaviors recorded during experimental trials

Behavior	Definition
Pen sector use	Time spent in each sector of trial pen, beginning when head and pectoral fins enter sector
Sociality	Within one body length of a second conspecific, either swimming in parallel or following (Myrberg & Gruber 1974, Guttridge et al. 2011)
Fright response	Rapid and sudden avoidance, defined as 'give way' or acceleration, similar to Myrberg & Gruber's (1974) 'explosive glide', but more generally a marked increase in swimming speed

bility, P) of discrete binary choices at any given time: either a display of the behavior (1) or absence of observed behavior (0). To examine and test for differences in exhibited behaviors as a function of size, abiotic experimental factors and presence/absence of a stimulus shark (solitary, size-matched conspecific, or predator treatments), the bounded condition of response data (0, 1) must be taken into consideration. For this purpose, a logit link function was applied to proportional response data (P) relative to n independent variables (X):

$$P = \frac{1}{1 + e^{-\beta_n X_n}} \quad (1)$$

which yields a linearized response, \dot{P} :

$$\dot{P} = \log\left(\frac{P}{1-P}\right) = \beta_n X_n + \varepsilon \quad (2)$$

where β is a regression coefficient for a corresponding explanatory variable and ε is stochastic error. Generalized linear models (GLM) using a logit link function and 'quasibinomial' error distribution were used to analyze proportional behavioral responses as a function of potential explanatory variables to best explain behavior responses and infer causation for variability in such behaviors. Proportion of behavior response for any time, P , was estimated through inclusion (and preclusion) of variables: test shark size, presence of a stimulus shark, side of the pen AMU units were on, and interactions, such that the full model may be represented as: $P \sim \text{Size} + \text{Treatment} + \text{AMU side} + \text{Interactions}$.

Variable inclusion was determined stepwise through deviance reduction using Wald's chi-square (χ^2). GLMs, model diagnostics and model selection were estimated using the R Computing Environment (R Core Team 2015).

RESULTS

Use of artificial mangrove structure

Test sharks paired with size-matched conspecifics spent a significantly smaller proportion of trial periods near AMUs than test sharks paired with predators ($p < 0.05$, PERMANOVA with post hoc pairwise comparisons; solitary trial [treatment 1]: $\bar{x} = 0.14 \pm 0.04$ SE; trial with size-matched conspecific [treatment 2]: $\bar{x} = 0.08 \pm 0.04$ SE;

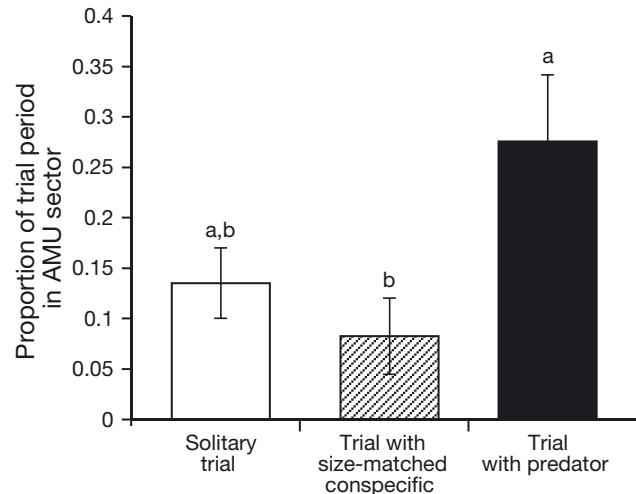


Fig. 2. Proportion of the trial period (mean \pm SE) test sharks spent near artificial mangrove units (AMUs) for each treatment. Different lowercase letters indicate statistical significance among treatments (PERMANOVA with post hoc pairwise comparisons, $p < 0.05$)

trial with predator [treatment 3]: $\bar{x} = 0.28 \pm 0.07$ SE; Fig. 2). However, while solitary test sharks spent less time near AMUs than when paired with a predator, the difference was not significant (Fig. 2). A priori analyses through the logit GLM approach examining salinity, temperature, depth and side of the pen in which AMUs were located inferred that those factors were not significant explanatory variables for either test sharks' AMU usage or displays of social behaviors ($p > 0.13$, Wald's χ^2). Test shark size was the primary variable explaining AMU usage ($p < 0.0001$, Wald's χ^2 ; Table 2). Treatment was also a significant explanatory factor in logit regression analyses ($p < 0.0001$, Wald's χ^2 ; Table 2). To examine specifically

Table 2. Results of logit generalized linear models explaining proportion of trial period using artificial mangrove units (AMUs) (P) as a function of test shark size (Size), treatment (Treatment) and interaction terms. Model structures included the collapse of the 3 experimental treatments into 2 categories, representing presence or absence of a predator (Large[0,1]). **Bold** type indicates best-fit model based on selection criteria

Logit regression model	df	De- viance reduced	Resi- dual df	Resi- dual de- viance	$p(>\chi^2)$
P	-	-	71	20.143	-
$P \sim \text{Size}$	1	5.2835	70	14.860	2.12×10^{-8}
$P \sim \text{Size} + \text{Treatment}$	2	3.724	68	11.136	1.57×10^{-5}
$P \sim \text{Size} + \text{Treatment} + (\text{Size} \times \text{Treatment})$	2	0.6991	66	10.437	0.1254
$P \sim \text{Size} + \text{Large}(0,1)$	1	3.3564	69	11.504	2.62×10^{-5}
$P \sim \text{Size} + \text{Large}(0,1) + (\text{Size} \times \text{Large}[0,1])$	1	0.079	68	11.425	0.5142

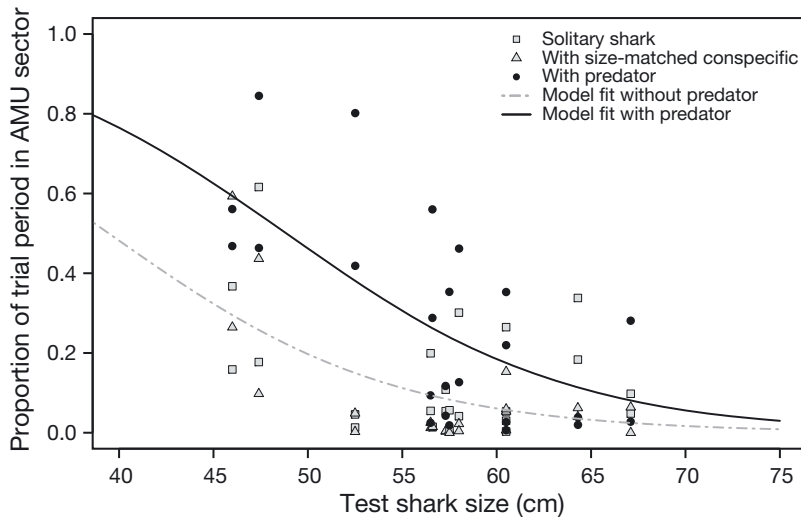


Fig. 3. Logit generalized linear model estimates of proportion of trial period test sharks spent using artificial mangrove units (AMUs) as a function of size (precaudal length), with presence or absence of a predator (large conspecific) as a factor

whether predator presence (treatment 3) was a significant factor driving AMU use, the 3 treatments were collapsed into 2, representing either presence (treatment 3) or absence (treatments 1 and 2) of a predator. Logit regression analyses adopting this approach determined that predator presence was a significant explanatory variable for AMU sector usage ($p < 0.0001$, Wald's χ^2 ; Table 2). While model deviance was lower when the 3 treatments were included as separate parameters, it was not reduced enough to warrant the extra degree of freedom and parameter estimate distinguishing the solitary treatment from the size-matched conspecific treatment (Table 2). Therefore, the model collapsing the 3 treatments into 2 statistical parameters (predator present or predator absent) was the most feasible. Hence, predator presence had an unequivocal effect on proportion of time test sharks spent near AMUs (Fig. 3).

Table 3. Results of logit generalized linear models explaining proportion of trial period exhibiting social behavior (P_{SOCIAL}) as a function of size (Size), presence/absence of a predator (Large[0,1]), trial (Trial) and interaction terms. **Bold** type indicates best-fit model based on selection criteria

Logit regression model	df	De- viance reduced	Resi- dual df	Resi- dual deviance	$p(>\chi^2)$
<i>P</i>	–	–	47	25.4111	–
<i>P</i> ~Size	1	2.3806	46	23.0304	0.000781
<i>P</i>~Size+Large(0,1)	1	14.4407	45	8.5897	$< 2.2 \times 10^{-16}$
<i>P</i> ~Size+Large(0,1)+Trial	1	0.0028	44	8.587	0.908604
<i>P</i> ~Size+Large(0,1)+(Size×Large[0,1])	1	0	44	8.5897	0.999186

Sociality and fright responses

Analyses implementing a logit GLM were used to test for significant effects of body size, predator presence or absence, and experimental trial on the proportion of trial time in which sharks exhibited sociality (Table 3). Test shark size ($p < 0.0001$, Wald's χ^2) and predator presence ($p < 0.0001$, Wald's χ^2) had profound significant effects on social behavior. Test sharks engaged in social swimming with size-matched conspecifics for much of the trial period, but did not do so with the predator (Fig. 4). Experimental trial, that is, in which side of the pen AMUs were situated, had no statistical significance ($p = 0.91$, Wald's χ^2) and contributed no bias to the results (Table 3). Interaction of body size and predator presence had no synergistic statistical effect ($p = 0.99$, Wald's χ^2) to explain sociality.

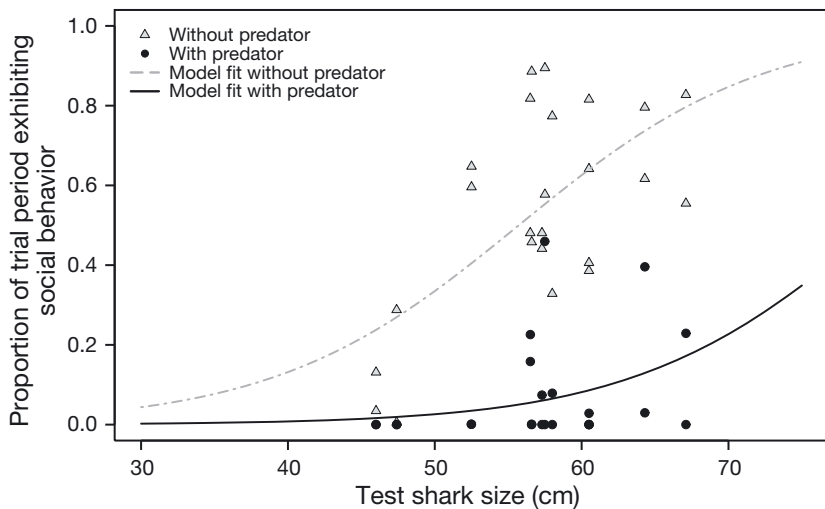


Fig. 4. Logit generalized linear model estimates of proportion of trial period test sharks engaged in social behavior as a function of size (precaudal length), with presence or absence of a predator (large conspecific) as a factor

Solitary test sharks (treatment 1) exhibited virtually no fright responses per trial period ($\bar{x} = 0.29 \pm 0.14$ SE), and test sharks paired with a size-matched conspecific (treatment 2) showed similarly low numbers of fright behaviors ($\bar{x} = 0.50 \pm 0.12$ SE; Fig. 5). However, when in the presence of the predator (treatment 3), test sharks demonstrated a significantly higher number of fright responses per trial ($\bar{x} = 7.92 \pm 0.19$ SE; PERMANOVA with post hoc pairwise comparisons, $p < 0.001$), with one individual peaking at 47 fright responses. Although the mean number of fright responses per trial in the presence of the predator (treatment 3) decreased between test sharks' first and second trial with the potential predator ($\bar{x}_{\text{trial1}} = 11.62 \pm 3.62$ SE, $\bar{x}_{\text{trial2}} = 4.15 \pm 0.88$ SE), no statistical significance was detected (Wilcoxon rank sum test, $p > 0.05$, $\log_{10}[x + 0.5]$ transformation).

DISCUSSION

This study examined the effects of perceived predation risk on the fine-scale habitat use of an elasmobranch in controlled experimental conditions. Results support the hypothesis that juvenile lemon sharks view larger conspecifics as potential predators, and this perception can lead to size-based patterns of habitat use. Test shark size and predator presence were significant drivers of AMU usage, and there was a negative relationship between body size and AMU use in the presence of a potential predator. When paired with the predator, smaller juveniles

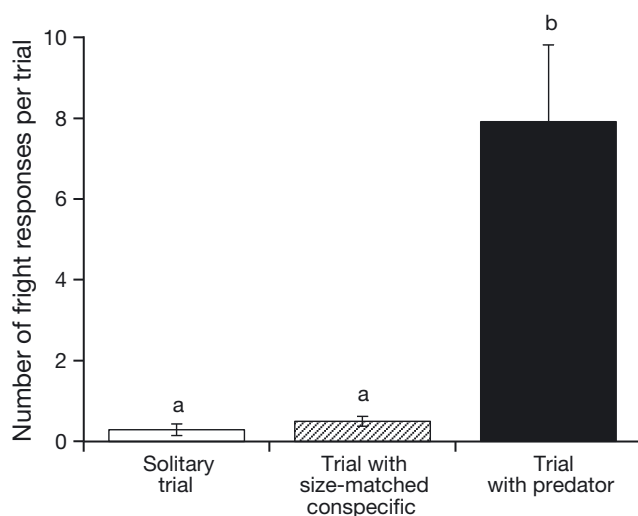


Fig. 5. Mean (\pm SE) number of fright responses test sharks exhibited per trial for each treatment. Different lowercase letters indicate significant differences among treatments (PERMANOVA with post hoc pairwise comparisons, $\log_{10}[x + 0.5]$ transformation, $p > 0.001$)

used the AMUs more than larger juveniles, indicating individual size-based sensitivity to predation risk. As social animals (Guttridge et al. 2009, 2011), juvenile lemon sharks exhibited a high degree of social swimming behaviors with size-matched conspecifics, but the presence of a large conspecific elicited fright responses.

Lima & Dill (1990) proposed that prey make habitat use decisions based on a combination of both prey abundance and predation risk. Since this early study, others have expanded on this concept, suggesting that predators' effects on prey habitat use are landscape-specific and vary with resource availability, predator abundance and habitat-specific escape tactics (Lima 1998, Heithaus et al. 2009). In our study, wild prey were physically excluded from the trial pen, and test sharks were fed to satiation to reduce confounding effects of foraging behavior while predation risk was manipulated within an experimental environment. No test shark was observed foraging within the trial pen during the experiment, nor did the large conspecific ever exhibit predatory behavior toward test sharks. Studies have shown that some prey are able to perceive whether a predator is in hunting mode and therefore a threat (see Preisser et al. 2007 for a review). Regardless of whether the test sharks in our experiment were able to make such a distinction, they did indeed seek the AMU structure more when the potential predator was present. Therefore, test sharks' habitat use was likely in response to individual perception of predation risk from the predator's presence.

One potential confounding issue is that the predator's use of the test pen could alter the test sharks' use of and/or access to AMUs. For example, if the predator were near the AMUs, test sharks might actively avoid the sector as a consequence of avoiding the predator. If the test sharks' movements reflect dynamic predator avoidance, refuge use may be confounded. However, despite varying predator position, including potential scenarios of the predator actively following a test shark or blocking access to AMUs, the models still show increased use of AMUs with a predator present, lending even more weight to our interpretation. During trials in the absence of a predator, the perceived risk of encountering a predator was as close to zero as possible in the semi-captive experimental setting. In the presence of a potential predator, however, the encounter was inevitable within the confines of the trial pen. As a result, fine-scale habitat use reflected the antipredatory behavioral decisions of each individual test shark to its own perceived risk of predation.

When paired with size-matched conspecifics, small juvenile lemon sharks spent less time in close proximity to the AMU refuge than when in the presence of a potential predator. The known advantages of grouping may explain why the proportion of time test sharks spent near AMUs was significantly lower when they were paired with a size-matched conspecific versus a large conspecific. Grouping behavior provides several advantages, including a decrease in individual risk to predation and earlier predator detection through shared vigilance (Krause & Ruxton 2002). Juvenile lemon sharks are known to be social in the wild, often forming small groups in mangrove inlets even when no predator is present (Guttridge et al. 2011). Therefore, the grouping behavior observed here may be responsible for the decrease in the small juveniles' use of the AMU refuge when paired with a size-matched conspecific in the form of a 'boldness' effect (Krause & Ruxton 2002). As there was no treatment pairing 2 size-matched juveniles with the predator, however, AMU use under such conditions is unknown. Interestingly, we did not detect a difference in time spent in proximity of the AMUs between our solitary test shark and predator treatments. In the wild, juvenile lemon sharks will often patrol the mangrove edge, but will rarely enter the complex root structures unless threatened or pursued by a predator (Guttridge et al. 2012). The reduced time spent in the AMU zone by the solitary shark could therefore reflect the typical patrolling behavior of a non-threatened juvenile lemon shark.

Test sharks spent more time engaged in social swimming with a size-matched conspecific than with a large conspecific (potential predator). Size-matched associations in lemon sharks are well known (Guttridge et al. 2009, 2011), and the high number of interactions observed in our study between size-matched conspecifics was expected. The nearly absent proximity between the test shark and the large conspecific, despite the small experimental enclosure, suggests avoidance likely due to a perceived predation risk. The existence of any period of sociality at all between test sharks and the large conspecific may reflect forced interactions within the confines of the 10 × 10 m trial pen during the 30 min trial period, or perhaps predator inspection behavior (Pitcher 1991).

Although there have been known occurrences of intraspecific predation on small juvenile lemon sharks by larger juveniles (Morrissey & Gruber 1993a,b), these interactions have rarely been observed in the wild. In Bimini, Guttridge et al. (2012) recorded 2 such wild predation attempts, and juveniles exhib-

ited strong fright responses on both occasions. In the present study, the high number of fright responses by small juveniles in the presence of a large conspecific, combined with the low (or nearly absent) levels of social swimming of test sharks with the large conspecific, suggest the Age-5 lemon shark was indeed considered to be a predation threat by the small juveniles up to Age-2 used in the trials.

In the wild, predator-prey interactions between small lemon sharks and larger conspecifics are likely to be short events (e.g. <60 s), ultimately resulting in 1 of 2 outcomes: consumption or escape. One limitation of our experimental setup is that it did not reflect this type of wild encounter, as both prey and predator were confined to a relatively small area and given 30 min to interact. It is likely that the prey, in our case, small lemon sharks, habituated to the predator, especially when we consider that no active attack or predatory type behavior by the large conspecific was observed. We found some possible evidence for such simple learning through a non-statistically significant reduction in fright responses by test sharks between their first and second trials with the predator. In contrast, no such evidence for habituation between trials was found for time spent in proximity to the AMUs during the predator treatment. One explanation for this might be that the small juvenile sharks were already habituated to the AMUs, having been exposed to them in previous trials prior to the predator treatment. Alternatively, the predator in this context was a more intense and dynamic stimulus, and in experiments on other taxa, such differences have been found to affect learning processes (Hollis 1984, Lieberman 1990).

Body size is an important determining factor for habitat use in numerous animal taxa (e.g. Werner et al. 1983, Stamps 1983, Wahle 1992). Prior to the present investigation, there have been few studies demonstrating a relationship between body size and antipredatory behavior in sharks. In one study, Guttridge et al. (2012) found strong correlations between juvenile lemon shark body size and time spent in a specific mangrove-fringed refuge area, where smaller juveniles used the refuge on more occasions and for longer periods than larger juveniles. Size-related refuge use may be due to a decrease in antipredator behavior with increased body size (Werner & Hall 1988, Bouskila et al. 1998). Our experimentally derived results support this idea, showing a negative relationship between body size and AMU refuge use in the presence of a predator. Increased AMU use by smaller juveniles in the presence of a large conspecific suggests a higher perception of

individual risk. Interestingly, there was no relationship between body size and either fright responses or sociality of test sharks within the predator treatment. That is, larger test sharks did not exhibit significantly more or fewer social behaviors or fright responses than smaller test sharks when the predator was present. This result may be due to small sample size, individual differences in experience with predators (as seen in other fishes; e.g. Mittelbach et al. 2014), or the fact that smaller sharks had fewer possible opportunities to interact with the predator due to spending more time seeking refuge among the AMUs. Despite these caveats, however, a significant negative relationship was indeed found between body size and AMU use. Therefore, juvenile lemon shark habitat use was strongly related to individual risk assessment, and staying near complex structure was a habitat-specific escape tactic used within the confines of the experimental configuration.

Mangrove prop roots are known to be important for predator avoidance in juvenile teleost fishes (Cocheuret de la Moriniere et al. 2004, Verweij et al. 2006, Nagelkerken & Faunce 2008), though they may actually be riskier at night (Hammerschlag et al. 2010). The present study is the first to use artificial mangrove structures to examine predator-prey interactions in a shark species. Here, the increased time spent in close proximity to AMUs by small sharks in the presence of a potential predator suggests that this type of structure might be an important habitat-specific refuge for juvenile lemon sharks, allowing them to avoid potential predators.

Several studies in Bimini investigating juvenile lemon shark habitat use have suggested sharks use the mangrove-fringed nursery areas during early development to avoid larger predatory sharks (Morrissey & Gruber 1993b, Franks 2007, Chapman et al. 2009). More recently, Guttridge et al. (2012) noted that wild juvenile lemon sharks dashed for shallow water and a mangrove inlet after detecting a larger conspecific. Our study provides experimental support for these observations in the wild, showing that body size and predator presence are significant drivers of habitat use. It further highlights the use of mangrove structure as a refuge and avoidance tactic for juvenile lemon sharks during encounters with potential predators.

Within Bimini, the North Sound is a 3 km² semi-enclosed lagoon naturally fringed by mangroves along its entire shoreline. It is an important nursery, where juvenile lemon sharks born within the area remain site-attached for their first several years of life (Morrissey & Gruber 1993a, Franks 2007, Chapman

et al. 2009). During all but the most extreme low tides, the North Sound mangrove shoreline provides complex subtidal habitats. Recently, the lagoon underwent large-scale anthropogenic mangrove removal via clear-cutting, dredging and filling, spanning approximately 37% (or 67 ha) of the mangrove-fringed shoreline within the nursery (Jennings et al. 2012). As a result, there was a significant reduction in availability of structurally complex subtidal habitat over a large area within the nursery. It is of critical importance to understand the full ecological function of these wetlands to best mitigate their loss.

Future studies on this topic should consider using a more realistic semi-natural set-up with a natural mangrove-fringed shoreline, as well as explore the interactions of size-matched juveniles together in the presence of a predator, the effects of learning, individual differences, timing of responses and behaviors within the trial period, but also shorter, more authentic predator-prey interactions. Our results underscore the importance of complex structural habitats as refugia for small lemon sharks. Further research is also warranted on the relationship between predator densities (and changes thereof) on mangrove habitat use and social behavior of juvenile sharks.

CONCLUSIONS

This study highlights the importance of subtidal mangrove structure as a habitat-specific escape option within a lemon shark nursery. It provides insights into mechanisms influencing ontogenetic habitat shifts out of natal nurseries and gives evidence that perceived predation risk is an important factor in determining the timing of these shifts. The negative correlation between body size and AMU use implies that juvenile lemon sharks are sensitive to individual predation risk based on size and assess accordingly the potential threat of a larger shark. The exact size and/or age difference at which a conspecific changes from a companion to a potential predator is unknown, but our results show that at least an Age-5 large juvenile lemon shark was perceived as a predation threat to up to Age-2 juveniles. Perceived predation risk was a driving force behind small-scale habitat use, and such behavioral decisions can have effects at local and larger scales. While the small scale of the experimental design may not fully explain population-level patterns, the results are instructive in that they help elucidate the underlying mechanisms driving such patterns. The sharks' use of artificial structures mimicking natural mangrove

shorelines has important management and mitigation implications, particularly in environments such as Bimini, where mangrove habitats have been and continue to be lost due to coastal development. Mangrove systems provide a wide variety of important ecosystem services (Barbier et al. 2011), and the present study strengthens the argument for conservation and restoration efforts. Natural habitats are desired for optimal ecosystem function, but if development does occur, it is important to consider restoration of the refuge function of mangrove shorelines, perhaps by requiring developers to add appropriate structural complexity to modified habitats.

Acknowledgements. This work was supported by grants from the Bimini Biological Field Station Foundation and the Guy Harvey Ocean Foundation. We thank the volunteers and staff at the Bimini Biological Field Station for their contribution. We thank the anonymous reviewers, whose thoughtful insights helped improve this manuscript. Thank you to Dr. E. A. Babcock for statistical support. This research was carried out under a permit from the Department of Marine Resources of the Commonwealth of The Bahamas.

LITERATURE CITED

- Barbier EB, Hacker SD, Kennedy C, Kock EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecol Monogr* 81:169–193
- Bouskila A, Robinson ME, Roitberg BD, Tenhumberg B (1998) Life-history decisions under predation risk: importance of a game perspective. *Evol Ecol* 12:701–715
- Boutin S (1995) Testing predator-prey theory by studying fluctuating populations of small mammals. *Wildl Res* 22: 89–100
- Chapman DD, Babcock EA, Gruber SH, DiBattista JD and others (2009) Long-term natal site-fidelity by immature lemon sharks (*Negaprion brevirostris*) at a subtropical island. *Mol Ecol* 18:3500–3507
- Clarke TA (1971) The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. *Pac Sci* 25:133–144
- Cocheret de la Moriniere E, van der Meij H, van der Velde G, Nagelkerken I (2004) What attracts juvenile coral reef fish to mangroves: habitat complexity or shade? *Mar Biol* 144:139–145
- Compagno LV (1984) FAO species catalogue, Vol. 4, Part 1. Sharks of the world: an annotated and illustrated catalogue of shark species known to date. FAO, Rome, p 250–655
- Creel S, Christianson D (2008) Relationships between direct predation and risk effects. *Trends Ecol Evol* 23:194–201
- 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
- Eberhardt LL, Garrott RA, Smith DW, White PJ, Peterson RO (2003) Assessing the impact of wolves on ungulate prey. *Ecol Appl* 13:776–783
- Faunce CH, Serafy JE (2006) Mangroves as fish habitat: 50 years of field studies. *Mar Ecol Prog Ser* 318:1–18
- Franks BR (2007) The spatial ecology and resource selection of juvenile lemon sharks (*Negaprion brevirostris*) in their primary nursery areas. PhD dissertation, Drexel University, Philadelphia, PA
- Grubbs RD (2010) Ontogenetic shifts in movements and habitat use. In: Carrier JC, Musick JA, Heithaus MR (eds) Sharks and their relatives II: biodiversity, adaptive physiology and conservation. CRC Press, Boca Raton, FL, p 319–350
- 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
- Gruber SH, de Marignac JRC, Hoenig JM (2001) Survival of juvenile lemon sharks at Bimini, Bahamas, estimated by mark–depletion experiments. *Trans Am Fish Soc* 130: 376–384
- Guttridge TL, Gruber SH, Gledhill KS, Croft DP, Sims DW, Krause J (2009) Social preferences of juvenile lemon sharks, *Negaprion brevirostris*. *Anim Behav* 78:543–548
- Guttridge TL, Gruber SH, DiBattista JD, Feldheim KA, Croft DP, Krause S, Krause J (2011) Assortative interactions and leadership in a free-ranging population of juvenile lemon shark *Negaprion brevirostris*. *Mar Ecol Prog Ser* 423:235–245
- Guttridge TL, Gruber SH, Franks BR, Kessel ST and others (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
- Hammerschlag N, Heithaus MR, Serafy JE (2010) Influence of predation risk and food supply on nocturnal fish foraging distributions along a mangrove–seagrass ecotone. *Mar Ecol Prog Ser* 414:223–235
- Hammerschlag N, Luo J, Irshick DJ, Ault JS (2012) A comparison of spatial and movement patterns between sympatric predators: bull sharks (*Carcharhinus leucas*) and Atlantic Tarpon (*Megalops atlanticus*). *PLOS ONE* 7: e45958
- Heithaus MR (2007) Nursery areas as essential shark habitats: a theoretical perspective. In: McCandless CT, Kohler NE, Pratt HLJ (eds) Shark nursery grounds of the Gulf of Mexico and the East Coast waters of the United States. American Fisheries Society, Bethesda, MD, p 3–13
- Heithaus MR, Dill LM (2002) Food availability and tiger shark predation risk influence bottlenose dolphin habitat use. *Ecol Soc Am* 83:480–491
- Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. *Trends Ecol Evol* 23:202–210
- Heithaus MR, Wirsing AJ, Burkholder D, Thomson J, Dill LM (2009) Towards a predictive framework for predator risk effects: the interaction of landscape features and prey escape tactics. *J Anim Ecol* 78:556–562
- Hollis KL (1984) Cause and function of animal learning processes. In: Marler P, Terrace HS (eds) The biology of learning. Dahlem Konferenzen. Springer, Berlin, p 357–371
- Jennings DE, DiBattista JD, Stump KL, Hussey NE, Franks BR, Grubbs RD, Gruber SH (2012) Assessment of the aquatic biodiversity of a threatened coastal lagoon at Bimini, Bahamas. *J Coast Conserv* 16:405–428
- Krause J, Ruxton GD (2002) Living in groups. Oxford University Press, Oxford
- Laegdsgaard P, Johnson C (2001) Why do juvenile fish

- utilise mangrove habitats? *J Exp Mar Biol Ecol* 257: 229–253
- Lieberman DA (1990) *Learning: behaviour and cognition*. Wadsworth, Belmont, CA
- Lima SL (1998) Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* 48:25–34
- ✦ Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- ✦ Mittelbach GG, Ballew NG, Kjelson MK (2014) Fish behavioral types and their ecological consequences. *Can J Fish Aquat Sci* 71:927–944
- ✦ Morrissey JF, Gruber SH (1993a) Home range of juvenile lemon sharks, *Negaprion brevirostris*. *Copeia* 425–434
- ✦ Morrissey JF, Gruber SH (1993b) Habitat selection by juvenile lemon sharks (*Negaprion brevirostris*). *Environ Biol Fishes* 38:311–319
- ✦ Myrberg AA, Gruber SH (1974) The behavior of the bonnethead shark, *Sphyrna tiburo*. *Copeia* 1974:358–374
- ✦ Nagelkerken I, Faunce CH (2008) What makes mangroves attractive to fish? Use of artificial units to test the influence of water depth, cross-shelf location, and presence of root structure. *Estuar Coast Shelf Sci* 79:559–565
- ✦ Newman SP, Handy RD, Gruber SH (2010) Diet and prey preference of juvenile lemon sharks *Negaprion brevirostris*. *Mar Ecol Prog Ser* 398:221–234
- ✦ Pitcher T (1991) Who dares, wins: the function and evolution of predator inspection behaviour in shoaling fish. *Neth J Zool* 42:371–391
- ✦ Preisser EL, Orock JL, Schmitz OJ (2007) Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. *Ecology* 88: 2744–2751
- R Core Team (2015) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. www.R-project.org/
- ✦ Rizzari JR, Frisch AJ, Hoey AS, McCormick MI (2014) Not worth the risk: apex predators suppress herbivory on coral reefs. *Oikos* 123:829–836
- ✦ Roff G, Doropoulos C, Rogers A, Bozec YM and others (2016) The ecological role of sharks on coral reefs. *Trends Ecol Evol* 31:395–407
- Snelson FF, Mulligan TJ, Williams SE (1984) Food habits, occurrence, and population structure of the bull shark (*Carcharhinus leucas*) in Florida coastal lagoons. *Bull Mar Sci* 34:71–80
- Springer S (1967) Social organization of shark populations. In: Gilbert PW, Matheson RF, Rall DP (eds) *Sharks, skates and rays*. Johns Hopkins University Press, Baltimore, MD, p 149–174
- ✦ Stamps JA (1983) The relationship between ontogenetic habitat shifts, competition and predator avoidance in a juvenile lizard (*Anolis aeneus*). *Behav Ecol Sociobiol* 12: 19–33
- ✦ Vaudo JJ, Heithaus MR (2013) Microhabitat selection by marine mesoconsumers in a thermally heterogeneous habitat: behavioral thermoregulation or avoiding predation risk? *PLOS ONE* 8:e61907
- ✦ Verweij MC, Nagelkerken I, de Graaff D, Peeters M, Bakker EJ, van der Velde G (2006) Structure, food and shade attract juvenile coral reef fish to mangrove and seagrass habitats: a field experiment. *Mar Ecol Prog Ser* 306: 257–268
- ✦ Wahle RA (1992) Body-size dependent anti-predator mechanisms of the American lobster. *Oikos* 65:52–60
- ✦ Werner EE, Hall DJ (1988) Ontogenetic habitat shifts in bluegill: the foraging rate-predation risk trade-off. *Ecology* 69:1352–1366
- ✦ Werner EE, Gilliam JF, Hall DJ, Mittelback GG (1983) An experimental test of the effects of predation risk on habitat use in fish. *Ecology* 64:1540–1548
- ✦ Yates PM, Heupel MR, Tobin AJ, Simpfendorfer CA (2015) Ecological drivers of shark distributions along a tropical coastline. *PLOS ONE* 10:e0121346

Editorial responsibility: Ivan Nagelkerken,
Adelaide, South Australia, Australia

Submitted: March 3, 2017; Accepted: May 7, 2017
Proofs received from author(s): June 21, 2017