

Planktonic predation risk varies with prey life history stage and diurnal phase

K. A. Kerr^{1,2,3,*}, A. Cornejo^{2,4}, F. Guichard^{1,3}, R. Collin^{2,3}

¹Department of Biology, McGill University, 1205 Docteur Penfield, Montréal, Québec H3A 1B1, Canada

²Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panamá, Republic of Panama

³McGill-STRI Neotropical Environment Option (NEO), Faculty of Science, McGill University, Montréal, Québec H3A 2T6, Canada

⁴Department of Biology and Chemistry, University of Bremen, Bibliothekstraße 1, Bremen 28359, Germany

ABSTRACT: Predation risk is believed to shape many aspects of the behavior, morphology and life history of marine organisms. The timing of synchronous larval release, postlarval migrations to adult habitat and diurnal vertical migrations are all considered adaptations to predictable variations in predation risk. However, despite the fact that predation risk is expected to vary predictably over time, this variation remains poorly understood for planktonic animals in the field. In this study, we conducted dock-based predation risk assays using tethered brine shrimp *Artemia franciscana*. We tested for the combined effects of prey life history stage and diurnal phase by measuring loss of adult and larval tethered individuals. We sampled during day and night, and during large and small amplitude tides on the Pacific coast of Panama. On the Caribbean coast of Panama and the Atlantic coast of Florida (where tidal amplitude is smaller), we sampled during day and night but did not test for an effect of tidal amplitude. Although predation risk differed between sites, the trends were the same at all 3 docks. Predation risk was significantly higher during the day than at night for larvae, whereas adults experienced the opposite trend in risk across the diurnal cycle, although the difference was not significant. Our results demonstrate a temporal gradient in planktonic predation risk across the diurnal cycle that depends on prey life history stage.

KEY WORDS: Predation risk · Plankton · Diurnal cycle · Size-dependent predation · Plankton tethering unit · Predator-prey interaction · Size refugia

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Behavioral, morphological and life history adaptations to reduce predation risk are widespread. Coloration patterns, body shape and protective armor or spines may all reduce predation, and some of these morphologies are induced merely by the perception of risk (Reimchen 1994, Morgan & Christy 1995, Werner & Anholt 1996, Morgan & Christy 1997, Bergstrom & Reimchen 2003, Hylander et al. 2012). Behaviorally, organisms respond to predation risk by adjusting foraging, courtship and selectivity of mates (Lima & Dill 1990, Berglund 1993, Sih 1994, Lima &

Bednekoff 1999, Kim et al. 2009). Further, many behaviors related to the timing of life history events such as synchronous spawning or larval release, timing of postlarval return to adult habitat and diurnal vertical migrations are considered adaptations to reduce predation risk, particularly by planktivorous fishes (Lampert 1989, Acosta & Butler 1999, De Robertis et al. 2000, Christy 2011). This diversity of responses to predation emphasizes its importance in shaping the lives of organisms.

Despite their importance, estimates of predation rates or overall mortality for plankton are still rare due to the difficulty of studying plankton mortality *in*

situ (Vaughn & Allen 2010). In addition, the few estimates that are available vary drastically from 2% to almost 100% of the population per day, with an average of 23% per day across 30 studies (Rumrill 1990, Morgan 1995, Vaughn & Allen 2010). Planktonic predation estimates near 0% were reported for natural plankton densities and communities studied in *in situ* corrals; however, only invertebrate planktonic predators were present (Johnson & Shanks 2003). On the other hand, predation rates exceeded 80% for live tethered prey when planktivorous fish were present (Acosta & Butler 1999, Bullard & Hay 2002, Motro et al. 2005, Allen & McAlister 2007, Bullard & Whitlatch 2008). Although these and other studies have provided much needed information on variation in predation risk, they have each focused on one environmental cycle (diurnal, lunar or seasonal), or on comparisons across habitats or prey sizes. Here, we measured predation risk at 3 tropical and subtropical sites to test for common influences of life stage category and diurnal state (day vs. night) on patterns of predation risk.

Many zooplankton behaviors that appear to have evolved to reduce predation risk are adjustments to timing of activities with respect to the diurnal cycle. Planktivorous fishes are visual hunters, and many of the most common nearshore species feed during the day (Morgan 1990). At night, when diurnal fish are not feeding, zooplankton migrate to surface waters, larvae are released and postlarvae migrate to adult habitats (Lampert 1989, Morgan & Christy 1995, Christy & Morgan 1998). In particular, the larvae of nearshore benthic invertebrates must pass through high densities of nearshore planktivores as they enter the plankton, and again when they return to settle in their adult habitat. Many carry out these migrations at night. The idea that planktonic animals are safer under the cover of darkness has been supported by some direct comparisons of risk during day and night. In a study using tethered brine shrimp, night trials were used as controls and resulted in 0% predation compared to up to 62% predation during the day (Motro et al. 2005). However, significant levels of predation at night have been reported from some nearshore habitats for large planktonic prey (i.e. postlarval spiny lobster; Acosta & Butler 1999, and polychaetes, decapods, and amphipods with an average size of 3.5 mm; Holzman & Genin

2003). Thus, we hypothesize that predation risk will be lower at night, but this temporal refuge may diminish as plankton grow. Indeed, we demonstrate that temporal variation in risk from day to night differs for different life stages of prey.

MATERIALS AND METHODS

Predation risk assays using tethered prey items

We deployed tethered prey from docks to assess patterns of relative predation risk. Since tethered prey cannot evade predation attempts, tethering studies do not measure actual mortality rates but instead measure risk of predation attempts. Thus, while our interest is in better understanding relative rates of mortality between day and night and different prey size and life history categories, here we refer to predation risk rather than mortality. Plankton tethering units (PTUs; Fig. 1) baited with brine shrimp allowed us to use a standardized method and prey item to assess relative predation risk across environmental cycles at several sites (Bullard & Whitlatch 2008). PTUs have been previously tested for artifacts including predator deterrence or attraction, hindrance of prey consumption by predators, loss of prey items not caused by predation, and the ability of prey to evade predation. PTUs were found to be free of these artifacts, with the exception of evasion of predators by prey, and were shown to be an effective method to assess relative predation pressure (Bullard & Hay 2002).

Brine shrimp *Artemia franciscana* were selected as prey to allow for standardized comparisons across areas that do not have the same plankton species.

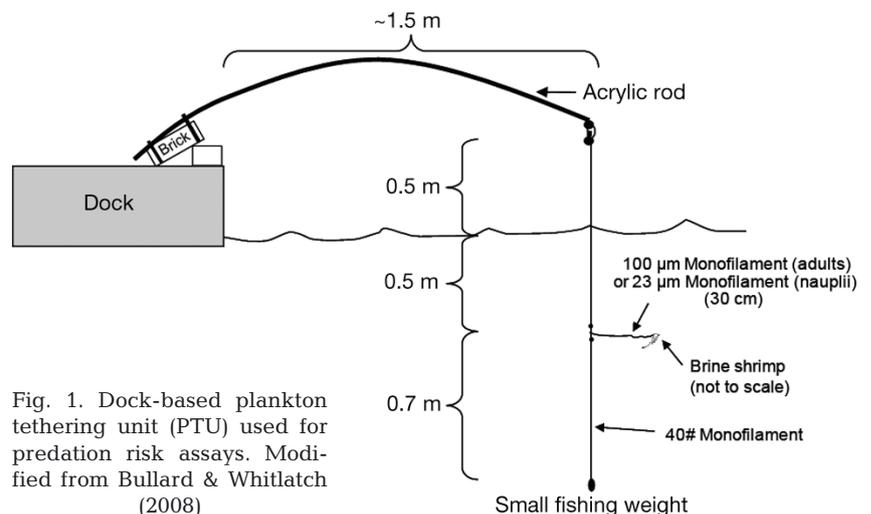


Fig. 1. Dock-based plankton tethering unit (PTU) used for predation risk assays. Modified from Bullard & Whitlatch (2008)

Adults and larvae (nauplii) were used to test our hypothesis that predation risk differs with life history stage. Brine shrimp were raised from eggs from a single supplier, except for assays conducted in Florida where adult brine shrimp were purchased from an aquarium supply store. Nauplii (2–4 d old) were used as our small prey category or life history stage (mean \pm SD: 0.62 ± 0.103 mm, $n = 80$ measured) (Fig. 2). Brine shrimp nauplii are similar in size to other newly hatched crustacean larvae, including crab zoea (Fig. 2). Sexually immature adults were used as our large prey category (avg. length = 3.7 ± 0.42 mm, $n = 209$) (Fig. 2) and are similar in size to some postlarval invertebrates that would be returning to nearshore habitats to settle and metamorphose. As brine shrimp grow, they not only increase in size but also undergo metamorphic changes resulting in morphological differences between larvae and adults. Such morphological changes with ontogeny are common among marine invertebrates, particularly crustaceans that have planktonic larvae, and thus changes in risk that accompany these changes in both morphology and size are also likely to be common.

Dock based predation risk assays

PTUs (Fig. 1) were each equipped with either an adult or nauplius brine shrimp *Artemia franciscana* and were deployed from docks. Methods for constructing and deploying the PTUs and for gluing the brine shrimp to tethers follow Bullard & Whitlatch (2008) and Bullard & Hay (2002). Briefly, in the laboratory, we placed live brine shrimp on a small screen made of plankton mesh and, with the aid of a dissecting microscope, glued each one to a 30 cm length of monofilament with cyanoacrylate glue (Loctite Super Bonder or Krazy Glue Gel). Different sized monofilament was used for each prey category: adults were glued to ~ 100 μ m invisible thread (available from sewing supply stores) and, due to their small size, nauplii were glued to 23 μ m diameter PET (polyester) micro-monofilament (Biogeneral Advanced Fiber Technology). Glued prey items were then placed in individual vials containing seawater and transported a few hundred meters from the laboratory to the dock for deployment.

Tethers made from nylon fishing line were attached to 2 m long acrylic rods extending ~ 1.5 m away from the edge of the dock (Fig. 1). The 30 cm monofilaments to which the *Artemia* were attached were located approximately 0.5 m below the water



Fig. 2. A tethered *Artemia franciscana* adult and nauplius and a recently hatched fiddler crab larva *Uca deichmanni* for size comparison

surface once deployed. Seven to 10 PTUs of each prey category were deployed 1.5 or 2 m apart in an alternating pattern beginning shortly after high tide. After 30 min, PTUs were recovered and each one was scored for absence or presence of the prey item. In some cases, partial corpses of the adults were recovered, providing clear evidence of predation. These instances were scored as absent. For 8 assays during which 77 adults were deployed, 3 partial corpses were recovered (4%). To determine if brine shrimp were alive and active during deployment, we scored adults as either alive or dead at the end of the deployment during a portion of our assays. 92% of recovered individuals were alive ($n = 771$).

Study sites

We sampled at 3 docks: the Smithsonian Tropical Research Institute's (STRI) Naos Marine Laboratories in the Bay of Panama (Panama, Pacific coast, $8^{\circ} 55.041' N$, $79^{\circ} 31.978' W$; hereafter Naos), at STRI's Bocas del Toro Research Station on Colon Island in Bocas del Toro (Panama, Caribbean coast, $9^{\circ} 21.066' N$, $82^{\circ} 15.444' W$; hereafter Bocas), and at the Smithsonian Marine Station (SMS) in Fort Pierce,

Florida (USA, Atlantic, 27° 27.360' N, 80° 18.570' W; hereafter Florida). These docks are located in different oceanographic conditions, biogeographic provinces and habitat types. We thus tested the effect of our treatment on predation risk within docks, and compared resulting patterns across docks without attempting to attribute differences in risk to particular characteristics of the sites.

Pacific: Isla Naos, Bay of Panama, Panama

On the Pacific coast of Panama, deployments were conducted during the rainy seasons between 4 August 2010 and 28 December 2011, when temperature and productivity are fairly constant and temperature averages ~28 or 29°C (D'Croz & O'Dea 2007, Robertson et al. 2009). Water clarity is variable (Table 1); tidal amplitude is large (Table 1) and tides are semi-diurnal. To test for an effect of tidal amplitude, we sampled during both diurnal and nocturnal high tides on 3 d during large amplitude tides and 3 d during small amplitude tides. The floating dock at Naos is located over muddy-sand habitat with rocky boulders located slightly inshore. Artificial lighting over the dock is present at night but was turned off during sampling. Streetlights located 50 m from the dock resulted in some artificial light even during sampling.

Caribbean and Atlantic: Bocas del Toro, Panama and Fort Pierce, Florida

At our other 2 sites where the range in tidal amplitude is small (Table 1), deployments were conducted daily at high tide for 3 to 4 wk. At Bocas, the dock is fixed and is located in a shallow, sheltered bay over a *Thalassia* seagrass bed. The shore is lined with red mangroves *Rhizophora mangle*. Water clarity is high, and the bottom was visible during all daytime assays (Table 1, Kaufmann & Thompson 2005). Tides are

mixed semidiurnal. Oceanographic conditions are relatively stable throughout the year with high temperature and low productivity (Kaufmann & Thompson 2005, Collin et al. 2009, Robertson et al. 2009). Physical monitoring data is available at http://biogeodb.stri.si.edu/physical_monitoring/research/bocas. Artificial lighting at the dock was turned off during assays.

At Fort Pierce, the dock is fixed and is located over muddy-sand substrate mixed with seagrass. Tides are semidiurnal. This site experiences seasonal changes in temperature and productivity; however, we sampled during summer when temperature was high and relatively constant (Table 1; physical monitoring data available at <http://nrmnhmp.riocan.com/site.php?siteIndex=1>). Artificial light is present at the dock at night.

Controls

Previous studies have demonstrated that losses of tethered prey items as a result of causes other than predation are rare (Bullard & Hay 2002, Motro et al. 2005). The small size of our prey items precluded direct observations of tethers during deployment, which would allow for a precise measurement of losses due to predation. Thus, prey losses in the field presented here are the result of predation events and other causes such as prey falling off the tethers. All methods to account for non-predation losses in the field aside from direct observations would have altered the flow regime around the tethers. Therefore, we tested for bias in losses resulting in different rates of the 2 prey categories falling off the tethers by deploying tethered prey in the lab. Control trials were conducted in a 90 × 90 × 30 cm water table in the outdoor aquarium area at STRI's Naos Marine Laboratories. Tethers, spaced 12 to 15 cm apart, were hung from 3 rods that spanned the width of the water table. Eight adults attached to 100 µm tethers and 8 nauplii

Table 1. Sampling dates and conditions at each site during sampling. Tidal amplitude and water temperature values are averages (±SD). Water depth (at high tide) and Secchi disk depth values are ranges

Site	Season	Sampling dates	Tidal amplitude (m)	Water temperature (°C)	Water depth (m)	Secchi disk depth (m)	Dock size (m)
Bocas	Wet	6–Oct–10 to 8–Nov–10	0.33	29.3 ± 0.51	1.5	0–bottom	3.5 × 10.5
Florida	Summer	13–Jul–11 to 2–Aug–11	0.44	29.2 ± 1.57	2.5–3	1.5–bottom	3 × 17
Naos	Wet	4–Aug–10 to 6–Dec–10 20–May–11 to 28–Dec–11	4.01 (range 2.03 to 6.37)	28.7 ± 0.82	5.5–9.5	0.5–>5 (bottom never visible)	7 × 21

attached to 23 μm tethers were deployed for 30 min during each trial. The spatial arrangement of adults and nauplii for each trial was determined using a random number generator. Prey items rarely fell off their tethers (average proportion lost: adults = 0.027, SD = 0.0566, nauplii = 0.075, SD = 0.0874; range of ind. lost trial⁻¹: adults = 0 or 1 out of 8 deployed, nauplii = 0 to 2; total losses: adults = 2 of 78, nauplii = 6 of 80) and we found no significant difference in losses between prey categories ($n = 10$ trials, generalized linear model [GLM, binomial] $p_{\text{prey}} = 0.15$).

We tested for a bias in predation risk due to the thread diameter used for each prey category in our regular assays (100 μm for adults and 23 μm for nauplii). We conducted 8 trials for each prey category. Assays were conducted with one prey type attached to both monofilament thicknesses, and were carried out on consecutive days during the daytime high tide at the Naos dock. Each assay consisted of the deployment of 5 to 10 PTUs with 100 μm monofilament and 5 to 10 PTUs with 23 μm monofilament. All other methods followed those described above for our predation risk assays. For the nauplii, losses did not differ significantly between the monofilament types (100 μm : avg. proportion lost = 0.215, SD = 0.103; 23 μm : avg. proportion lost = 0.168, SD = 0.127; $n = 8$ trials; Wilcoxon signed rank test, paired, $p = 0.35$). For the adults, losses were lower with the 100 μm monofilament than with the 23 μm monofilament (100 μm : avg. proportion lost = 0.09, SD = 0.186, 23 μm : avg. proportion lost = 0.25, SD = 0.146). The difference was nearly significant ($n = 8$ trials, Wilcoxon signed rank test, paired, $p = 0.051$), potentially indicating that either the adults were not securely held by the thinner monofilament or that predators were more likely to attack adult brine shrimp attached to the thinner monofilament. These results demonstrate that the generally higher rate of predation on adults attached to 100 μm monofilament compared to nauplii attached to 23 μm monofilament that we observed during our regular assays (see 'Results') is not due to thread diameter.

Data analysis

Statistical analyses were conducted in R (version 2.14.1; www.R-project.org). Counts of prey items lost and recovered were analysed using GLMs using a binomial error distribution and logit link function. Predictor variables included diurnal phase (day vs. night), prey life history stage category (adults vs. nauplii), site (Naos, Bocas, Florida) and their inter-

actions. Naos data were also analysed separately to examine the effects of diurnal phase, prey category (adults vs. nauplii), tidal amplitude and interactions. Overdispersion was detected, so standard errors were corrected using a quasi-GLM (Crawley 2007, Zuur et al. 2009). Model selection was conducted by comparing nested models with and without individual predictor variables or their interactions using F -tests (Crawley 2007). Only the reduced model is presented. Multiple pairwise comparisons were made using Tukey tests in the 'multcomp' package in R (Hothorn et al. 2008). Since the number of prey items deployed differed among assays, proportion of prey items lost (hereafter 'risk') is presented in all figures.

Counts of prey items lost and recovered with respect to water visibility (as measured by Secchi disk depth) were analysed using generalized additive models (GAMs) with a binomial error distribution and logit link function. Secchi disk data were collected throughout sampling only at Naos and Bocas. At Bocas, the bottom was visible during the day during all samples (Table 1). Thus, only samples collected at night at Bocas and during the day and night at Naos were included in the analysis.

RESULTS

Patterns of risk consistently differed between the prey life history stage categories ($p_{\text{prey}} < 0.001$; Fig. 3, Table 2) despite differences in overall risk among sites ($p_{\text{site}} < 0.001$; Table 2). Further, a significant interaction between diurnal phase and prey was observed ($p_{\text{diurnal:prey}} < 0.001$; Fig. 3, Table 2). More specifically, risk was significantly lower for nauplii at night than for nauplii during the day (multiple comparison Tukey test, $p = 0.03$) and was lower than for adults during both day ($p < 0.01$) and night ($p < 0.01$). Risk for adults did not differ significantly between day and night ($p = 0.99$). We expected that if non-predation losses were significant in the field, the number of losses would increase with the strength of the tidal current; however, tidal amplitude had no significant effect on the proportion of prey items lost and thus was not included in the final model. Our experiments indicate that nauplii experienced a small-size or early life stage refuge at night, and that this refuge is consistent across all 3 geographical locations of our study.

To examine the possibility of size- or life stage-selective predation, we plotted the proportion of adults lost versus the proportion of nauplii lost for each assay. During the day, we found no consistent

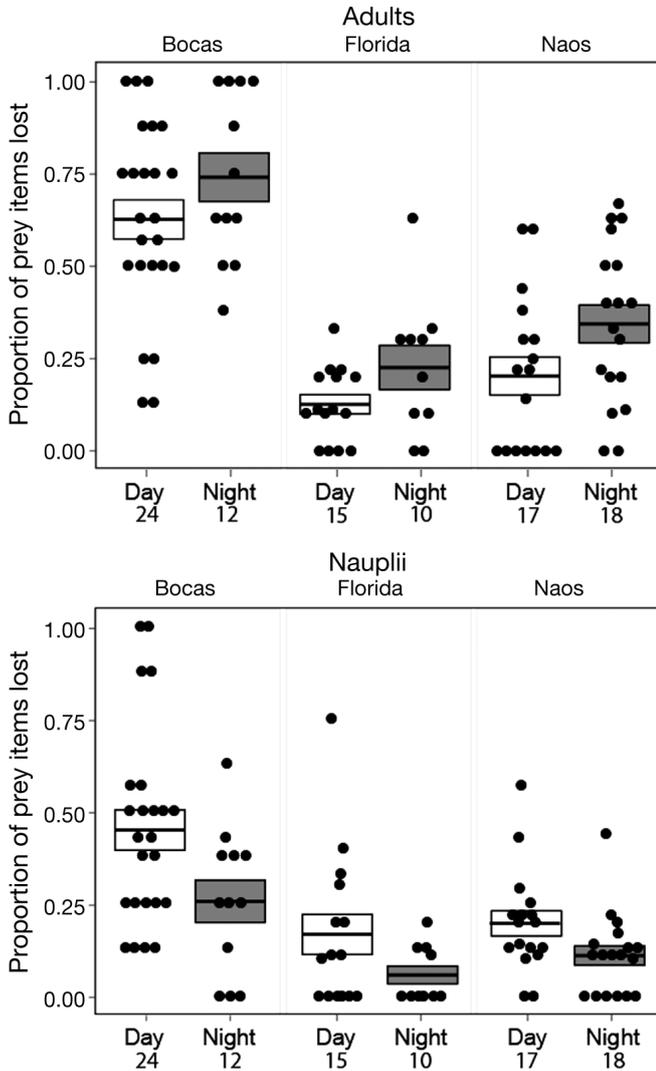


Fig. 3. Contrasting patterns in proportion of *Artemia franciscana* prey lost across the diurnal cycle for the 2 prey categories (nauplii vs. adults) at 3 sites. Boxes represent the mean (center line) and standard error for each group. Shading of the boxes represent diurnal state (white = day, gray = night). Circles are individual data points. Sample sizes (number of assays) are provided at the bottom of the graph. Significant effects of site, prey category and the interaction between diurnal state and life stage were detected (see Table 2). Risk for nauplii at night was significantly lower than risk during the day, or for adults during the day or night

directional loss of prey items with respect to their life stage category at any of the sites (Fig. 4). In contrast, at night, a consistently larger proportion of adults compared to nauplii were lost at all 3 sites (Fig. 4).

If the dominant predators at a site select their prey using vision, there may be a relationship between risk and water visibility. Secchi disk data demonstrate a non-linear significant increase in risk for adults with increasing visibility during the

Table 2. Analysis of deviance table for generalized linear models of prey items lost (deployed – recovered, recovered) using a binomial error distribution adjusted for overdispersion (quasi-binomial)

Model term	df	De- viance	Residual df	Residual deviance	F	p
NULL			191	674.69		
diurnal	1	2.88	190	671.81	1.63	0.203
prey	1	48.60	189	623.21	27.52	<0.001
site	2	206.79	187	416.42	58.55	<0.001
diurnal:prey	1	30.43	186	385.99	17.23	<0.001
prey:site	2	9.81	184	376.19	2.78	0.065

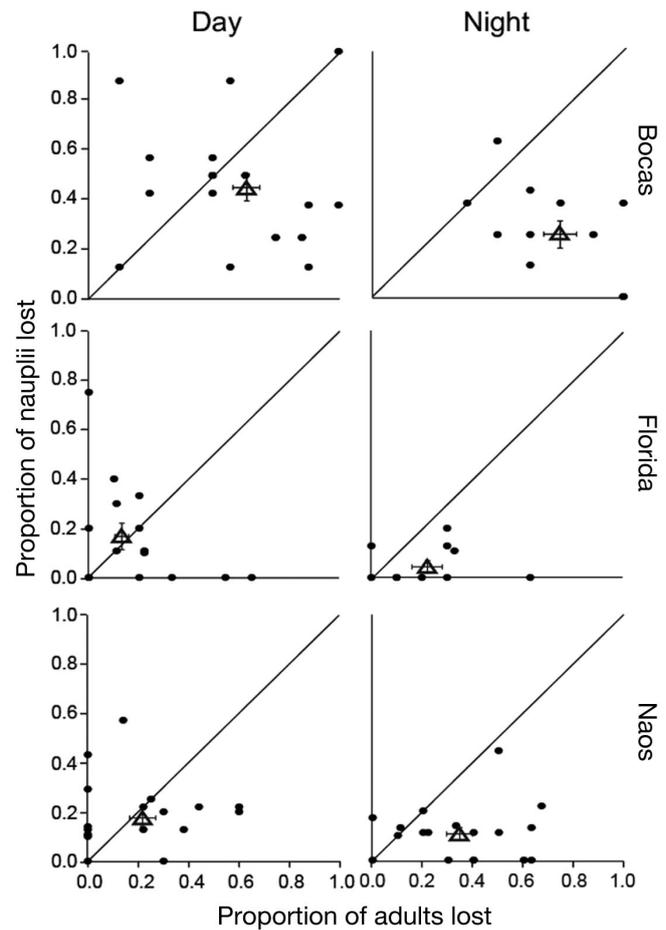


Fig. 4. Proportion of *Artemia franciscana* nauplii lost with respect to proportion of adults lost per assay during day and night at the 3 sites. The diagonal line represents the line of equality, or 1-to-1 relationship between these proportions. Black circles represent individual assays. Open triangles and error bars represent average and standard error of all assays at that site for that diurnal phase. Error bars that are not visible are smaller than the triangle. Points above the line indicate a higher loss of nauplii relative to the loss of adults, while points below the line indicate a higher loss of adults relative to nauplii

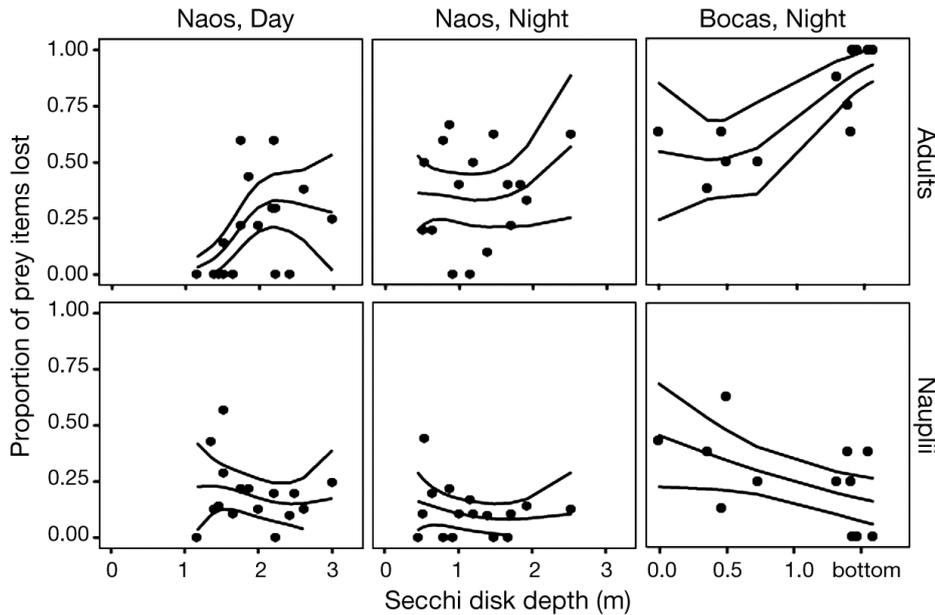


Fig. 5. Proportion of prey items lost relative to Secchi disk depth measurements at Naos during the day and night, and at Bocas at night. Daytime at Bocas was not included because the bottom was visible during all assays. Relationships were fitted with generalized additive models with a binomial error structure. A significant effect of Secchi disk depth was found for adults during the day at Naos ($p = 0.02$), adults at night at Bocas ($p = 0.001$) and nauplii at night at Bocas ($p = 0.03$)

day at Naos and at night at Bocas (Fig. 5, Table 3). At Naos during the day, the relationship was strongest between 1 and 2 m visibility. Increased visibility beyond 2 m did not result in increased risk. At Bocas at night, loss of adult prey was 100% when the Secchi disk was still visible once it reached the bottom (visibility of 1.5 m or greater). For nauplii, risk decreased with increasing visibility at Bocas at night, but no significant relationship was detected at Naos (Fig. 5, Table 3). However,

the significant effect of visibility on risk for adults at Naos during the day ($p = 0.02$) and for nauplii at Bocas at night ($p = 0.03$) should be interpreted with caution. p -values for smoothed factors in GAMs are approximate and may be underestimates when smoothing parameters are uncertain, thus p -values less than 0.001 or larger than 0.2 can be trusted, but smoothers with p -values near 0.05 cannot be confidently assigned as significant or not (Zuur et al. 2009).

Table 3. Generalized additive model (GAM) fits to lost prey (deployed – recovered, recovered) with respect to water visibility (Secchi disk depth), prey life stage category and diurnal phase (Naos only) using a binomial error distribution. Smoothed variables are denoted by $s(\text{variable})$. Effective degrees of freedom (edf), a representation of the level of nonlinearity, is provided for smoothed variables (linear = 1, nonlinear > 1). p -values for smoothed variables are approximate (Zuur et al. 2009), so values between 0.1 and 0.01 should be interpreted with caution (denoted by *italics*). For Bocas, daytime assays were excluded since the bottom was always visible

	Model term	df/edf	χ^2	p
Naos (day & night)	diurnal	1	12.99	<0.001
	prey	1	2.92	0.086
	diurnal:prey	1	13.58	<0.001
	<i>s(Secchi:adults:day)</i>	1.86	8.38	0.021
	<i>s(Secchi:adults:night)</i>	2.36	1.76	0.604
	<i>s(Secchi:nauplii:day)</i>	1.90	0.37	0.890
	<i>s(Secchi:nauplii:night)</i>	1.91	1.24	0.622
Bocas (night only)	prey	1	40.31	<<0.001
	s(Secchi:adults)	1.90	14.19	0.001
	s(Secchi:nauplii)	1.00	4.52	0.033

DISCUSSION

Responses to predictable spatial, temporal and ontogenic patterns of predation risk allow organisms to balance risk with daily needs and life history events such as reproduction and recruitment. Differences in risk from day to night or with size are fairly well documented, but for marine plankton, data on how risk varies across both (potentially interacting) variables are still relatively sparse. Here, we demonstrate that patterns of risk across environmental cycles can change or reverse during ontogeny: the temporal refuge experienced by nauplii at night was not observed for adult brine shrimp.

Our results for *Artemia* nauplii support the hypothesis that planktonic predation risk is higher during the day than at night. Many behaviors of marine organisms are adjustments to reduced predation risk at night. Zooplankton make diurnal vertical migrations away from the

surface waters to escape higher predation pressure during the day, and some only carry out activities such as foraging and molting at night (Ohman 1988). Numerous intertidal crab species release their larvae at night during large amplitude ebbing tides, and species that do so tend to be less morphologically protected from visual predators than species that do not release larvae at these times (Morgan & Christy 1995, Christy 2011, Kerr et al. 2012, Kerr 2012). Similarly, some species that must cross areas with high concentrations of planktivorous fishes upon their return to adult habitats, do so during large amplitude nocturnal flood tides, in some cases only during the new moon (Christy & Morgan 1998, Acosta & Butler 1999). These are all examples of responses to diurnal patterns in risk that are driven by temporal patterns in activity and foraging by predators (Ohman 1988). The most abundant fishes in nearshore habitats are often planktivorous fishes that are active during the day (Baxter 2004, Dominici-Arosemena & Wolff 2005, 2006). Evidence from stomach contents, abundance surveys and tethering experiments have shown that several of the most common fish species found in estuaries and on reefs exert higher predation pressure during the day (Morgan 1990, Clark et al. 2003, Motro et al. 2005, Yahel et al. 2005a). Thus, in nearshore habitats, diurnal predation pressure by planktivorous fishes can be significant.

Adult brine shrimp did not experience a predictable temporal refuge from predators. Risk for adults did not significantly differ between day and night, and adults were more likely to be eaten than nauplii during nighttime assays. One important change associated with ontogeny of many planktonic larvae is body size. It is important to consider whether the differences in risk between the prey life stage categories are the result of size/life stage-selective predation during individual assays or if risk simply varies among assays. A directional bias in loss towards one prey life stage category during most assays — as we found here for adults at night — may indicate size or stage-selective predation. Size-specific predation has been well documented in terrestrial, freshwater and marine habitats, but the direction of size selectivity of prey depends on the type of predator (Rumrill 1990, Allen 2008). Many predators select smaller prey items, and much of the size-selective predation literature supports the 'bigger is better' hypothesis for predator avoidance (Sogard 1997, Pechenik 1999, Allen 2008). However, the idea that 'bigger is better' has been challenged by other studies (Litvak & Leggett 1992, Scharf et al. 2000). If the dominant predators are fish, larger zoo-

plankton may be at higher risk than smaller plankton throughout the diurnal cycle since diurnally feeding planktivorous fish select for larger prey if available (Hobson 1991, Allen 2008), and nocturnal planktivorous fish cannot detect small plankton. Nocturnal planktivorous fishes are visual predators that require minimal light levels for foraging (Hobson 1975, Holzman & Genin 2003, Holzman et al. 2007). However, in contrast to diurnal planktivores, nocturnal planktivores have low visual resolution and therefore can only detect relatively large (>1 mm) prey items (Holzman & Genin 2003, 2005). Consequently, smaller plankton are less vulnerable to predation by planktivorous fishes at night.

Previous nearshore studies have documented relatively high levels of nocturnal predation risk for large plankton, but risk is variable with habitat and location within the water column. Dungeness crab *Cancer magister* postlarvae tethered in the plankton were at relatively low, but similar risk, during the day and night, but experienced significantly higher risk at night compared to during the day when tethered in a benthic habitat (Allen & McAlister 2007). Acosta & Butler (1999) recorded mortality of up to 80% for spiny lobster *Panulirus argus* postlarvae attached to drifting tethers at night. Thus, significant risk of predation at night, as we observed here for adult brine shrimp, may be more common than is generally appreciated.

The size bias in prey detection by nocturnal planktivorous fish may explain the significant difference in nocturnal predation risk between nauplii and adult brine shrimp and the evidence for bias in risk towards adults at night at all 3 sites. An interaction between size and diurnal variation in predation risk appears to drive the timing of diurnal vertical migrations of different plankton size categories among and within species. The general pattern is that smaller plankton come to the surface earlier and descend later apparently due to lower risk at night relative to larger plankton (Alldredge & King 1980, De Robertis et al. 2000, Yahel et al. 2005b). However, the effects of differential predation risk on the timing of vertical migration have been inferred from differences in timing for different size classes and, in some cases, have been related to fish abundance and behavior. Our results support these studies with direct measures of relative predation risk across the diurnal cycle.

Size preferences and activity patterns of the community of predators are expected to drive diurnal patterns of risk via size-dependent predation. Our results point towards a dominant influence of visual predators that select for larger prey. The lowest over-

all levels of risk were for nauplii at night; a time period when visual predators would be least able to detect these small prey. Adults were at higher risk than nauplii regardless of diurnal phase. Our Secchi disk results indicate that risk for adults increased with water visibility up to about 2 m. Reaction distances by fish towards prey >1 mm in length tend to be on the order of 10s of cm rather than metres (Walton et al. 1997, Holzman & Genin 2003). It therefore makes sense that visibility increases of more than 1 or 2 m would not increase prey detection and predation risk. We found no increase in risk with visibility for nauplii; however, given their small size and the tendency for reaction distances by fishes to increase with prey size, it is unlikely that visual predators would be targeting these prey from more than a few centimeters away. In fact, at night at Bocas, risk for nauplii decreased with increased visibility. Further, the assays during which visibility was the highest coincided with the largest bias in risk towards adults.

While we do not know the identities of the predators of our tethered prey, planktivorous fishes were present near the docks at our sites during both day and night. Sergeant majors *Abudefduf troschelii*, diurnal planktivores, were consistent residents near the dock at Naos and Bocas. Silversides, a group of species of small schooling fish, were observed at all sites. Many species of silversides are planktivorous and can eat plankton the size of brine shrimp (Skibinski 2005, Holzman et al. 2007). At least one of the species of silversides found in the waters of each of our study sites is nocturnal (Froese & Pauly 2011). Potential predators that are both non-visual and are present at our sites include chateognaths and cnidarian medusa. Chateognaths are abundant and ubiquitous predatory plankton that are regularly found in plankton samples at Naos (K.A.K. & R.C. pers. obs.). These animals detect prey based on vibrations, enabling them to feed regardless of light conditions, and some authors have reported that they feed primarily at night (Feigenbaum & Reeve 1977, Feigenbaum 1991). However, chateognaths are gape-limited and tend to prefer smaller prey, and are therefore more likely to feed on our small prey category (Canino & Grant 1985, Allen 2008, Lie et al. 2012). Given the low rates of predation on nauplii relative to adults particularly at night, non-visual predators did not appear to play a strong role in the risk of predation during our sampling.

Tethering studies cannot provide quantitative estimates of true risk to natural prey, but they can provide an effective relative measure of predation risk. Unlike gut content, feeding preference or enclosure

studies, which focus on specific predator types, tethering studies allow detection of trends in overall risk (Bullard & Hay 2002, Bullard & Whitlatch 2008). Conducting predation risk assays from docks also provide several benefits. Docks are located in nearshore habitats where many marine organisms spend their adult lives. Further, they provide a substrate for sessile marine invertebrates that release eggs and larvae into the plankton and to which postlarvae may settle to begin their adult lives. These habitats also shelter many fish species that may feed on zooplankton. Thus, although risk may be higher in the habitat surrounding docks than in some other nearshore habitats, docks provide a convenient sampling location to examine patterns in predation risk in a habitat where many marine organisms begin or end their planktonic larval stages. In addition, unlike the use of boats or SCUBA, deployment of tethering units from docks involves minimal disturbance of the water column. Since adult brine shrimp are similar in size to many late-stage meroplankton when they return to nearshore to settle into adult habitat, and larval brine shrimp are similar in size to many decapod larvae, we expect that the suite of predators that would normally feed on decapod larvae and postlarval invertebrates of similar size to *Artemia* would also eat *Artemia*. That said, PTUs have only been tested for artifacts with respect to fish predators (Bullard & Hay 2002). Since it is not known how PTUs affect the ability of non-fish predators to feed on tethered prey, our results should be interpreted cautiously with respect to risk from invertebrate predators. Chaetognaths and other predators that swallow their prey whole may have difficulty consuming tethered prey. Thus, our results for nauplii, in particular, may underestimate planktonic predation. However, rates of predation by invertebrate predators, including chaetognaths, under natural plankton densities appear to be low (Johnson & Shanks 2003).

Despite our 3 sampling sites being located in different oceans and habitat types, as well as having different communities of potential predator species, we found consistent patterns in variation in risk across diurnal phase and life stage categories. This robust result indicates this pattern in risk may be general across different environmental and biotic conditions.

The larvae of nearshore benthic marine invertebrates must migrate away from the adult habitat as small plankton and return as much larger plankton. This research increases knowledge of important shifts in diurnal patterns of meroplankton predation risk for differing life history stages. As others have predicted, we demonstrate that generalized state-

ments of variation in planktonic predation risk with respect to either diurnal phase or prey life history stage fail to capture the complexity of interacting environmental and biological drivers of predation risk.

Acknowledgements. We thank the following people at STRI for their assistance in the field and in the laboratory: M. Salazar, C. Bonilla, K. McDonald, A. Velarde, Sr. Ramos, M. Lesoway and J. Luque. We also thank S. dos Santos for providing data from the STRI long-term monitoring program, J. Christy for equipment and several hours of discussion on zooplankton predation risk and M. Boyle for helpful comments on the manuscript. We are indebted to I. Koev, Director of Research at Biogeneral Advanced Fiber Technology (www.biogeneral.com), for donating hundreds of metres of 23 µm micro-monofilament. We thank the administrative and security staff at STRI's Naos Marine Laboratories and Bocas Research Station and the Smithsonian Marine Station (SMS) at Fort Pierce for logistical assistance and access to the docks during all hours of the day and night. Funding for this research was provided by a Smithsonian Marine Science Network Grant awarded to R.C. and funding of F.G. by the Natural Sciences and Engineering Research Council of Canada (NSERC). This is Smithsonian Marine Station at Fort Pierce Contribution No. 937. The manuscript was improved by the comments of 2 anonymous reviewers.

LITERATURE CITED

- Acosta CA, Butler MJ (1999) Adaptive strategies that reduce predation on Caribbean spiny lobster postlarvae during onshore transport. *Limnol Oceanogr* 44:494–501
- Allredge AL, King JM (1980) Effects of moonlight on the vertical migration patterns of demersal zooplankton. *J Exp Mar Biol Ecol* 44:133–156
- Allen JD (2008) Size-specific predation on marine invertebrate larvae. *Biol Bull* 214:42–49
- Allen JD, McAlister JS (2007) Testing rates of planktonic versus benthic predation in the field. *J Exp Mar Biol Ecol* 347:77–87
- Baxter L (2004) A study of the reef fish communities of Las Perlas Archipelago, Panama. MSc thesis, Heriot-Watt University, Edinburgh
- Berglund A (1993) Risky sex: male pipefishes mate at random in the presence of a predator. *Anim Behav* 46:169–175
- Bergstrom CA, Reimchen TE (2003) Asymmetry in structural defenses: insights into selective predation in the wild. *Evolution* 57:2128–2138
- Bullard SG, Hay ME (2002) Plankton tethering to assess spatial patterns of predation risk over a coral reef and seagrass bed. *Mar Ecol Prog Ser* 225:17–28
- Bullard SG, Whitlatch RB (2008) Seasonal variation in planktivory risk in a southern New England coastal habitat. *J Exp Mar Biol Ecol* 357:1–6
- Canino MF, Grant GC (1985) The feeding and diet of *Sagitta tenuis* (Chaetognatha) in the lower Chesapeake Bay. *J Plankton Res* 7:175–188
- Christy JH (2011) Timing of hatching and release of larvae by brachyuran crabs: patterns, adaptive significance and control. *Integr Comp Biol* 51:62–72
- Christy JH, Morgan SG (1998) Estuarine immigration by crab postlarvae: mechanisms, reliability and adaptive significance. *Mar Ecol Prog Ser* 174:51–65
- Clark KL, Ruiz GM, Hines AH (2003) Diel variation in predator abundance, predation risk and prey distribution in shallow-water estuarine habitats. *J Exp Mar Biol Ecol* 287:37–55
- Collin R, D'Croz L, Gondola P, Del Rosario JB (2009) Climate and hydrological factors affecting variation in chlorophyll concentration and water clarity in the Bahia Almirante, Panama. *Smithson Contrib Mar Sci* 38:323–334
- Crawley MJ (2007) *The R book*. John Wiley & Sons, West Sussex
- D'Croz L, O'Dea A (2007) Variability in upwelling along the Pacific shelf of Panama and implications for the distribution of nutrients and chlorophyll. *Estuar Coast Shelf Sci* 73:325–340
- De Robertis A, Jaffe JS, Ohman MD (2000) Size-dependent visual predation risk and the timing of vertical migration in zooplankton. *Limnol Oceanogr* 45:1838–1844
- Dominici-Arosemena A, Wolff M (2005) Reef fish community structure in Bocas del Toro (Caribbean, Panama): gradients in habitat complexity and exposure. *Caribb J Sci* 41:613–637
- Dominici-Arosemena A, Wolff M (2006) Reef fish community structure in the Tropical Eastern Pacific (Panamá): living on a relatively stable rocky reef environment. *Helgol Mar Res* 60:287–305
- Feigenbaum D (1991) Food and feeding behaviour. In: Bone Q, Kapp H, Pierrot-Bults AC (eds) *The biology of chaetognaths*. Oxford University Press, Oxford, p 45–54
- Feigenbaum D, Reeve MR (1977) Prey detection in the chaetognatha: response to a vibrating probe and experimental determination of attack distance in large aquaria. *Limnol Oceanogr* 22:1052–1058
- Froese R, Pauly D (2011) FishBase. Available at www.fishbase.org
- Hobson ES (1975) Feeding patterns among tropical reef fishes. *Am Sci* 63:382–392
- Hobson ES (1991) Trophic relationships of fishes specialized to feed on zooplankters above coral reefs. In: Sale PF (ed) *The ecology of fishes on coral reefs*. Academic Press, San Diego, CA, p 69–95
- Holzman R, Genin A (2003) Zooplanktivory by a nocturnal coral-reef fish: effects of light, flow, and prey density. *Limnol Oceanogr* 48:1367–1375
- Holzman R, Genin A (2005) Mechanisms of selectivity in a nocturnal fish: a lack of active prey choice. *Oecologia* 146:329–336
- Holzman R, Ohavia M, Vaknin R, Genin A (2007) Abundance and distribution of nocturnal fishes over a coral reef during the night. *Mar Ecol Prog Ser* 342:205–215
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363
- Hylander S, Souza MS, Balseiro E, Modenutti B, Hansson LA (2012) Fish-mediated trait compensation in zooplankton. *Funct Ecol* 26:608–615
- Johnson KB, Shanks AL (2003) Low rates of predation on planktonic marine invertebrate larvae. *Mar Ecol Prog Ser* 248:125–139
- Kaufmann KW, Thompson RC (2005) Water temperature variation and the meteorological and hydrographic environment of Bocas del Toro, Panama. *Caribb J Sci* 41:392–413
- Kerr KA (2012) Timing it right: reproductive timing during variable temperature and implications for risk of preda-

- tion on larvae. PhD thesis, McGill University, Montreal
- Kerr K, Christy J, Collin R, Guichard F (2012) Reducing error in reproductive timing caused by temperature variation: interspecific differences in behavioural adjustment by fiddler crabs. *Mar Ecol Prog Ser* 459:1–16
- Kim TW, Christy JH, Dennenmoser S, Choe JC (2009) The strength of a female mate preference increases with predation risk. *Proc R Soc Lond B Biol Sci* 276:775–780
- Lampert W (1989) The adaptive significance of diel vertical migration of zooplankton. *Funct Ecol* 3:21–27
- Lie AAY, Tse P, Wong CK (2012) Diel vertical migration and feeding of three species of chaetognaths (*Flaccisagitta enflata*, *Aidanosagitta delicata* and *Aidanosagitta neglecta*) in two shallow, subtropical bays in Hong Kong. *J Plankton Res* 34:670–684
- Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am Nat* 153:649–659
- Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- Litvak MK, Leggett WC (1992) Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. *Mar Ecol Prog Ser* 81:13–24
- Morgan SG (1990) Impact of planktivorous fishes on dispersal, hatching, and morphology of estuarine crab larvae. *Ecology* 71:1639–1652
- Morgan SG (1995) Life and death in the plankton. In: McEdward L (ed) *Ecology of marine invertebrate larvae*. CRC Press, Boca Raton, FL, p 279–321
- Morgan SG, Christy JH (1995) Adaptive significance of the timing of larval release by crabs. *Am Nat* 145:457–479
- Morgan SG, Christy JH (1997) Planktivorous fishes as selective agents for reproductive synchrony. *J Exp Mar Biol Ecol* 209:89–101
- Motro R, Ayalon I, Genin A (2005) Near-bottom depletion of zooplankton over coral reefs: III: Vertical gradient of predation pressure. *Coral Reefs* 24:95–98
- Ohman MD (1988) Behavioral responses of zooplankton to predation. *Bull Mar Sci* 43:530–550
- Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Mar Ecol Prog Ser* 177:269–297
- Reimchen TE (1994) Predators and evolution in threespine stickleback. In: Bell MA, Foster SA (eds) *The evolutionary biology of the threespine stickleback*. Oxford University Press, Oxford, p 240–276
- Robertson DR, Christy JH, Collin R, Cooke RG and others (2009) The Smithsonian Tropical Research Institute: marine research, education, and conservation in Panama. *Smithson Contrib Mar Sci* 38:73–93
- Rumrill SS (1990) Natural mortality of marine invertebrate larvae. *Ophelia* 32:163–198
- Scharf FS, Juanes F, Rountree RA (2000) Predator size-prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Mar Ecol Prog Ser* 208:229–248
- Sih A (1994) Predation risk and the evolutionary ecology of reproductive behaviour. *J Fish Biol* 45:111–130
- Skibinski H (2005) Light positively affects foraging success of the night feeding zooplanktivorous fish hardyhead silverside (*Atherinomorus lacunosus*). BSc thesis, Uppsala University
- Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull Mar Sci* 60:1129–1157
- Vaughn D, Allen JD (2010) The peril of the plankton. *Integr Comp Biol* 50:552–570
- Walton WE, Emiley JA, Hairston NG Jr (1997) Effect of prey size on the estimation of behavioral visual resolution of bluegill (*Lepomis macrochirus*). *Can J Fish Aquat Sci* 54:2502–2508
- Werner EE, Anholt BR (1996) Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology* 77:157–169
- Yahel R, Yahel G, Genin A (2005a) Near-bottom depletion of zooplankton over coral reefs: I: Diurnal dynamics and size distribution. *Coral Reefs* 24:75–85
- Yahel R, Yahel G, Berman T, Jaffe JS, Genin A (2005b) Diel pattern with abrupt crepuscular changes of zooplankton over a coral reef. *Limnol Oceanogr* 50:930–944
- Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York, NY

Editorial responsibility: Steven Morgan,
Bodega Bay, California, USA

Submitted: July 9, 2013; Accepted: January 15, 2014
Proofs received from author(s): April 2, 2014