Predators Induce Phenotypic Plasticity in Echinoderms across Life History Stages

Danielle K. Barnes[1,2] **and Jonathan D. Allen**[2,*]

[1] Department of Biological Science, Florida State University, Tallahassee, Florida
[2] Biology Department, William & Mary, Williamsburg, Virginia

---

Abstract

*Marine invertebrates with biphasic life cycles feature life history transitions that coincide with habitat changes from benthic adults to planktonic embryos and larvae, then a return to the benthos as a juvenile at metamorphosis. The metamorphic transition exposes animals to a new suite of benthic predators, and high mortality often occurs in the hours and days following settlement. Juvenile invertebrates may produce phenotypically plastic morphological defenses when predator cues are detected. However, time lags inherent to phenotypic plasticity may delay the production of defenses until after the period of highest vulnerability. It should, therefore, be beneficial for planktonic larvae approaching settlement to detect waterborne cues from benthic predators and produce juvenile phenotypes appropriate for postmetamorphic survival. Echinoderms are useful models for testing transhabitat and trans–life history stage phenotypic plasticity because many species have larvae that construct their juvenile phenotype while still in the water column. In this study, we tested whether planktonic echinoderm larvae exposed to cues from benthic predators modified their juvenile phenotypes at settlement. Green urchin (*Strongylocentrotus droebachiensis*) and Pacific sand dollar (*Dendraster excentricus*) larvae were exposed to predatory green crab (*Carcinus maenus*) or red rock crab (*Cancer productus*) cues, respectively, from their early-stage juvenile rudiment formation through settlement. Green urchin larvae exposed to predator cues settled with significantly more juvenile spines compared to unexposed controls. Sand dollars exhibited earlier settlement, larger disk area, fewer spines, and shorter spines when exposed to benthic predator cues. Sand dollar larvae were also exposed to cues from planktonic crab larvae and in response settled sooner and larger, with even fewer and shorter spines than those exposed to benthic predator cues. These results suggest that echinoderm larvae alter their juvenile phenotype in response to predator cues, but the response varies between species, and responses to planktonic threats may be prioritized over benthic ones.*

Introduction

Most marine invertebrates exhibit a biphasic life cycle, consisting of a benthic adult stage and a planktonic larval stage (Thorson, 1950; Jagersten, 1972; Rieger, 1994). Invertebrates exhibiting complex life histories are particularly vulnerable during the transition from planktonic larva to benthic juvenile because of the drastic changes in both habitat and morphology as larvae settle from the water column to the ocean floor (Strathmann, 1985; Gosselin and Qian, 1997; Hadfield *et al.*, 2001; Allen and McAlister, 2007). Diverse predator communities on the benthos pose novel threats to recruits adapted to larval life in the water column (Thorson, 1966), and as a result, juvenile benthic invertebrates experience high mortality in the hours and days following settlement (reviewed by Gosselin and Qian, 1997; Hunt and Scheibling, 1997). In just one of many examples, juvenile bryozoans experience mortality exceeding 90% within the first 7 days following settlement onto the benthos as a result of predation by fish (Keough, 1986).

Many species of echinoderms also experience high mortality after their transition to the benthic juvenile stage. For example, juvenile purple sea urchins (*Strongylocentrotus*

Received 12 February 2023; Accepted 25 April 2023; Published online 26 July 2023.

* Corresponding author; email: jdallen@wm.edu.

Abbreviations: ASW, artificial seawater; dpf, days postfertilization; FHL, Friday Harbor Laboratories; FSW, filtered seawater; MANOVA, multivariate analysis of variance.

The Biological Bulletin, April 2023, volume 244, number 2: 000–000. https://doi.org/10.1086/725633

purpuratus) experience greater than 50% mortality in the first 3 weeks following settlement (Rowley, 1990) and juvenile ochre sea stars (*Pisaster ochraceus*) experience greater than 97% mortality within the first year (Sewell and Watson, 1993). Without a refuge, juvenile green sea urchin (*Strongylocentrotus droebachiensis*) can suffer greater than 70% mortality within 48 h when exposed to crustacean predators (Feehan *et al.*, 2014). Similarly, juvenile sand dollars (*Dendraster excentricus*) are heavily preyed upon by crustaceans in laboratory experiments (Highsmith, 1982), and during their first year of life in the field, they experience approximately 88% mortality (Cameron and Rumrill, 1982). High mortality of early postmetamorphic juveniles poses a significant population bottleneck for marine invertebrates in general (Osman and Whitlatch, 2004), and the high risk of predation in the benthic environment may lead to strong selective pressure for juvenile invertebrates to adopt defensive strategies, as it does for predator-induced phenotypic plasticity more generally (Harvell, 1990).

Inducible morphological defenses are a common mechanism to protect organisms from predation (Tollrian and Harvell, 1999). Inducible defenses result from phenotypic plasticity (Pigliucci, 2001) and are most common when reliable cues are available (Harvell, 1990). In the marine environment, predators often exude chemicals that disperse throughout the water column (Ruther *et al.*, 2002), allowing for detection of cues well in advance of physical contact. Cues composed of water-soluble peptides have the potential to be advected a considerable distance from the source, depending on the turbulence of the environment (Turner *et al.*, 1994; Weissburg and Zimmer-Faust, 1994). Organisms that perceive cues from potential predatory threats may be able to produce a phenotype with superior defensive morphological traits relative to those that cannot perceive or respond. Inducible defenses have been documented across many taxa of marine invertebrates, including barnacles (Lively, 1986), bryozoans (Harvell, 1986), mussels (Leonard *et al.*, 1999; Freeman and Byers, 2006), and snails (Appleton and Palmer, 1988; Trussell and Nicklin, 2002; Bourdeau, 2009).

Echinoderms have also been observed to produce morphological defenses in response to predators. For example, adult *S. droebachiensis* grow heavier and thicker skeletons when exposed to cues from predatory crabs (Selden *et al.*, 2009). Larval echinoderms also exhibit inducible defenses in response to predator cues. For example, sand dollar larvae clone when exposed to fish mucus (Vaughn and Strathmann, 2008) apparently in order to reduce size and avoid detection from visual predators (Vaughn, 2010). However, we are unaware of any studies reporting either that juvenile echinoderms deploy inducible morphological defenses in response to predators despite their high vulnerability to predation after settlement or that cues detected during the planktonic period may be used to modify juvenile phenotype.

One limitation of phenotypic plasticity is the time lag between an environmental change and the phenotypic response (Padilla and Adolph, 1996; DeWitt *et al.*, 1998). Plastic responses can be slow to initiate since gene transcription must be altered in order to express a different phenotype (DeWitt and Scheiner, 2004). Therefore, predator cues detected by juvenile echinoderms only after settlement may initiate a delayed plastic response that cannot be manifested until after the period of highest vulnerability. If larvae undergo metamorphosis into an environment with abundant predators, then they may be consumed before effectively employing a defensive response. It would be beneficial, therefore, if planktonic larvae could detect predator presence in their future benthic environment prior to settlement. Because the juvenile body plan is constructed within the planktonic larva (Cameron and Hinegardner, 1978; Gosselin and Jangoux, 1998; Heyland *et al.*, 2014), echinoderms may be more likely than other phyla to exhibit predator-induced plasticity in juvenile morphology if these cues are detected in the late larval stage. As larvae approach settlement, they interact with the benthos by probing the substrate to find a suitable settlement site (Burke, 1980). The proximity of competent larvae to the benthos, where the waterborne chemical cues from benthic predators are the most concentrated, may allow larvae to reliably sense benthic predator presence prior to settlement into that environment.

Another limitation of phenotypic plasticity is information reliability. Plastic organisms may produce maladaptive phenotypes if they incorrectly interpret environmental cues (DeWitt *et al.*, 1998). Late-stage larvae that are temporally and spatially closer to settling in the benthic environment may more reliably detect the benthic habitat than their parents or early developmental stage larvae and, therefore, produce the most accurate response.

Additionally, cues from benthic predators may trigger a different plastic response (across life history stages) than those from planktonic predators. If exposed to multiple predators, organisms may prioritize a response that defends themselves from the predator that poses the largest or most immediate risk (Sih *et al.*, 1998; Bourdeau, 2009). Cues from predators in different habitats represent threats from different stages in the complex life history. Larvae may tailor their response to predators that pose the more immediate threat if the cues from the planktonic and benthic predator are indistinguishable from each other.

In this study, we tested whether cues from predators in a future habitat (the benthos) can be perceived by echinoderm larvae in their current habitat (the plankton) and result in a modification of juvenile phenotypes immediately after they metamorphose and settle onto the benthos. We studied the responses of two species of echinoderms (the green sea urchin, *S. droebachiensis*, and the Pacific sand dollar *D. excentricus*) to cues from predatory crustaceans (cues from the European green crab, *Carcinus maenus*, or the red rock crab, *Cancer productus*, respectively). We hypothesized that late-stage echinoderm larvae exposed to predatory benthic crab cues would (1) delay settlement, (2) settle with more spines, (3) settle with longer spines, and (4) settle as larger juveniles in order to reduce mortality. We also studied the response of late-stage *D. excentricus* larvae to cues from predatory crab larvae to test if the plastic response would differ between exposure to planktonic or benthic predator cues.

Materials and Methods

Green urchins

For the first experiment, we studied the juvenile phenotypes of *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) after they were exposed as larvae to waterborne cues from the European green crab (*Carcinus maenas* (Linnaeus, 1758)). Green crabs were selected as a cue source because they are a known predator of juvenile sea urchins (Fagerli *et al.*, 2014) and are abundant benthic predators across New England (Baillie and Grabowski, 2019). Two trials were conducted in the laboratory in Williamsburg, Virginia, in the spring of 2018 and 2019, using two separate male-female pairs to account for differences in genotype and laboratory conditions.

Adult *S. droebachiensis* and green crabs were shipped overnight from the Marine Biological Laboratory at Woods Hole, Massachusetts, in March of 2018 (trial 1) and 2019 (trial 2). Adult urchins were spawned using standard methods by injecting 1 mL of 0.5 mol L^{-1} KCl into the coelomic cavity (Strathmann, 1987). To collect eggs, female urchins were inverted over glass beakers filled with artificial seawater (ASW; Instant Ocean, Spectrum Brands, Blacksburg, VA) at a temperature of 12 °C and a salinity of 32. Eggs collected from a single female were rinsed in ASW and immediately fertilized with dilute sperm collected from a single male. Each trial consisted of offspring from one male-female pair. Eggs were checked to ensure high (>90%) fertilization and then transferred into 1-L glass bowls filled with ASW, kept in aquaria at 12 °C, and manually stirred multiple times per day.

The *S. droebachiensis* embryos developed in these conditions until 3 days after fertilization, when they had reached the gastrula stage. Swimming gastrulae were separated into 10, 250-mL glass beakers filled with 200 mL of ASW at a salinity of 32 and a density of 100 embryos per beaker. Beakers were kept in a 12 °C water bath and stirred by paddles connected to a 10-rpm motor in order to suspend the larvae and microalgal food (Strathmann, 1987). Beaker locations were rotated every other day in order to minimize potential effects of position in the water bath. Three times per week until settlement, 100 mL of culture water in each beaker was reverse filtered through a 35-μm mesh and replenished with 100 mL of ASW at a salinity of 32. After each water change, larvae were fed a mixed algal diet consisting of *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Rhodomonas lens* at concentrations of 2500 cells mL^{-1} for each species.

Each trial consisted of two treatments: (1) waterborne cues from a benthic predator and (2) a control with no cue. There were five replicate beakers per treatment (10 beakers total). Adult green crabs were used to generate the benthic predator cue. Upon arrival, crabs were separated into aerated individual 20-L tanks containing ASW at a salinity of 32 in a cold room maintained at 15 °C. Carapace lengths of the crabs ranged from 51 to 59 mm (trial 1) and from 46 to 59 mm (trial 2), and the wet weight of the crabs was estimated to range from 30.3 to 46.3 g (trial 1) and from 22.4 to 46.3 g (trial 2; estimated using the following conversion: wet weight (g) $= 0.26$ [carapace width (cm)]$^{2.92}$, $R^2 = 0.99$, from Menge, 1983). Crabs were fed a diet of frozen shrimp every 2 days, except for the window where they were used to create predator cue water. Every 2 days, one of the crabs was randomly chosen to generate the predator cue water. To generate water with waterborne cues, an individual crab was housed in an aerated tank filled with 6 L of ASW for 24 h. The cue water was then filtered through a 5-μm bag filter in order to remove any particulate waste and larger microorganisms. The water for the control treatment was collected from a replicate tank with 6 L of ASW without crabs. The pH, dissolved oxygen, temperature, and salinity of the predator cue and control water were monitored using a YSI Pro 1030 conductivity, pH, and temperature probe (Yellow Springs Instrument, Yellow Springs, OH).

The predator cue water was introduced to each of the beakers beginning when the development of spines as part of the juvenile rudiment was first observed using polarized light under a compound microscope. During water changes, after 100 mL of the original culture water was filtered out, 100 mL of the predator cue water was added in place of the ASW. Predator cues were added to the beakers in the same manner for each subsequent water change (*i.e.*, every other day) until all urchins had settled. Larval settlement was induced by adding a blue mussel shell (*Mytilus edulis*) to each beaker beginning at 28 days after fertilization (Pearce and Scheibling, 1990).

Upon metamorphosis, urchin juveniles were removed from each beaker, and morphological measurements were taken at ×100 magnification using a compound microscope fitted with an ocular micrometer. Mean spine length was calculated by averaging the length of each juvenile's three longest spines. The total number of spines was also counted. Disk area was determined by measuring the length of the body at the longest axis and then the width of the body at its widest point perpendicular to the length. These two measures were then used to calculate the area of an ellipse as a proxy for sea urchin disk area.

Pacific sand dollars

For the second experiment, we studied the juvenile phenotypes of Pacific sand dollars (*Dendraster excentricus*

(Eschscholtz, 1831)) after they were exposed as larvae to waterborne cues from the red rock crab (*Cancer productus* Randall, 1840). *Cancer productus* is commonly used in experiments as a predator of larval *D. excentricus* (Rumrill *et al.*, 1985; Pennington *et al.*, 1986; Allen, 2008), in addition to being frequently associated with sand dollar beds and being observed feeding on adult sand dollars in the field (Merrill and Hobson, 1970). Two trials were conducted at the University of Washington's Friday Harbor Laboratories (FHL) on San Juan Island, Washington, in the summer of 2019. For each trial a unique male-female pair was used to account for differences in genotypes and laboratory conditions.

Adult *D. excentricus* individuals were collected from a large sand dollar bed in East Sound, Orcas Island, Washington (48°41′40″ N, 122°53′45″ W) in June 2019. Sand dollars were transported to FHL, where they were housed in tanks connected to a flow-through seawater system. The sand dollars were spawned using standard methods (Strathmann, 1987) by injecting 1 mL of 0.53 mol L^{-1} KCl into the coelomic cavity. To collect the eggs, female sand dollars were inverted over 50-mL glass beakers filled with 1-μm filtered seawater (FSW) at a salinity of 32. Eggs collected from a single female were rinsed in FSW and immediately fertilized with dilute sperm collected from a single male. Eggs were checked to ensure high (>90%) fertilization and then transferred into 1-L glass bowls filled with FSW and kept in a flow-through seawater table at ambient temperature (range: ~12 °C–14 °C) and manually stirred multiple times per day.

Two days after fertilization, when embryos had developed into the prism larval stage, larvae were sorted into 20 replicate 250-mL glass beakers containing 200 mL of FSW at a salinity of 33 with 100 larvae per beaker. Beakers were kept in a flow-through sea table at ambient temperature (range: 12 °C–14 °C). The beakers were stirred by paddles connected to a 10-rpm motor in order to suspend the larvae and microalgal food (Strathmann, 1987). Beaker locations were rotated every other day in order to minimize potential effects of position in the water bath. Every other day until settlement, 100 mL of culture water in each beaker was reverse filtered through a 35-μm mesh and replenished with 100 mL of FSW. After water changes, larvae were fed a mixed algal diet consisting of *D. tertiolecta*, *I. galbana*, and *R. lens* at concentrations of 2500 cells mL^{-1} for each species.

The first trial consisted of two treatments: (1) a benthic predator cue and (1) a control without cue. There were 10 replicate beakers per treatment (20 beakers total). Adult red rock crabs (*C. productus*) were used to create the benthic predator cue. Red rock crabs were collected from the intertidal shoreline in front of FHL in July of 2019 and housed in flow-through seawater tanks at ambient salinity and temperature. Red rock crabs are abundant benthic predators (1–5 m^{-2}) in intertidal habitats surrounding our study area (Yamada and Boulding, 1996). The crabs' carapace lengths ranged from 53 to 61 mm, and their masses ranged from 16.8 to 23.6 g. To generate water with a predator cue, a single crab was housed in a closed jar filled with 3 L of FSW for 24 h. The crab was then removed, and the water was filtered through a 5-μm bag filter, reoxygenated by vigorously shaking for 1 min, and diluted with an additional 3 L of FSW. Every other day, a different crab was used to produce the predator cue. The control water came from a jar in the same conditions without a crab. The pH, dissolved oxygen, temperature, and salinity of the predator cue and control water were monitored as done for the green urchin experiment.

The second trial consisted of three treatments: (1) a benthic predator cue, (2) a planktonic predator cue, and (3) a control. There were 20 total beakers, with seven replicate beakers in the benthic predator cue treatment, seven in the planktonic predator cue treatment, and six in the control. The same population of red rock crabs used in the first trial was used to create the benthic predator cue. To create the planktonic predator cue, *C. productus* megalopae were collected from the waters off the dock at FHL where they congregate during the day at densities of up to tens of larvae per liter (JDA, pers. obs.). Crab larvae were identified to species, using the criteria described in Trask (1970). The average mass of an individual megalopa was determined to be 0.019 g, which was used to calculate the number of megalopae needed for a given cue batch, such that the total mass of all megalopae combined was equivalent to the mass of the adult crab used in the corresponding benthic predator cue batch (~150–200 megalopae per 1 L of FSW). The control water came from a jar in the same conditions.

Benthic predator cue water, planktonic predator cue water, or control water was added to the beakers at 14 days after fertilization, when the sand dollar larvae had developed spines on the juvenile rudiment that could be observed with polarized light. After the culture water was filtered, 100 mL of predator cue water or control water was added. Predator cues were added to the culture beakers in each subsequent water change until all larvae had settled. Settlement was induced starting at 18 days after fertilization by adding into each beaker approximately 0.2 g of sand from the flow-through sand dollar tanks at FHL that had been conditioned by adult sand dollars for over a year (Highsmith, 1982).

On the day each juvenile metamorphosed, it was removed from the beaker, and morphological measurements were taken at ×100 magnification using a compound microscope. Mean spine length was calculated by averaging each juvenile's three longest spines. The total number of spines was also counted. Disk area was determined by measuring the length of the body at the longest axis and then the width of the body at its widest point perpendicular to the length under a compound microscope at ×100 magnification. These two measures were then used to calculate the area of an ellipse as a proxy for sand dollar disk area.

Statistical analysis

For all experiments, the data were analyzed in IBM SPSS Statistics (ver. 22; IBM, Armonk, NY). The residuals of the data were tested for normality using a Shapiro-Wilk test, and all were found to be normally distributed ($P > 0.05$). Multivariate analysis of variance (MANOVA) tests were conducted with treatment and trial as the independent variables and average age at settlement, average disk area, average spine number, and average spine length as the response variables. These tests were used to determine whether there were differences in age at settlement, disk area, spine number, and spine length between juveniles from the predator cue and control treatment. The data from the second trial of the sand dollar experiment were also analyzed using a MANOVA test with treatment as the independent variable and average age at settlement, average disk area, average spine number, and averages spine length as the response variables. Where significant effects were found, a Bonferroni *post hoc* test was run to test for differences between the three treatments. Linear regression tests were to explore the possible relationships between the juvenile features. Age at settlement was the independent variable, and disk area, spine number, and spine length were the separate dependent variables for each treatment. Linear regressions were also run using disk area as the independent variable and spine number and spine length as the separate dependent variables for each treatment. Additionally, linear regressions were run using spine number as the independent variable and spine length as the dependent variable for each treatment.

Results

Green urchins

In the first trial, *Strongylocentrotus droebachiensis* larvae began to settle 27 days postfertilization (dpf) and continued until 49 dpf. Across all beakers, 20.55% of the larvae successfully settled into the juvenile stage. In the second trial, *S. droebachiensis* larvae began to settle 32 dpf and continued until 53 dpf. In total, 80.45% of the larvae in the second trial successfully settled.

The average age at settlement of *S. droebachiensis* was 32.95 ± 1.43 dpf (mean ± SE) in the predator cue treatment and 35.41 ± 1.43 dpf in the control treatment (Fig. 1A) in the first trial. In the second trial, the average age at settlement of *S. droebachiensis* was 41.46 ± 0.52 dpf in the predator cue treatment and 42.81 ± 1.57 dpf in the control treatment (Fig. 1A). Overall, *S. droebachiensis* in the first trial settled sooner than in the second trial, but there was no significant effect of cue treatment on age at settlement (Fig. 1A; Table 1A).

Strongylocentrotus droebachiensis in the first trial settled with an average juvenile disk area of 99,618 ± 2902 μm^2 in the predator cue treatment and 98,800 ± 4503 μm^2 in the control treatment (Fig. 1B). In the second trial, the average juvenile disk area of *S. droebachiensis* was 77,367 ± 1372 μm^2 in the predator cue treatment and 75,709 ± 2057 μm^2 in the control treatment (Fig. 1B). Overall, the *S. droebachiensis* in the first trial settled as significantly larger juveniles than those in the second trial, but there was no significant effect of treatment on the average disk area of juveniles (Fig. 1B; Table 1B). There was, however, a significant negative relationship between juvenile disk area and age at settlement across trials for both the predator cue treatment and the control treatment (Fig. 2A).

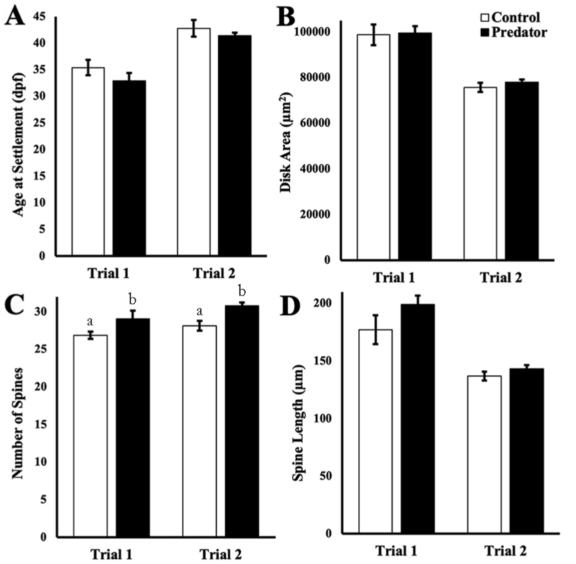

Figure 1. Effects of predator cue on age at settlement (A), disk area (B), spine number (C), and spine length (D). Each bar represents the mean ± SE. White bars represent the control treatment, and black bars represent the predator cue treatment. Different letters above bars represent significant differences between treatments.

The average juvenile spine number of *S. droebachiensis* in the first trial was 29.07 ± 1.07 in the predator cue treatment and 26.88 ± 0.49 in the control treatment (Fig. 1C). In the second trial, the average juvenile spine number of *S. droebachiensis* was 30.78 ± 0.46 in the predator cue treatment and 28.12 ± 0.65 in the control treatment (Fig. 1C). However, *S. droebachiensis* exposed to predator cues settled as juveniles with more spines than the juveniles in the control treatment (Fig. 1C; Table 1C). Juvenile spine number was also positively correlated with juvenile disk area for both the predator cue and control treatments (Fig. 2B).

The average juvenile spine length of *S. droebachiensis* in the first trial was 199.08 ± 7.86 μm in the predator cue treatment and 177.19 ± 12.49 μm in the control treatment (Fig. 1D). In the second trial, the average juvenile spine length of *S. droebachiensis* was 143.26 ± 3.12 μm in the predator cue treatment and 136.99 ± 3.88 μm in the control treatment (Fig. 1D). Overall, *S. droebachiensis* juveniles in the first trial had longer spines than those in the second trial (Fig. 1D; Table 1D). There was no significant difference

Table 1

Multivariate analysis of variance (MANOVA) table for results of the green urchin experiment for age of settlement (A), juvenile disk area (B), juvenile spine number (C), and juvenile spine length (D) between trials (1 and 2) and treatments (benthic predator cue and control)

Factor	df	*F*	*P*
A. Trial	1	36.819	**<0.001**
Treatment	1	2.12	0.165
Trial × treatment	1	0.179	0.678
B. Trial	1	59.035	**<0.001**
Treatment	1	0.176	0.680
Trial × treatment	1	0.020	0.888
C. Trial	1	4.280	0.049
Treatment	1	11.555	**0.004**
Trial × treatment	1	0.110	0.745
D. Trial	1	38.007	**<0.001**
Treatment	1	3.275	0.089
Trial × treatment	1	1.009	0.330

Significant effects ($P < 0.05$) are indicated in bold.

in juvenile spine length between the predator cue treatment and the control treatment (Fig. 1D; Table 1D). Juvenile spine length was positively correlated with juvenile disk area across trials in the predator cue treatment and the control treatment (Fig. 2C).

Pacific sand dollars

In the first trial, *Dendraster excentricus* larvae began settlement at 21 dpf and continued until 48 dpf. In total, 28.15% of the larvae successfully settled into the juvenile stage. In the second trial, larvae began settlement at 24 dpf and continued until 38 dpf. In the second trial, 21.40% of the larvae successfully settled into the juvenile stage.

The average age at settlement of *D. excentricus* in the first trial was 38.64 ± 1.33 dpf (mean ± SE) in the benthic predator cue treatment and 42.18 ± 1.09 dpf in the control treatment (Fig. 3A). In the second trial, the average age at settlement was 27.65 ± 0.56 dpf in the benthic predator cue treatment, 27.65 ± 0.56 dpf in the planktonic predator cue treatment, and 34.95 ± 0.99 dpf in the control treatment (Fig. 3A). Overall, sand dollars settled later in the first trial than in the second trial (Fig. 3A; Table 2A). *Dendraster excentricus* in the benthic predator cue treatment settled sooner than in the control treatment (Fig. 3A; Table 2A). *Dendraster excentricus* in the planktonic predator cue treatment settled sooner than in the control treatment, but it was not significantly different in the benthic predator cue treatment (Fig. 3A; Table 3A).

The average juvenile disk area of *D. excentricus* in the first trial was 67,714 ± 1120 μm^2 in the benthic predator cue treatment and 63,140 ± 778 μm^2 in the control treatment (Fig. 3B). In the second trial, the average juvenile disk area was 58,999 ± 1374 μm^2 in the benthic predator cue treatment, 58,665 ± 1870 μm^2 in the planktonic predator cue treatment, and 50,366 ± 1596 μm^2 in the control treatment (Fig. 3B). Overall, *D. excentricus* in the first trial settled as larger juveniles than in the second trial, and juveniles in the benthic predator cue treatment were larger than in the control treatment (Fig. 3B; Table 2B). *Dendraster excentricus* in the planktonic predator cue treatment in the second trial settled larger than in the control treatment, but there was no significant difference in size between juveniles in the planktonic predator cue treatment and the benthic predator cue treatment (Fig. 3B; Table 3B).

The average spine number of *D. excentricus* in the first trial was 28.63 ± 0.69 in the benthic predator cue treatment and 32.16 ± 0.56 in the control treatment (Fig. 3C). In the second trial, the average spine number of *D. excentricus* was 24.89 ± 0.48 in the benthic predator cue treatment, 22.62 ± 0.57 in the planktonic predator cue treatment, and 27.90 ± 0.70 in the control treatment (Fig. 3C). Overall, the *D. excentricus* juveniles in the first trial had more spines than in the second trial (Fig. 3C; Table 2C). *Dendraster excentricus* juveniles in the benthic predator cue treatment had fewer spines than juveniles in the control treatment (Fig. 3C; Table 2C). *Dendraster excentricus* juveniles in

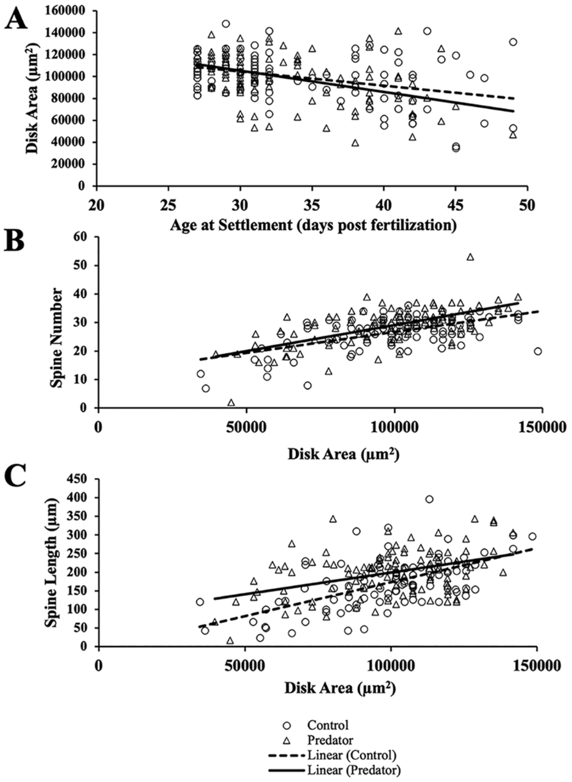

Figure 2. Linear regressions for green urchin trials between juvenile disk area and age at settlement for the predator cue treatment (A; solid line, $y = -1937.4x + 163{,}451$, $R^2 = 0.189$, $P < 0.001$) and the control treatment (dashed line, $y = -1303.8x + 143{,}936$, $R^2 = 0.130$, $P < 0.001$); between spine number and juvenile disk area for the predator cue treatment (B; solid line, $y = 0.0002x + 10.851$, $R^2 = 0.395$, $P < 0.001$) and the control treatment (dashed line, $y = 0.0001x + 12.037$, $R^2 = 0.331$, $P < 0.001$); and between spine length and juvenile disk area for the predator cue treatment (C; solid line, $y = 0.0012x + 82.818$, $R^2 = 0.179$, $P < 0.001$) and the control treatment (dashed line, $y = 0.0018x - 9.9177$, $R^2 = 0.368$, $P < 0.001$). Each symbol represents one juvenile. Triangles represent juveniles from the predator cue treatment, and circles represent juveniles from the control.

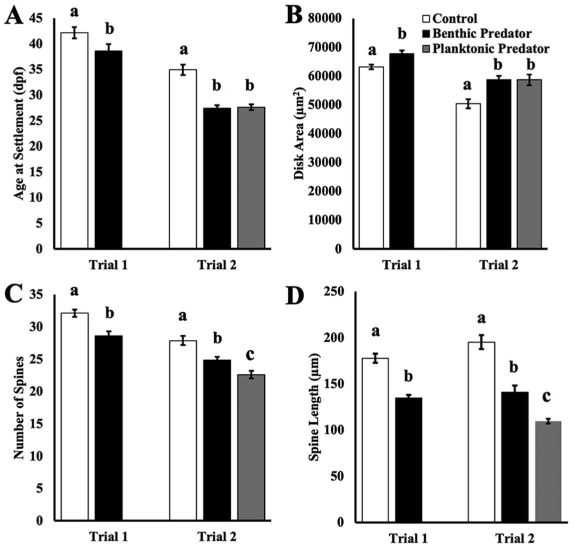

Figure 3. Effects of predator cue treatments on age at settlement (A), disk area (B), spine number (C), and spine length (D). Each bar represents the mean ± 1 SE. Different lowercase letters above bars represent significant differences between treatments. White bars represent the control treatment, black bars represent the benthic predator cue treatment, and gray bars represent the planktonic predator cue treatment.

the planktonic predator cue treatment had fewer spines than the juveniles in the benthic predator cue treatment and the control treatment. Juvenile spine number was positively correlated with age at settlement across trials for both the benthic predator cue treatment and the control treatment (Fig. 4A).

In the first trial, the average juvenile spine length of *D. excentricus* was 134.66 ± 3.43 μm in the benthic predator cue treatment and 177.7 ± 4.89 μm in the control treatment (Fig. 3D). In the second trial, the average juvenile spine length of *D. excentricus* was 140.91 ± 7.23 μm in the benthic predator cue treatment, 109.76 ± 2.64 μm in the planktonic predator cue treatment, and 195.07 ± 7.57 μm in the control treatment (Fig. 3D). Overall, *D. excentricus* in the first trial settled with shorter spines than in the second trial, and juveniles in the benthic predator cue treatment settled with shorter spines than in the control treatment (Fig. 3D; Table 2D). *Dendraster excentricus* juveniles in the planktonic predator cue treatment had fewer spines than the juveniles in the benthic predator cue treatment and the control. Juvenile spine length was positively correlated with juvenile spine number across trials for both the benthic predator cue treatment and the control treatment (Fig. 4B).

Discussion

Our study tested whether planktonic echinoderm larvae respond plastically to encounters with waterborne cues from benthic predators by altering their juvenile phenotype at settlement. As predicted, we found that when exposed to cues from benthic predators, *Strongylocentrotus droebachiensis* larvae metamorphosed into juveniles with enhanced defensive structures (greater numbers of spines). However, in opposition to our predictions, these larvae metamorphosed at the same time and at the same size as unexposed larvae. We also found that, as predicted, sand dollar (*Dendraster excentricus*) larvae exposed to predator cues metamorphosed into larger juveniles. However, these larger juveniles had reduced defensive structures (fewer and shorter spines) and also settled sooner than unexposed larvae, both the opposite of our initial predictions. Last, we found that sand dollar larvae exposed to cues from planktonic predators exhibited similarly accelerated settlement and larger juvenile size as those exposed to benthic predator cues but exhibited even greater reductions in defensive structures.

We demonstrated in this study that echinoderm larvae can detect and respond to cues from their future benthic habitat, which may be beneficial as they navigate the threat of predation in both environments. The planktonic environment has been demonstrated to pose a high risk of predation for larval marine invertebrates (Thorson, 1950; Young and Chia, 1987, Rumrill, 1990; Morgan, 1995; Vaughn and Allen, 2010). Laboratory studies have demonstrated that echinoderm larvae are preyed upon by crab megalopae (Rumrill *et al.*, 1985; Allen, 2008) as well as a suite of other planktonic predators including amphipods, chaetognaths, copepods, cnidarian medusae, ctenophores, euphausiids, and fish (Pennington *et al.*, 1986). In response to these planktonic predator cues, echinoderm larvae are known to employ a broad array of behavioral and morphological defenses. For example, sand dollar larvae attempt to avoid capture by predatory crab zoeae by adjusting their vertical distribution when they encounter the predator either physically (Rumrill *et al.*, 1985) or when they detect waterborne chemical cues (Metaxas and Burdett-Coutts, 2006). Sand dollar larvae have also been shown to clone in response to predator

Table 2

Multivariate analysis of variance (MANOVA) table for the sand dollar experiments showing effects of predator cue on age at settlement (A), juvenile disk area (B), juvenile spine number (C), and juvenile spine length (D) between trials (1 and 2) and treatments (benthic predator cue and control)

Factor	df	*F*	*P*
A. Trial	1	62.723	**<0.001**
Treatment	1	22.537	**<0.001**
Trial × treatment	1	2.893	0.100
B. Trial	1	84.082	**<0.001**
Treatment	1	31.067	**<0.001**
Trial × treatment	1	2.564	0.120
C. Trial	1	36.618	**<0.001**
Treatment	1	26.305	**<0.001**
Trial × treatment	1	0.079	0.781
D. Trial	1	4.644	0.049
Treatment	1	78.613	**<0.001**
Trial × treatment	1	1.027	0.319

Significant effects ($P < 0.05$) are indicated in bold.

Table 3

Multivariate analysis of variance (MANOVA) table for results of the second trial of the sand dollar experiment showing effects of predator cue on age at settlement, juvenile disk area, juvenile spine number, and juvenile spine length between treatments (control, benthic predator cue, and planktonic predator cue)

Factor	df	F	P	Treatment	Bonferroni P
Age	2	35.798	<**0.001**		
				Control *vs.* benthic	<**0.01**
				Control *vs.* planktonic	<**0.01**
				Benthic *vs.* planktonic	>0.05
Disk area	2	8.328	**0.003**		
				Control *vs.* benthic	<**0.01**
				Control *vs.* planktonic	<**0.01**
				Benthic *vs.* planktonic	>0.05
Spine number	2	21.127	<**0.001**		
				Control *vs.* benthic	<**0.01**
				Control *vs.* planktonic	<**0.01**
				Benthic *vs.* planktonic	<**0.01**
Spine length	2	51.603	<**0.001**		
				Control *vs.* benthic	<**0.01**
				Control *vs.* planktonic	<**0.01**
				Benthic *vs.* planktonic	<**0.05**

Significant effects ($P < 0.05$) are indicated in bold.

cues (Vaughn and Strathmann, 2008), possibly in order to evade detection by visual fish predators (Allen, 2008; Vaughn, 2010).

As larvae reach competency to settle and their opportunities for growth in the plankton are limited, transitioning to the benthos may offer a greater scope for growth (Werner, 1986). Organisms with biphasic life cycles often transition habitats in order to maximize their growth relative to mortality risk (Werner, 1988). The benthos, however, poses its own set of risks because it hosts a suite of novel predators that larvae have not previously encountered (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Comparisons of threats to developing organisms from benthic and planktonic predators are few but suggest that benthic habitats may be riskier than planktonic ones (Allen and McAlister, 2007). The metamorphic transition to the benthos may be especially challenging to organisms because settlers need to gather information about their new habitat and respond to it nearly simultaneously. Echinoderm larvae possess the advantage of developing juvenile phenotypes within their larval body while still in the plankton (Cameron and Hinegardner, 1978; Gosselin and Jangoux, 1998; Heyland *et al.*, 2014), potentially allowing the implementation of plastic juvenile responses if waterborne cues from the benthos can be recognized prior to metamorphosis. In this study, green urchin and sand dollar larvae both reacted to benthic predator cues, although the response from each of the species differed, which could be accounted for by variation in their juvenile ecology.

Once on the benthos, juvenile green urchins are typically solitary, partially as a result of being repelled by cues from conspecifics (Scheibling and Hamm, 1991). Juveniles live cryptically under rocks and in small crevices (Keats *et al.*, 1985; Scheibling and Raymond, 1990) that function as potential refuges from predators (Ojeda and Dearborn, 1991; Scheibling, 1996; Feehan *et al.*, 2014). Laboratory experiments have shown that juveniles exhibit cryptic behavior in the presence of predators (Scheibling and Hamm, 1991); however, larvae do not preferentially settle in cryptic microhabitats, and they do not immediately seek out these habitats as new juveniles (Scheibling and Robinson, 2008). Therefore, immediately upon settlement, juveniles may be vulnerable in the open, alone and exposed to benthic predators. In our study, we found that green urchins exposed to benthic predator cues as larvae increased their defensive structures at settlement by growing more and longer spines. These spines could serve as a morphological defense against predation. In adult green urchins, spines protect the tests from failure under impact due to predation attempts (Strathmann, 1981) by absorbing the energy and spreading the load away from the test (Tsafnat *et al.*, 2012). Additionally, studies have shown that urchins that have lost their spines are more susceptible to predation (Shears and Ross, 2010) and that longer spines make it more difficult for crustacean predators to handle them and thus influenced their choice of prey (Tegner and Levin, 1983).

While urchins exhibited increased defensive structures, we found that, contrary to our predictions, green urchin juvenile body size was not affected by predator cues. Green urchin populations commonly feature a bimodal size distribution (Scheibling and Stephenson, 1984; Scheibling, 1986, 1996; Ojeda and Dearborn, 1991). Small juveniles, when provided a spatial refuge, remain cryptic and experience

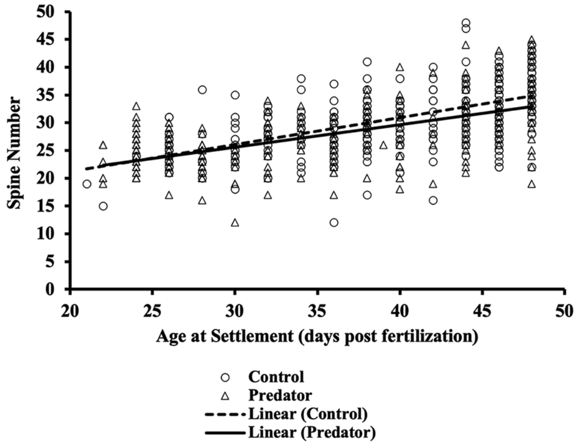

Figure 4. Linear regressions for sand dollar trials between juvenile spine number and age at settlement for the benthic predator cue treatment (A; solid line, $y = 0.4062x + 13.395$, $R^2 = 0.406$, $P < 0.001$) and the control treatment (dashed line, $y = -0.488x + 11.401$, $R^2 = 0.337$, $P < 0.001$) and between spine length and juvenile spine number for the benthic predator cue treatment (B; solid line, $y = 3.7149x + 35.371$, $R^2 = 0.309$, $P < 0.001$) and the control treatment (dashed line, $y = 4.1228x + 51.702$, $R^2 = 0.309$, $P < 0.001$). Each symbol represents one juvenile. Triangles represent juveniles from the predator cue treatment, and circles represent juveniles from the control.

lower rates of predation than more intermediate-sized urchins that have outgrown cobble refuges (Tegner and Dayton, 1981; Tegner and Levin, 1983; Andrew and MacDiarmid, 1991; Scheibling and Hamm, 1991; Scheibling, 1996). An increase in body size at settlement may make juvenile green urchins less cryptic and, subsequently, more vulnerable to predation. Green urchins therefore may not benefit from constructing larger juvenile bodies when exposed to benthic predator cues. In the future, tests isolating the effect of body size on postsettlement mortality would make a fruitful area for studies of juvenile urchin ecology.

The response of *D. excentricus* to predator cues varied considerably from *S. droebachiensis*. Sand dollars exhibit a more gregarious postmetamorphic ecology than urchins and, therefore, may employ a different predator defense strategy. Sand dollar larvae preferentially settle on sand that has been conditioned by adults (Highsmith, 1982; Burke, 1984). Unlike the mainly solitary *S. droebachiensis* juveniles, *D. excentricus* juveniles enter the benthos surrounded by dense aggregations of larger adults that offer protection from benthic predators (Highsmith, 1982), possibly reducing the need for investment in enhanced morphological defenses. In our study, we found that *D. excentricus* settled with fewer and shorter spines when exposed to benthic predator cues. Rather than producing morphological defenses, *D. excentricus* appeared to be investing in larger body sizes instead. Sand dollars exposed to either benthic or planktonic predator cues settled as larger juveniles. Settling at a larger size could aid in defense if they are too large to be gripped by small crustacean predators (Highsmith, 1982). Alternatively, reduced defensive structures in sand dollars exposed to predator cues could be a by-product of early settlement. We found that the sooner a sand dollar larva settled to the benthos, the fewer spines it had as a juvenile, regardless of treatment. Additionally, the fewer spines a juvenile sand dollar had, the shorter those spines were. Sand dollars could be settling earlier at the cost of reduced defensive structures. If sand dollars allocate more energy toward growing spines or remaining in the water column, they may need to tap into energetic reserves at the cost of reduced juvenile body size.

Dendraster excentricus larvae exposed to either planktonic or benthic predator cues settled earlier compared to unexposed larvae. The apparent lack of discrimination between predator cues, regardless of the habitat of the predator, could be due to substantial similarities in the molecular structures of waterborne cues produced by crabs regardless of life history stage (Rittschof and Cohen, 2004). Earlier settlement could be the most beneficial response for a sand dollar larva if there was an imminent threat of predation in the water column (*i.e.*, a planktonic predator was present), but this seems counterintuitive if the cue came from a benthic predator and it settled earlier into that environment where the predator was. Exposing larvae to cues from two distinct life stages of the same organism is broadly analogous to experiments testing how organisms respond to cues from multiple predators. When exposed to multiple predators, organisms may prioritize their responses toward the predator that poses the largest risk (Sih *et al.*, 1998; Bourdeau, 2009). For example, when exposed to cues from both crabs and sea stars simultaneously, snails produce the phenotype expressed when exposed to cues from crabs alone (Bourdeau, 2009). In this case, sand dollar larvae may generalize responses to predator cues from the same species regardless of life history stage and assume the benthic predator cue indicates a more imminent planktonic threat because the risk of predation when a planktonic predator cue is detected by planktonic larvae is high and immediate. It would be useful to test the response of sand dollar larvae to predator cues if no positive settlement cue is provided to indicate a safe benthic refuge to settle in (in this case, adult-conditioned sand).

Despite the lack of discrimination between cues for some responses, such as the timing of settlement and disk area, there was a distinction for others such as the length and number of spines. When exposed to benthic predator cues, sand dollar larvae had shorter and fewer spines compared to the control but longer and more spines compared to the planktonic predator exposure. The differential response to benthic and planktonic predator cues raises the possibility that larvae may be attempting to restore some of the physical protections against benthic predators that had been lost in the response to planktonic predators.

Other limitations of our experimental design and our current understanding of larval and juvenile biology may complicate interpretation of our results. For example, a larva may approach the benthos and receive a stimulus from a benthic predator well in advance of actual metamorphosis and settlement. This can lead to a mismatch between benthic cue and eventual benthic habitat, limiting the effectiveness of any plastic response (for a review of the limits of plastic responses, see DeWitt *et al.*, 1998). Additionally, a larva may either delay (*e.g.*, Johnson and Strathmann, 1989) or accelerate (Ferner *et al.*, 2019) metamorphosis and settlement in response to predators and physical cues, respectively. These changes in settlement timing likely have knock-on effects on juvenile phenotype that may impact postmetamorphic survival. Alternatively, the cues of benthic predators may actually be attractive in some cases, such as in barnacles (Raimondi, 1988), where the utility of predatory gastropod cue to indicate the presence of adult barnacles may outweigh any risks of postsettlement consumption. General lack of knowledge of the identities of benthic predators of newly settled juveniles may also mean that our choice of cues was inappropriate. For example, we used decapod cues when tanaid crustaceans may be more important predators in sand dollars in particular (Highsmith, 1982), although responses to kairomones from all crustaceans may be similar. Nonarthropod predators (*e.g.*, annelids, molluscs, nemerteans, *etc.*) may be equally likely to prey upon newly settled juveniles and would be well worth exploring as signals of benthic mortality. Last, even if our choice of predators was appropriate, the densities of predator and prey in our experiments may not match well with those found in nature. For example, the larval abundance we chose (0.5 mL^{-1}) was about an order of magnitude higher than the maximum reported for the site where we worked (Emlet, 1986). Similarly, while crab adults and megalopae can be locally comparable to the densities used here, the mean densities for the regions we worked can also be lower (Yamada and Boulding, 1996; Baillie and Grabowski, 2019), especially for megalopae (Jamieson and Phillips, 1988).

Overall, however, our study showed that planktonic echinoderm larvae detect benthic predator cues and respond by plastically altering their benthic juvenile phenotype. The variation in responses between the two species may be explained by their specific postmetamorphic ecology and strategies for predator defense. Future studies can address the adaptive utility of these responses by manipulating defensive structures in juveniles and measuring the resulting mortality due to predation. Regardless of the adaptiveness of these responses and potentially different results with different environmental cues, we now know from our study that predator-induced plasticity can cross boundaries of life history stages and habitats in these two echinoderm species.

Acknowledgments

This work was supported by the National Science Foundation through grant 1850837 to JDA and an Honors Fellowship through the William & Mary Charles Center and Sigma Xi Grant in Aid of Research to DKB. DKB also received travel support from the William & Mary Office of Leadership Development to present the results of this study. The University of Washington's Friday Harbor Laboratories provided housing and use of their marine laboratory facilities to conduct this research. We thank Amanda Yeo and Brianna Peterson for their invaluable assistance in the laboratory, as well as Augie Kalytiak-Davis for his expertise on juvenile marine invertebrates. Last, we thank Orissa Moulton, Rowan Lockwood, and two anonymous reviewers for their helpful comments on prior versions of the manuscript. Both authors (DKB and JDA) contributed equally to experimental design, the conduct of the work itself, and the writing of the manuscript. Both authors consent to the submission and publication of this work.

Literature Cited

Allen, J. D. 2008. Size-specific predation on marine invertebrate larvae. *Biol. Bull.* **214:** 42–49.

Allen, J. D., and J. S. McAlister. 2007. Testing rates of planktonic *versus* benthic predation in the field. *J. Exp. Mar. Biol. Ecol.* **347:** 77–87.

Andrew, N. L., and A. B. MacDiarmid. 1991. Interrelations between sea urchins and spiny lobsters in northeastern New Zealand. *Mar. Ecol. Prog. Ser.* **70:** 211–222.

Appleton, R. D., and A. R. Palmer. 1988. Waterborne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. *Proc. Natl. Acad. Sci. USA* **85:** 4387–4391.

Baillie, C., and J. H. Grabowski. 2019. Invasion dynamics: interactions between the European green crab *Carcinus maenas* and the Asian shore crab *Hemigrapsus sanguineus*. *Biol. Invasions* **21:** 787–802.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. *Ecology* **90:** 1659–1669.

Burke, R. D. 1980. Podial sensory receptors and the induction of metamorphosis in echinoids. *J. Exp. Mar. Biol. Ecol.* **47:** 223–234.

Burke, R. D. 1984. Pheromonal control of metamorphosis in the Pacific sand dollar, *Dendraster excentricus*. *Science* **225:** 442–443.

Cameron, R. A., and R. T. Hinegardner. 1978. Early events in sea urchin metamorphosis, description and analysis. *J. Morphol.* **157:** 21–31.

Cameron, R. A., and S. S. Rumrill. 1982. Larval abundance and recruitment of the sand dollar *Dendraster excentricus* in Monterey Bay, California, USA. *Mar. Biol.* **71:** 197–202.

DeWitt, T. J., and S. M. Scheiner. 2004. *Phenotypic Plasticity: Functional and Conceptual Approaches*. Oxford University Press, Oxford.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13:** 77–81.

Emlet, R. B. 1986. Larval production, dispersal and growth in a fjord: a case study on larvae of the sand dollar *Dendraster excentricus*. *Mar. Ecol. Prog. Ser.* **31:** 245–254.

Fagerli, C. W., K. M. Norderhaug, H. Christie, M. F. Pedersen, and S. Fredriksen. 2014. Predators of the destructive sea urchin *Strongylocentrotus droebachiensis* on the Norwegian coast. *Mar. Ecol. Prog. Ser.* **502:** 207–218.

Feehan, C. J., F. T. Y. Francis, and R. E. Scheibling. 2014. Harbouring the enemy: kelp holdfasts protect juvenile sea urchins from predatory crabs. *Mar. Ecol. Prog. Ser.* **514:** 149–161.

Ferner, M. C., J. Hodin, G. Ng, and B. Gaylord. 2019. Brief exposure to intense turbulence induces a sustained life-history shift in echinoids. *J. Exp. Biol.* **222:** jeb187351.

Freeman, A. S., and J. E. Byers. 2006. Divergent induced responses to an invasive predator in marine mussel populations. *Science* **313:** 831–833.

Gosselin, P., and M. Jangoux. 1998. From competent larva to exotrophic juvenile: a morphofunctional study of the perimetamorphic period of *Paracentrotus lividus* (Echinodermata, Echinoida). *Zoomorphology* **118:** 31–43.

Gosselin, L., and P. Qian. 1997. Juvenile mortality in benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* **146:** 265–282.

Hadfield, M. G., E. J. Carpizo-Ituarte, K. Del Carmen, and B. T. Nedved. 2001. Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. *Am. Zool.* **41:** 1123–1131.

Harvell, C. D. 1986. The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs, and consequences. *Am. Nat.* **128:** 810–823.

Harvell, C. D. 1990. The ecology and evolution of inducible defenses. *Q. Rev. Biol.* **65:** 323–340.

Heyland, A., J. Hodin, and C. Bishop. 2014. Manipulation of developing juvenile structures in purple sea urchins (*Strongylocentrotus purpuratus*) by morpholino injection into late stage larvae. *PLoS One* **9:** e113866.

Highsmith, R. C. 1982. Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. *Ecology* **63:** 329–337.

Hunt, H., and R. Scheibling. 1997. Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* **155:** 269–301.

Jagersten, G. 1972. *Evolution of the Metazoan Life Cycle*. Academic Press, London.

Jamieson, G. S., and A. C. Phillips. 1988. Occurrence of *Cancer* crab (*C. magister* and *C. oregonensis*) megalopae off the west coast of Vancouver Island, British Columbia. *Fish Bull.* **86:** 525–542.

Johnson, L. E., and R. R. Strathmann. 1989. Settling barnacle larvae avoid substrata previously occupied by a mobile predator. *J. Exp. Mar. Biol. Ecol.* **128:** 87–103.

Keats, D. W., G. R. South, and D. H. Steele. 1985. Ecology of juvenile green sea urchins (*Strongylocentrotus droebachiensis*). Pp. 295–302 in *Proceedings of the Fifth International Echinoderm Conference: Echinodermata*, B. F. Keegan, B. D. S. O'Connor, eds. CRC, London.

Keough, M. J. 1986. The distribution of a bryozoan on seagrass blades: settlement, growth, and mortality. *Ecology* **67:** 846–857.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. *Ecology* 80: 1–14.

Lively, C. M. 1986. Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. *Evolution* **40:** 232–242.

Menge, B. A. 1983. Components of predation intensity in the low zone of the New England rocky intertidal region. *Oecologia* **58:** 141–155.

Merrill, R. J., and E. S. Hobson. 1970. Field observations of *Dendraster excentricus*, a sand dollar of western North America. *Am. Midl. Nat.* **83:** 595–624.

Metaxas, A., and V. Burdett-Coutts. 2006. Response of invertebrate larvae to the presence of the ctenophore *Bolinopsis infundibulum*, a potential predator. *J. Exp. Mar. Biol. Ecol.* **334:** 187–195.

Morgan, S. G. 1995. Life and death in the plankton: larval mortality and adaptation. Pp. 279–321 in *Ecology of Marine Invertebrate Larvae*, L. R. McEdward, ed. CRC, Boca Raton, FL.

Ojeda, F. P., and J. H. Dearborn. 1991. Feeding ecology of benthic mobile predators: experimental analyses of their influence in rocky subtidal communities of the Gulf of Maine. *J. Exp. Mar. Biol. Ecol.* **149:** 13–44.

Osman, R. W., and R. B. Whitlatch. 2004. The control of the development of a marine benthic community by predation on recruits. *J. Exp. Mar. Biol. Ecol.* **311:** 117–145.

Padilla, D. K., and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evol. Ecol.* **10:** 105–117.

Pearce, C. M., and R. E. Scheibling. 1990. Induction of metamorphosis of larvae of the green sea urchin, *Strongylocentrotus droebachiensis*, by coralline red algae. *Biol. Bull.* **179:** 304–311.

Pennington, J. T., S. S. Rumrill, and F. S. Chia. 1986. Stage-specific predation upon embryos and larvae of the Pacific sand dollar, *Dendraster excentricus*, by 11 species of common zooplanktonic predators. *Bull. Mar. Sci.* **39:** 234–240.

Pigliucci, M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. JHU, Baltimore.

Raimondi, P. T. 1988. Settlement cues and determination of the vertical limit of an intertidal barnacle. *Ecology* **69:** 400–407.

Rieger, R. M. 1994. The biphasic life cycle—a central theme of metazoan evolution. *Am. Zool.* **34:** 484–491.

Rittschof, D., and J. H. Cohen. 2004. Crustacean peptide and peptide-like pheromones and kairomones. *Peptides* **25:** 1503–1516.

Rowley, R. J. 1990. Newly settled sea urchins in a kelp bed and urchin barren ground: a comparison of growth and mortality. *Mar. Ecol. Prog. Ser.* **62:** 229–240.

Rumrill, S. S. 1990. Natural mortality of invertebrate larvae. *Ophelia* **32:** 163–198.

Rumrill, S. S., J. T. Pennington, and F. S. Chia. 1985. Differential susceptibility of marine invertebrate larvae: laboratory predation of sand dollar, *Dendraster excentricus* (Eschscholtz), embryos and larvae by zoeae of the red crab, *Cancer productus*. Randall. *J. Exp. Mar. Biol. Ecol.* **90:** 193–208.

Ruther, J., T. Meiners, and J. L. Steidle. 2002. Rich in phenomena-lacking in terms: a classification of kairomones. *Chemoecology* **12:** 161–167.

Scheibling, R. 1986. Increased macroalgal abundance following mass mortalities of sea urchins (*Strongylocentrotus droebachiensis*) along the Atlantic coast of Nova Scotia. *Oecologia* **68:** 186–198.

Scheibling, R. 1996. The role of predation in regulating sea urchin populations in eastern Canada. *Oceanol. Acta* **19:** 421–430.

Scheibling, R. E., and J. Hamm. 1991. Interactions between sea urchins (*Strongylocentrotus droebachiensis*) and their predators in field and laboratory experiments. *Mar. Biol.* **110:** 105–116.

Scheibling, R. E., and B. G. Raymond. 1990. Community dynamics on a subtidal cobble bed following mass mortalities of sea urchins. *Mar. Ecol. Prog. Ser.* **63:** 127–145.

Scheibling, R. E., and M. C. Robinson. 2008. Settlement behaviour and early post-settlement predation of the sea urchin *Strongylocentrotus droebachiensis*. *J. Exp. Mar. Biol. Ecol.* **365:** 59–66.

Scheibling, R. E., and R. L. Stephenson. 1984. Mass mortality of *Strongylocentrotus droebachiensis* (Echinodermata: Echinoidea) off Nova Scotia, Canada. *Mar. Biol.* **78:** 153–164.

Selden, R., A. S. Johnson, and O. Ellers. 2009. Waterborne cues from crabs induce thicker skeletons, smaller gonads and size-specific changes in growth rate in sea urchins. *Mar. Biol.* **156:** 1057–1071.

Sewell, M. A., and J. C. Watson. 1993. A “source” for asteroid larvae? Recruitment of *Pisaster ochraceus, Pycnopodia helianthoides* and *Dermasterias imbricata* in Nootka Sound, British Columbia. *Mar. Biol.* **117:** 387–398.

Shears, N. T., and P. M. Ross. 2010. Toxic cascades: multiple anthropogenic stressors have complex and unanticipated interactive effects on temperate reefs. *Ecol. Lett.* **13:** 1149–1159.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* **13:** 350–355.

Strathmann, M. F. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press, Seattle.

Strathmann, R. R. 1981. The role of spines in preventing structural damage to echinoid tests. *Paleobiology* **7:** 400–406.

Strathmann, R. R. 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annu. Rev. Ecol. Syst.* **16:** 339–361.

Tegner, M. J., and P. K. Dayton. 1981. Population structure, recruitment and mortality of two sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*) in a kelp forest. *Mar. Ecol. Prog. Ser.* **5:** 68.

Tegner, M. J., and L. A. Levin. 1983. Spiny lobsters and sea urchins: analysis of a predator-prey interaction. *J. Exp. Mar. Biol. Ecol.* **73:** 125–150.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25:** 1–45.

Thorson, G. 1966. Some factors influencing the recruitment and establishment of marine benthic communities. *Neth. J. Sea Res.* **3:** 267–293.

Tollrian, R., and C. D. Harvell. 1999. *The Ecology and Evolution of Inducible Defenses*. Princeton University Press. Princeton, Princeton, NJ.

Trask, T. 1970. A description of laboratory-reared larvae of *Cancer productus* Randall (Decapoda: Brachyura) and a comparison to larvae of *Cancer magister* Dana. *Crustaceana* **18:** 133–146.

Trussell, G. C., and M. O. Nicklin. 2002. Cue sensitivity, inducible defense, and trade-offs in a marine snail. *Ecology* **83:** 1635–1647.

Tsafnat, N., J. D. F. Gerald, H. N. Le, and Z. H. Stachurski. 2012. Micromechanics of sea urchin spines. *PLoS One* **7:** e44140.

Turner, E. J., R. K. Zimmer-Faust, M. A. Palmer, M. Luckenbach, and N. D. Pentchef. 1994. Settlement of oyster (*Crassostrea virginica*) larvae: effects of water flow and a water-soluble chemical cue. *Limnol. Oceanogr.* **39:** 1579–1593.

Vaughn, D. 2010. Why run and hide when you can divide? Evidence for larval cloning and reduced larval size as an adaptive inducible defense. *Mar. Biol.* **157:** 1301–1312.

Vaughn, D., and J. D. Allen. 2010. The peril of the plankton. *Integr. Comp. Biol.* **50:** 552–570.

Vaughn, D., and R. R. Strathmann. 2008. Predators induce cloning in echinoderm larvae. *Science* **319:** 1503–1503.

Weissburg, M. J., and R. K. Zimmer-Faust. 1994. Odor plumes and how blue crabs use them in finding prey. *J. Exp. Biol.* **197:** 349–375.

Werner, E. E. 1988. Size, scaling, and the evolution of complex life cycles. Pp. 60–81 in *Size-Structured Populations*, B. Ebenman and L. Persson, eds. Springer, Berlin.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.* **128:** 319–341.

Yamada, S. B., and E. G. Boulding. 1996. The role of highly mobile crab predators in the intertidal zonation of their gastropod prey. *J. Exp. Mar. Biol. Ecol.* **204:** 59–83.

Young, C. M., and F. S. Chia. 1987. Abundance and distribution of pelagic larvae as influenced by predation, behavior and hydrographic factors. Pp. 385–463 in *Reproduction of Marine Invertebrates*, Vol. 9, *Seeking Unity in Diversity*, A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell/Boxwood, Palo Alto, CA.