

# Eastern oysters use predation risk cues in larval settlement decisions and juvenile inducible morphological defenses

Jessica L. Pruettt\*, Marc J. Weissburg

School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

---

**ABSTRACT:** Predation is an important factor affecting the recruitment of marine benthic populations. Larvae can reduce their risk of predation by avoiding settlement near predators, whereas juveniles can resist predation through induced morphological defenses. We performed a 2 mo field study to understand the nature of the predation risk cues used by settling eastern oyster larvae and developing juveniles to decrease predation threat during recruitment. We found that oyster recruitment was highest on caged tiles near juvenile oysters, but lowest on tiles near active mud crab predators that produce a combination of predator metabolites and crushed conspecific alarm cues. Chemical cues from crushed conspecifics alone did not affect recruitment in the field, yet oyster larvae avoided either mud crab cues or crushed conspecific cues when presented separately in lab behavior experiments. Comparing the field to lab results suggests alarm cues may be unreliable under natural environmental conditions or were not present at high enough concentrations in the relatively high turbulent flow environment of our field site to convey the predation threat necessary to deter oyster settlement. Oyster spat grew most quickly when exposed to crushed conspecifics and may employ different strategies in response to unique risk cues or predation risk levels by avoiding settlement near actively feeding future predators but growing quickly to reach a size refuge in the presence of alarm cues. These non-consumptive predator effects at different life stages may have important demographic consequences for oyster populations that need to be considered in conjunction with predator consumptive effects.

**KEY WORDS:** Alarm cue · *Crassostrea virginica* · Non-consumptive effect · *Panopeus herbstii* · Phenotypic plasticity · Predation risk · Settlement cue

---

— Resale or republication not permitted without written consent of the publisher —

## 1. INTRODUCTION

Predators have large impacts on community structure through consumption of prey. However, prey can reduce their risk of predation through plastic antipredator responses (Lima 1998), which also have important non-consumptive effects on communities (Werner & Peacor 2003, Schmitz et al. 2008). Predation events release predator cues and injured prey cues that cause prey to modify behavioral, morphological, or life-history traits (Chivers & Smith 1998, Kats & Dill 1998, Ferrari et al. 2010). However, antipredator responses have costs (Lima & Dill 1990),

so prey often react to predation risk in a graded manner dependent on the level of perceived threat (Chivers et al. 2001, Weissburg et al. 2014). The role of predation event risk cues is generally understood for behavioral and morphological responses (Chivers & Smith 1998, Schoeppner & Relyea 2009, Bourdeau 2010), but is less clear for other non-consumptive effects.

Recruitment is important for sustaining marine benthic populations (Roughgarden et al. 1988, Pineda et al. 2009), but newly settled larvae and juveniles experience heavy losses through predation (Gosselin & Qian 1997). Larvae and juveniles conse-

\*Corresponding author: [jpruettt7@gatech.edu](mailto:jpruettt7@gatech.edu)

quently counteract the threat of predation with behavioral and morphological antipredator responses. Larvae avoid settling near predator cues (Ellrich et al. 2015) and juveniles induce morphological defenses to become more resistant to predation (Robinson et al. 2014). Chemical cues associated with predation events mediate these responses but the relative importance of predator versus injured prey cues remains unclear.

Larvae avoid settlement when exposed to cues indicative of predation risk. Several species of crustacean and fish larvae avoid or decrease settlement in response to chemical cues from predators alone (Johnson & Strathmann 1989, Boudreau et al. 1993, Welch et al. 1997, Dixon et al. 2012, Ellrich et al. 2015) or predators actively consuming conspecific prey (Vail & McCormick 2011). Prey-derived cues (e.g. injured prey metabolites, referred to as alarm cues) are used by organisms to react to predation risk (Chivers & Smith 1998, Ferrari et al. 2010), but no studies have examined larval settlement in the presence of these cues. For example, fish embryos and larvae use injured conspecific alarm cues to learn predation threats (Holmes & McCormick 2010, Atherton & McCormick 2015). The use of alarm cues in a variety of contexts, the natural occurrence of cues from injured prey during predation events, and evidence that settling larvae actively avoid future predators all suggest alarm cues also may mediate settlement.

Yet alarm cues from injured prey are not always the result of a predation event and can be an unreliable cue that does not always induce antipredator responses without predator cues (Schoepner & Relyea 2009, Bourdeau 2010). Conspecifics may release metabolites if injured or stressed by factors other than predation, and so this cue may be only weakly related to predation risk. The larvae of many sessile adult marine species also use adult metabolites as settlement cues because fertilization success and overall survival rates are increased when in conspecific aggregations (Crisp 1984, Burke 1986, Pawlik 1992, Hadfield & Paul 2001). It is unclear whether such cues are perceived differently from cues from injured conspecifics as a result of qualitative or quantitative differences, or both. Still, larvae confronted with chemical cues from injured adults must balance the cost of forgoing settlement in a suitable habitat with the benefit of potentially reduced predation risk. Barnacle larvae did not reduce settlement near dogwhelk predators when adult barnacles were present but did when adults were absent, which suggests the positive cue of adult conspecifics outweighs

the negative cue of future predators (Ellrich et al. 2016). However, this study did not allow dogwhelks to forage on adult barnacles, so it is unknown if injured adult alarm cues in combination with predator cues can heighten perceived predation risk enough to reduce settlement by barnacle larvae.

Settlement decisions are not the only way predation risk cues produce non-consumptive effects during recruitment. Newly attached juveniles can undergo morphological changes in response to predation events to decrease predation risk (Lively 1986b). These inducible defenses are beneficial in environments where the risk of predation is high but unpredictable and defenses are costly (Lively 1986a, Harvell 1990). In marine invertebrates, shell formation is energetically costly and detracts from other growth parameters. Thus, many gastropod and bivalve species produce thicker shells in response to reliable predation risk cues only (Brookes & Rochette 2007, Sherker et al. 2017). For example, mussels grow thicker shells and are harder to crush when exposed to cues from predators or crushed conspecifics (Leonard et al. 1999, Sherker et al. 2017). Predators fed conspecific diets also induce more types of morphological defenses in snails and oysters compared to predators fed heterospecific diets (Bourdeau 2010, Bible et al. 2017). Studies on morphological induction in juvenile bivalves have been performed either in the lab (Leonard et al. 1999, Newell et al. 2007) or using juveniles from the field that settled under unknown predator risk levels (Johnson & Smee 2012, Sherker et al. 2017), so the importance of predator presence at the time of settlement in these responses is undetermined.

In this study, we used the eastern oyster *Crassostrea virginica* to examine larval settlement in response to chemical cues associated with predation events in natural and controlled settings, and to determine whether risk cues modify morphology immediately post settlement. Oysters are gregarious settlers that respond positively to chemical cues from conspecific juveniles and adults in the lab (Hidu 1969, Keck et al. 1971, Tamburri et al. 1992, Tamburri et al. 1996) and the field (Smee et al. 2013). Aggregations of adult oysters form large reefs that provide ecosystem services and offer refugia for a wide range of species (Wells 1961, Lenihan & Peterson 1998, Newell 2004, Grabowski et al. 2012). Juvenile oyster spat respond to chemical cues from predators by increasing shell mass and resistance to crushing measured as shell crushing force (Robinson et al. 2014, Scherer et al. 2016). Notably, the mud crab *Panopeus herbstii*, that lives within oyster reefs and

is a voracious predator of juvenile oysters, induces shell morphological responses (Johnson et al. 2014, Scherer & Smee 2017). However, oyster larvae settlement response to negative chemical cues has not been experimentally examined. The only tests of larval predator avoidance with bivalves have been in mussel species, and found that mussel larvae avoid predator cues in the lab (Morello & Yund 2016) but show either avoidance or no response in settlement near predators in the field (von der Meden et al. 2015, Ehlers et al. 2018).

We determined the effect of future predation risk on oyster recruitment using a 2 mo field study and predicted that oyster larvae would avoid settling near chemical cues typical of those released from predators actively foraging on juvenile oysters (i.e. predator metabolites and cues from damaged conspecifics). We also measured shell growth rates in the newly settled oyster spat to test if the chemical risk cues induced morphological responses. Our goal was to specifically examine the role of damaged conspecific metabolites by presenting them with and without predator metabolites. We hypothesized that the potential ambiguity of cues from injured conspecifics in natural environments would make this a less effective cue in the absence of cues from predators themselves. We assessed the behavioral response of oyster larvae to individual cues in still-water behavior experiments to provide additional evidence regarding the role of single versus multiple cues in mediating settlement and post-settlement responses.

## 2. MATERIALS AND METHODS

### 2.1. Animal collection and maintenance

All crab species and oysters used in both experiments for cue treatments were collected by hand from oyster reefs located within Wassaw Sound (Savannah, GA, USA) and associated tributaries. Collections were done under the approval of a scientific collection permit issued by the Georgia Department of Natural Resources. For the field recruitment experiment, animals were held in separate flow-through systems at the Skidaway Institute of Oceanography prior to their deployment. For the laboratory behavior experiments, animals were transported to Georgia Institute of Technology (GT) and each species was separately housed in 28 l aquaria. Aquaria were filled with artificial seawater maintained at conditions similar to the collection site (25 ppt salinity, 22–24°C water temperature). Eyed,

pediveliger oyster larvae tested in the lab behavior experiments were obtained from Auburn University Shellfish Laboratory (AUSL) oyster hatchery and shipped overnight to GT. Larvae were maintained in aerated sterile artificial seawater at conditions that matched AUSL conditions (20 ppt salinity, 22–24°C water temperature) and used in behavior experiments within 2 d.

### 2.2. Field recruitment experiment

We monitored recruitment onto tiles placed in the field to determine the effect of predation risk cues on larval settlement and post-settlement growth rates. The field experiment was performed on intertidal mud flats at Priest Landing, Skidaway Island, GA, which is located in the Wilmington River adjacent to Wassaw Sound. Tidal range in this area is between 2 and 3 m. This site is characterized by loose oyster clusters and patch oyster reefs bordered by *Spartina alterniflora* salt marshes. The average water temperature during the duration of the experiment ranged between 26 and 29°C; salinity range was 25–28 ppt. Flow in this area is tidally driven and primarily unidirectional during ebb and flood tides. Relative to other sites in Wassaw Sound, flow velocity is moderate at this site (8–10 cm s<sup>-1</sup>) but with high turbulent kinetic energy (TKE) ( $0.3 \times 10^{-3}$ – $0.65 \times 10^{-3}$  m<sup>2</sup> s<sup>-2</sup>) (Wilson et al. 2013).

Cages were used to both restrict predator access to recruitment tiles and to surround settling tiles with a constant source of cue. Cages had an interior tile enclosure (15 cm diam.) that was contained within, but separated with mesh from, an exterior treatment enclosure (30 cm diam.) (Fig. 1). Cages were constructed out of 1 cm<sup>2</sup> vexar mesh and had a 15 cm height. Slate tiles (10 × 10 × 0.5 cm) were provided as a settlement substrate for oyster recruitment and placed within the interior tile enclosure. Tiles were attached to 15 cm long PVC poles with marine adhesive sealant so that they could be staked into the mud and raised slightly (ca. 2 cm) above the substrate to limit sedimentation. The exterior treatment enclosure contained 1 of 4 cue treatments: crushed juvenile oysters, whole juvenile oysters, mud crabs foraging on crushed juvenile oysters, and crushed dead oyster shells. This set of cues reflects that predation in natural conditions will result in the release of both injured conspecific metabolites and predator cues, whereas conspecific metabolites can be released as a result of other injuries or stress. Whole oysters are a positive control and crushed shells a control for the experimental treatment involving crushed oysters.

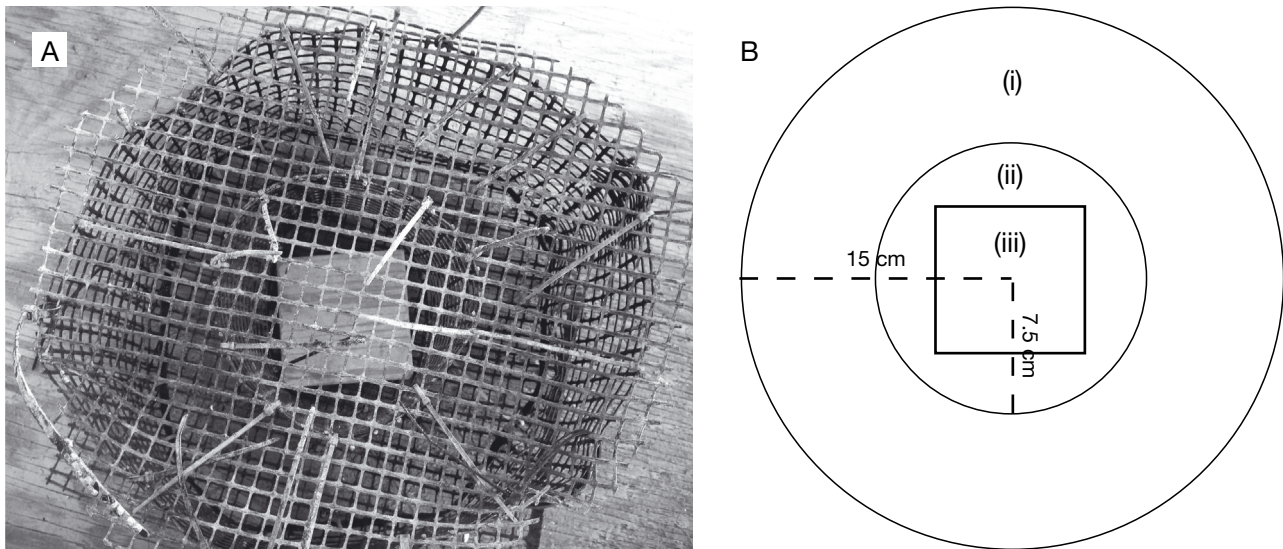


Fig. 1. Eastern oyster field recruitment cage setup. (A) Top view of recruitment cage; (B) diagram of recruitment cage showing (i) exterior treatment enclosure that contained either mud crabs foraging on crushed juvenile oysters, crushed oysters, whole oysters, or crushed oyster shell control, (ii) interior tile enclosure, and (iii) the recruitment tile (10 × 10 cm)

A total of 5 adult mud crabs *Panopeus herbstii* of either sex (carapace width: 20–25 mm) were added to the exterior treatment enclosure for the mud crabs foraging on crushed oyster treatment. Mud crabs were fed oyster flesh ad libitum for 48 h prior to the start of the experiment. Mud crabs in the cages were fed 15 crushed oysters (hinge length: 20–40 mm, wet tissue weight: ~0.5–1 g) every 3–4 d throughout the experiment to equal a predation rate of 1 oyster d<sup>-1</sup> crab<sup>-1</sup>. This rate was chosen based on preliminary laboratory feeding trials and equivalent to consumption in other field trials (Hill & Weissburg 2013, Pruett & Weissburg 2018). Any missing or dead mud crabs were replaced during semiweekly checks. The crushed oyster treatment and whole oyster treatment also received 15 oysters each that were replaced each time the mud crabs were fed. Oysters were crushed by striking the oysters with the blunt end of an oyster knife several times and scoring the flesh to simulate crab predation. Dead oyster shells in the control treatment were obtained from the field experiment site, crushed in the same manner as previously described, and replaced at the same intervals as the other treatments.

Cages were placed on mudflats in between naturally occurring intertidal oyster patch reefs. Treatments were deployed in blocks and treatment cages within a block were at least 1.5 m away from each other and oyster reefs in the direction of tidal flow. Blocks were separated by natural oyster reefs that were at least 15 m wide with 9 blocks total. Photographs were taken of each tile approximately

every week in order to assess recruitment processes throughout the experiment. The experiment began in early June 2017 and ended 55 d later in late July.

The total number of oyster recruits on each tile were counted at the end of the experiment with the aid of a dissecting microscope, when we also measured mortality as the number of scars on each tile. Weekly photographs were analyzed to identify and count new recruits in each weekly cohort. Individual oyster recruits were mapped throughout the experiment to assess survival and calculate shell growth rate. The final shell area of oyster recruits that arrived in the cohorts between Days 20 and 33 was measured using ImageJ software (version 1.51; National Institutes of Health). Growth rate was then calculated as area/cohort age (Day 55 cohort arrival day). We used a 1-way ANOVA with a block effect to assess differences in total recruitment between cue treatments. The data were square-root transformed to meet homogeneity of variances assumptions. An ANCOVA was used to analyze for an effect of cue treatment on mortality using the total number of oyster recruits as a covariate. Block did not have a significant effect on mortality so was dropped from the analysis and all data were pooled. We used a mixed-effects model fit by restricted maximum likelihood (REML) to determine differences in oyster growth rate, with cue treatment and cohort arrival day as fixed effects and block as a random effect. The growth data were square-root transformed to meet normality assumptions.



All analyses were performed in R (version 3.4.2; R Core Team 2017). The 'lme4' package (Bates et al. 2015) was used for mixed-effects model analysis and degrees of freedom and p-values were based on Kenward-Roger approximations using the 'lmerTest' package (Kuznetsova et al. 2017). Post hoc comparisons were performed using the 'lsmeans' package (Lenth 2016).

### 2.3. Laboratory behavior experiments

We observed larval behavioral responses when exposed to individual cues in behavior assays to clarify the role of mud crab and crushed oyster cues and better understand how separate cues affect recruitment in the field. Laboratory experiments were conducted at GT using assay chambers that were designed based on Morello & Yund (2016). Assay chambers consisted of two 3.75 cm long  $\times$  0.1 cm wide  $\times$  0.4 cm deep channels connected by a center well (0.5 cm diam.  $\times$  0.4 cm depth) and with 2 opposite end wells (Fig. 2). These assay chambers were milled into a 1.27 cm thick acrylic plastic sheet and the total volume of the chamber was 12 ml. The center well served as the starting position for the oyster larvae. The end wells, which were 3.75 cm diameter and 0.8 cm deep with a rounded bottom, contained either the treatment cue or control seawater. Dye studies indicated that the channel within 0.5 cm of the center chamber remained free of cues for the duration of the experiment. Therefore, we defined the region within 0.5 cm of the center well edges on each side as the 'no-response' zone using lines marked on the underside of the acrylic sheet.

The treatment well contained one of the following cues: seawater conditioned with whole adult oysters,

crushed adult oysters, or metabolites from mud crabs or hermit crabs. Seawater conditioned with whole adult oysters was expected to attract oyster larvae as indicated by both the field experiment and previous work (Tamburri et al. 1992, Barnes et al. 2010). The field results were ambiguous regarding the salience of cues from crushed adult oysters because alarm cues did not deter larval settlement but did induce a growth response in recently settled juveniles (see below). Mud crab conditioned seawater was anticipated to deter oyster larvae based on juvenile induction responses (Johnson et al. 2014, Scherer & Smee 2017). Seawater conditioned with hermit crabs *Clibanarius vittatus* was included as a neutral control to assess how oyster larvae respond to general crab metabolites. Mud crabs were fed oyster flesh *ad libitum* for 48 h prior to cue-conditioning, whereas hermit crabs were fed commercial algae wafers. The treatment water was made by bathing either 2 crushed adult oysters (hinge length: 60–70 mm, wet tissue weight: ~6 g), 2 whole adult oysters (hinge length: 65–75 mm, wet tissue weight: ~6 g), 1 mud crab (carapace width: 29–30 mm), or 1 hermit crab (carapace width: 13–15 mm) in 500 ml of autoclaved artificial seawater (20 ppt salinity, 22–24°C water temperature) for 8 h. The crushed oyster treatment was prepared in the same fashion as the field experiment using the blunt end of an oyster knife. Organisms were removed after conditioning and water was passed through a 6  $\mu$ m filter. Cue-conditioned seawater was then used immediately in behavior experiments. Fresh batches of cue-conditioned seawater were made using different organisms for each day of trials.

Behavior experiment trials were conducted under infrared (IR) illumination to eliminate the potential for phototaxis (Thorson 1964, Wheeler et al. 2017).

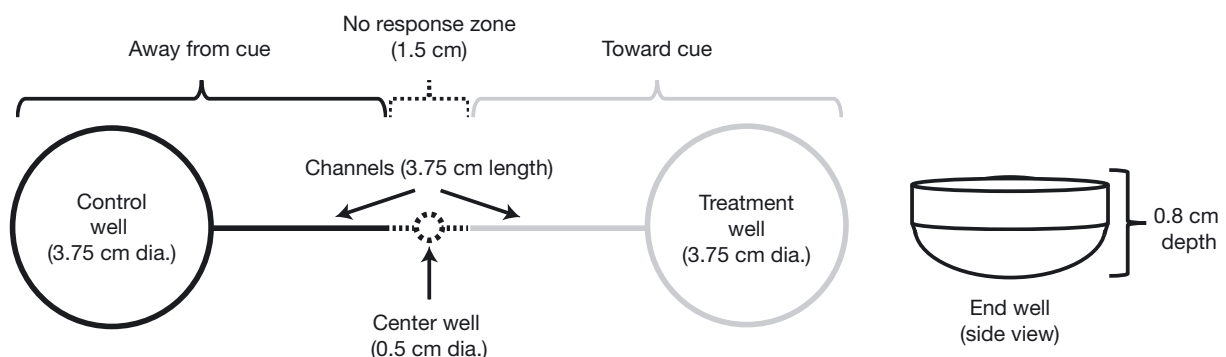


Fig. 2. Diagram of assay chamber (based on Morello & Yund 2016). A single pediveliger eastern oyster larvae was pipetted into the center well and cue-conditioned seawater (see Section 2.3 for treatments) was injected into the randomly chosen right or left end well (shown as right well above). The location of the oyster larvae after 30 min was recorded as either away from cue, no response, or toward cue

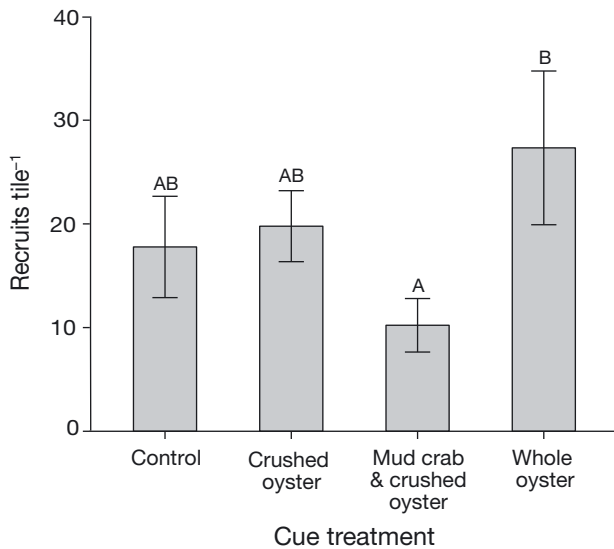


Fig. 3. Mean ( $\pm$ SE) number of eastern oyster recruits on caged tiles surrounded by different cue treatments in the field after 8 wk ( $n = 9$ ). Different letters denote means that are significantly different based on Tukey's post hoc tests ( $p < 0.05$ )

Assay chambers were first filled with 8 ml of sterile artificial seawater. Then, a single eyed pediveliger oyster larvae was added to the center well and confirmed to be actively swimming. One well was randomly predetermined as the treatment well and 1 ml of cue-conditioned seawater was slowly injected into the bottom of the well using a 1 ml syringe with a 26 gauge needle. The same volume (1 ml) of sterile artificial seawater was then injected in the opposite well. The position of the larvae was recorded after 30 min

as away from treatment well, no response (within the 'no-response zone' defined above), or toward the treatment well (Fig. 2). Trials were ended at 30 min because dye visualization revealed the dye had diffused to the borders of the no-response zone by this time. One trial of each treatment cue, including a sterile seawater control, was run simultaneously as a block. Each assay chamber was rinsed thoroughly with distilled water after every trial. Blocks were repeated 17 times across 2 d with the same batch of larvae.

The behavioral response of larvae to move away or toward a cue treatment was tested against a random response prediction (1:1 ratio) to determine if larvae were attracted or deterred by a given cue treatment. Individual exact 1-sided binomial tests were performed for behavior experiments with cue treatments that were predicted to elicit a directional response in larvae (see above; crushed adult oyster cue, mud crab cue, whole oyster cue) and 2-sided binomial tests were used for behavior experiments for cue treatments with no predicted response (hermit crab cue, seawater control).

### 3. RESULTS

#### 3.1. Field recruitment experiment

Oyster recruitment on caged tiles depended on cue treatment ( $F_{3,24} = 3.04$ ,  $p = 0.048$ ; Fig. 3), with the block effect showing marginal significance ( $F_{8,24} = 2.25$ ,  $p = 0.06$ ). Oyster recruitment was lowest in the presence of mud crabs foraging on crushed oysters (i.e. predator metabolites and alarm cue) but was only significantly different from recruitment near whole juvenile oysters. Recruitment on tiles surrounded by either crushed juvenile oysters (alarm cue only) or control crushed oyster shell was intermediate between these 2 treatments.

Weekly monitoring of oyster recruitment revealed 2 major recruitment events on Days 26 and 55 (Fig. 4). The pattern that occurred during each pulse was consistent with the overall trends obtained from the counts at the end of the experiment. Generally, oyster recruitment each week was highest on the tiles surrounded by whole oysters, and during the major recruitment events averaged  $13 \pm 2$  new oyster recruits tile<sup>-1</sup> wk<sup>-1</sup>. Weekly recruitment was lowest on tiles surrounded by mud crabs foraging

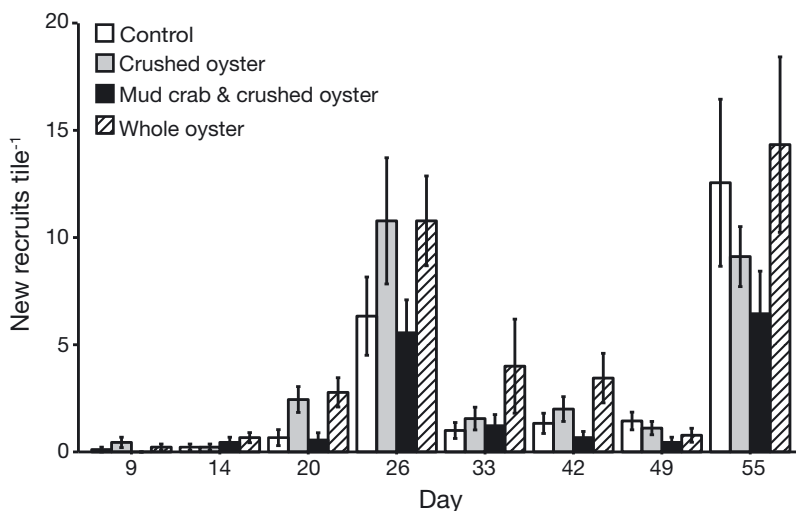


Fig. 4. Mean ( $\pm$ SE) number of new eastern oyster recruits on caged tiles surrounded by each cue treatment on different days during the 55 d field experiment

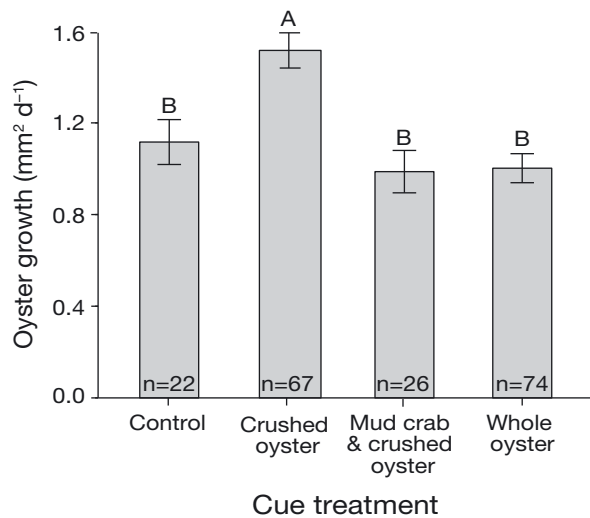


Fig. 5. Mean ( $\pm$ SE) growth rate of oyster recruits on caged tiles surrounded by different cue treatments in the field. Different letters denote means that are significantly different based on Tukey's post hoc tests ( $p < 0.05$ )

on crushed oysters and averaged  $6 \pm 1$  new recruits  $\text{wk}^{-1}$  during the major recruitment events. Weekly recruitment during the major recruitment events to tiles surrounded by control crushed oyster shells or crushed adult oysters was not consistently high or low relative to the other treatments and averaged about  $10 \pm 2$  new oyster recruits during these events  $\text{wk}^{-1}$  (Fig. 4).

Oyster recruit mortality was determined as the number of scars on the tiles at the end of the experiment and was not affected by cue treatment ( $F_{3,28} = 0.41$ ,  $p = 0.75$ ). However, the covariate (number of recruits) significantly affected mortality ( $F_{1,28} = 23.67$ ,  $p < 0.0001$ ). This density-dependence in the absence of a treatment effect suggests resource competition had strong impacts on juvenile survival. There was no significant interaction between cue treatment and the covariate ( $F_{3,28} = 0.44$ ,  $p = 0.73$ ).

Growth rate of the oyster recruits for the 3 cohorts that arrived between Days 20 and 33 (and survived until the end of the experiment) was significantly affected by cue treatment ( $F_{3,174.1} = 4.88$ ,  $p < 0.01$ ) and cohort arrival date ( $F_{2,171.8} = 15.18$ ,  $p < 0.0001$ ). There was no significant interaction between cue treatment and cohort arrival date ( $F_{6,172.2} = 1.15$ ,  $p = 0.33$ ). Oyster recruits on tiles surrounded by crushed adult oysters on average gained shell area 47.5% faster than oyster recruits exposed to the other cue treatments (Fig. 5). Also, oyster recruits in the older cohorts (arrival Day 20 and 26) had higher growth rates than the youngest cohort (arrival Day 33).

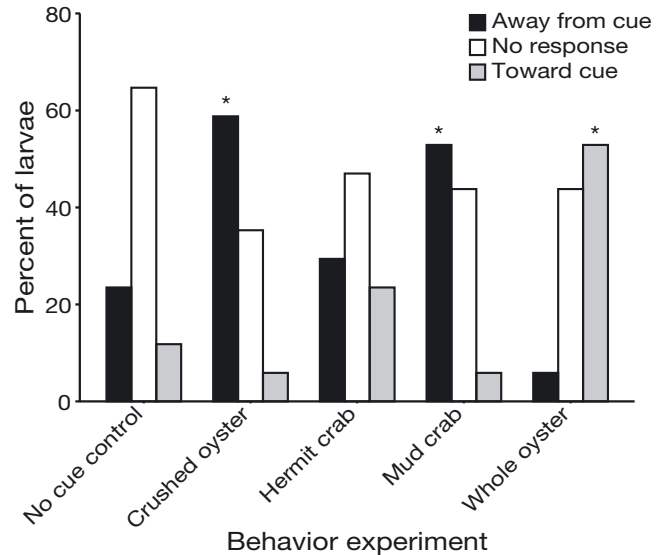


Fig. 6. Percent of eastern oyster larvae that moved away from cue, remained in center of assay chamber (i.e. no response), or moved toward cue treatment for each behavior experiment which presented larvae with cue-conditioned seawater versus seawater control ( $n = 17$ ). Asterisks: oyster larvae moved away from or toward cue treatment with significantly higher than expected frequency relative to a 1:1 ratio ( $p < 0.05$ )

### 3.2. Laboratory behavior experiments

Oyster larvae moved away from wells that contained seawater conditioned with either crushed adult oysters or mud crabs with significantly higher frequencies than a null 1:1 response ratio (exact 1-sided binomial test; crushed oyster,  $p < 0.01$ ; mud crab,  $p = 0.01$ ) (Fig. 6). In contrast, oyster larvae moved toward wells with seawater conditioned with whole adult oysters (exact 1-sided binomial test;  $p = 0.01$ ) (Fig. 6). Oyster larvae showed no aversion or attraction to wells that contained hermit crab conditioned seawater or no cue seawater control (exact 2-sided binomial test; hermit crab,  $p = 1.0$ ; no cue,  $p = 0.69$ ) (Fig. 6).

## 4. DISCUSSION

Mortality due to predation is high during marine invertebrate recruitment (Gosselin & Qian 1997) but larvae can reduce their risk of predation by avoiding settlement near predation risk cues (Ellrich et al. 2015, Ehlers et al. 2018). We found that oyster recruitment in our field experiment was reduced only when chemical cues from both foraging predators and crushed conspecifics were present and there was

no effect of conspecific alarm cues alone. Oyster recruitment was highest in the presence of juvenile oysters, but this positive effect was mitigated by the combined negative effects of mud crabs foraging on crushed oysters (Fig. 3). Weekly recruitment patterns also showed that the number of oyster recruits was always lowest near the combination of predator and injured prey cues (Fig. 4). Oyster larvae were deterred by crushed oyster chemicals in the absence of flow and background odors in the lab behavior experiment (Fig. 6), but these alarm cues by themselves were insufficient to affect recruitment under natural conditions in our field experiment.

Several taxa of larvae avoid future predator-associated cues during settlement (Ellrich et al. 2015, Benkwitt 2017, Ehlers et al. 2018) but we believe this is the first demonstration that oyster larvae also are deterred by predation risk cues. Further, reduced settlement in the field required a combination of injured conspecific and predator cues even though both seem aversive in the laboratory assays. The effect of crushed conspecific cue in the lab may reflect high and persistent alarm cue concentrations due to the lack of flow and background chemical noise. Waterborne cues are mixed by turbulent flow in the field, which creates filaments of cues with variable concentrations that larvae transiently encounter (Koehl & Hadfield 2010). Crushed conspecific cues might not have been present in high enough concentrations to be a reliable signal for predation under our flow conditions in the field. However, our field site was characterized by high turbulence levels relative to other sites in the area (Wilson et al. 2013), which suggests that crushed conspecific cues may be available in enough concentration to affect settlement at lower flow sites. Barnacle recruitment was reduced by predator cues in wave-sheltered environments but not wave-exposed conditions, which also supports that larval ability to detect negative cues may be hindered in high flow conditions (Ellrich & Scrosati 2016).

Alternatively, due to the high cost of delayed metamorphosis (Pechenik et al. 1998, Bishop et al. 2006), oyster larvae may require a more reliable indication of high predation risk to avoid settlement than alarm cues encode, which only signal prey are being damaged but not necessarily by a foraging predator. Generally, more costly defenses, such as morphological changes, are not induced by alarm cues alone, but require cues from predators fed conspecific prey (Schoeppner & Relyea 2009, Bourdeau 2010). It is not clear if this is because predator diet cues provide qualitatively different information that conveys

greater risk than alarm cues or if the quantitative concentration of both cues work additively (Scherer & Smee 2016). Thus, settlement in our field experiment was reduced either because the combination of mud crab and crushed conspecific cues produced a greater concentration of predation risk cues or the addition of predator cues with the alarm cue encodes a higher level of predation threat.

As in other systems, predator identity and diet affect the salience of predator-derived cues (Weissburg et al. 2014, Scherer & Smee 2016) for oysters. Oyster larvae were seemingly able to distinguish between crab species that reflect different levels of predation threat in the lab behavior experiments. Larvae were about equally as likely to move away from or towards seawater conditioned with hermit crabs (Fig. 6), which do not represent a threat to oysters because they are primarily grazers and have weak claws that are unable to crush oyster shells (Williams 1984). It is unclear if oyster larvae encoded this difference in risk by using predator-specific chemical cues or diet-associated cues, since hermit crabs were fed an algae diet. Diet cues contained in predator exudates are often used by prey in combination with predator odor to assess the level of predation risk (Chivers & Mirza 2001, Scherer & Smee 2016). Fish larvae avoid chemical cues released by either carnivorous or herbivorous fish fed fish diets when settling (Dixon et al. 2012). Juvenile oysters also only induce production of stronger shells when exposed to blue crabs fed fresh oyster flesh and do not respond to starved blue crabs or blue crabs fed aged oyster flesh (Scherer et al. 2016, 2017), suggesting that oyster larvae may use dietary cues as well. Further research is needed to determine if oyster larvae can identify and respond to other predators during settlement decisions and if risk is encoded through predator diet. Regardless, these results show that consumption of oyster flesh is at the very least necessary for larvae to interpret metabolites released from predators as indicative of predation risk.

Oyster larvae that settled near crushed conspecifics grew faster (i.e. increased shell area more quickly) than any of the other treatments despite a lack of reduced recruitment (Fig. 5). This may reflect a less costly strategy to respond to predation risk when it is perceived as less certain. Frog tadpoles only increase hiding behavior in response to crushed conspecifics but decrease activity and alter morphology in response to the riskier cue of an actively foraging predator (Schoeppner & Relyea 2009). Oyster larvae may avoid settling near actively feeding future predators that convey immediate risk but disregard



ambiguous conspecific alarm cues during settlement, while still defending themselves at least partially once settled. The faster shell growth in response to crushed conspecifics may be a generalized response to reach a size refuge more quickly by utilizing less energetically expensive inorganic calcium carbonate to rapidly attain larger sizes more resistant to predation (Scherer et al. 2018).

Alternatively, the growth rate increase in the presence of crushed oysters may not reflect an antipredator response but may be the result of increased dissolved nutrients in the water column that could benefit growth in several ways. First, crushed oysters leak dissolved organic matter, such as amino acids (Carr et al. 1996, Zimmer et al. 1999), that oysters can uptake directly and potentially use to supplement metabolism and enhance growth (Ferguson 1982, Langdon & Newell 1996, Wendt & Johnson 2006). Secondly, the dissolved organic matter leaked from crushed oysters may be rapidly mineralized by bacteria, which provides a secondary source of nutrition for oyster spat leading to increased growth (Crosby et al. 1990). Although either dissolved nutrients or bacteria may be utilized by bivalves as a dietary supplement it is still unclear if this benefits shell growth, especially under field conditions (Knauer & Southgate 1999). Lastly, the increase in food availability may stimulate feeding activity (Higgins 1980), but little is known about chemical feeding stimulants in suspension-feeders and no isolated waterborne compounds have been shown to increase oyster feeding rates (Ward & Targett 1989, Tamburri & Zimmer-Faust 1996).

The combination of mud crab and injured oyster metabolites had no effect on shell growth despite previous lab studies that show both mud (Scherer & Smee 2017) and blue crab predator cues (Robinson et al. 2014, Scherer et al. 2016) increase shell size. However, other studies have shown that oyster spat can increase shell strength without noticeable changes in shell size or weight. Predator cues from mud crabs may increase the proportion of energetically costly organic shell material to add strength independent of shell mass (Newell et al. 2007, Robinson et al. 2014). Thus, our results suggest that predation risk encoded by actively feeding mud crab predators causes juvenile oysters to respond by investing in stronger organic shell material that is more resistant to crushing. Alarm cues alone may convey a lesser level of risk so that oysters respond with a less costly strategy of using cheaper inorganic shell content to reach a size refuge rapidly. Direct measurements of shell strength are required to evaluate fully the responses

of oysters, and perhaps other bivalves, to aversive cues singly and in combination.

Inducible morphological defenses are costly and thus may not be produced when resources are scarce (Harvell 1990). Recently settled oysters may have been resource-limited in our field experiment resulting in a lack of growth response in the combination mud crab and crushed oyster cue treatment. Yet 3 wk old oysters induced the same intensity of morphological responses to mud crab predators under both low and high resource availability in an 8 wk laboratory experiment (Scherer & Smee 2017), which suggests oysters may respond morphologically to predation risk even when resources are limited. The duration of our field experiment may not have been long enough to detect morphological changes in the mud crab with crushed oyster treatment (i.e. only 3 to 5 wk exposure) or perhaps juvenile oysters must reach a certain point in development before changes in shell structure due to predation risk occur.

Predation has a strong influence on oyster recruitment and population structure (Knights et al. 2012, Johnson et al. 2014, Carroll et al. 2015). Our study demonstrates that an important juvenile oyster predator also may affect population densities through non-consumptively deterring oyster larvae settlement. Additionally, oysters that still settle in the presence of predators may alter their shell morphology to resist predation attempts (Newell et al. 2007, Robinson et al. 2014, Scherer et al. 2016). These induced morphological changes may come at the cost of reduced fecundity (Harvell 1990, Johnson & Smee 2012, Scherer et al. 2018), which will further modify oyster populations. Thus, consumptive and non-consumptive predator effects at each life stage of the oyster need to be considered to understand the persistence of this foundation species.

*Acknowledgements.* We thank Jeff Beauvais and Nolan Hubbard for field assistance. We also appreciate the help and support of the staff at the Skidaway Institute of Oceanography and the Shellfish Research Lab at UGA Marine Extension and Georgia Sea Grant. This research was funded by NSF grant Bio-OCE #1234449 awarded to M.J.W.

#### LITERATURE CITED

- ✦ Atherton JA, McCormick MI (2015) Active in the sac: damselfish embryos use innate recognition of odours to learn predation risk before hatching. *Anim Behav* 103:1–6
- ✦ Barnes BB, Luckenbach MW, Kingsley-Smith PR (2010) Oyster reef community interactions: the effect of resident fauna on oyster (*Crassostrea* spp.) larval recruitment. *J Exp Mar Biol Ecol* 391:169–177
- ✦ Bates D, Machler M, Bolker BM, Walker SC (2015) Fitting

- linear mixed-effects models using lme4. *J Stat Softw* 67: 1–48
- ✦ Benkwitt CE (2017) Predator effects on reef fish settlement depend on predator origin and recruit density. *Ecology* 98:896–902
- ✦ Bible JM, Griffith KR, Sanford E (2017) Inducible defenses in Olympia oysters in response to an invasive predator. *Oecologia* 183:809–819
- ✦ Bishop CD, Huggett MJ, Heyland A, Hodin J, Brandhorst BP (2006) Interspecific variation in metamorphic competence in marine invertebrates: the significance for comparative investigations into the timing of metamorphosis. *Integr Comp Biol* 46:662–682
- ✦ Boudreau B, Bourget E, Simard Y (1993) Behavioural responses of competent lobster postlarvae to odor plume. *Mar Biol* 117:63–69
- ✦ Bourdeau PE (2010) Cue reliability, risk sensitivity and inducible morphological defense in a marine snail. *Oecologia* 162:987–994
- ✦ Brookes JJ, Rochette R (2007) Mechanism of a plastic phenotypic response: predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*. *J Evol Biol* 20: 1015–1027
- Burke RD (1986) Pheromones and the gregarious settlement of marine invertebrate larvae. *Bull Mar Sci* 39:323–331
- ✦ Carr WES, Netherton JC, Gleeson RA, Derby CD (1996) Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organisms. *Biol Bull (Woods Hole)* 190: 149–160
- ✦ Carroll JM, Marion JP, Finelli CM (2015) A field test of the effects of mesopredators and landscape setting on juvenile oyster, *Crassostrea virginica*, consumption on intertidal reefs. *Mar Biol* 162:993–1003
- Chivers DP, Mirza RS (2001) Predator diet cues and the assessment of predation risk by aquatic vertebrates: a review and prospectus. In: Marchlewska-Koj A, Lepri JJ, Müller-Schwarze D (eds) *Chemical signals in vertebrates*, Vol 9. Springer, Boston, MA, p 277–284
- ✦ Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience* 5:338–352
- ✦ Chivers DP, Mirza RS, Bryer PJ, Kiesecker JM (2001) Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. *Can J Zool* 79:867–873
- Crisp DJ (1984) Overview of research on marine invertebrate larvae, 1940–1980. In: Costlow JD, Tipper RC (eds) *Marine biodeterioration: an interdisciplinary study*. Naval Institute Press, Annapolis, MD, p 103–126
- ✦ Crosby MP, Newell RIE, Langdon CJ (1990) Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. *Limnol Oceanogr* 35:625–639
- ✦ Dixon DL, Pratchett MS, Munday PL (2012) Reef fishes innately distinguish predators based on olfactory cues associated with recent prey items rather than individual species. *Anim Behav* 84:45–51
- ✦ Ehlers SM, Scrosati RA, Ellrich JA (2018) Nonconsumptive predator effects on prey demography: dogwhelk cues decrease benthic mussel recruitment. *J Zool (Lond)* 305: 240–245
- ✦ Ellrich JA, Scrosati RA (2016) Water motion modulates predator nonconsumptive limitation of prey recruitment. *Ecosphere* 7:e01402
- ✦ Ellrich JA, Scrosati RA, Molis M (2015) Predator nonconsumptive effects on prey recruitment weaken with recruit density. *Ecology* 96:611–616
- ✦ Ellrich JA, Scrosati RA, Romoth K, Molis M (2016) Adult prey neutralizes predator nonconsumptive limitation of prey recruitment. *PLOS ONE* 11:e0154572
- ✦ Ferguson JC (1982) A comparative study of the net metabolic benefits derived from the uptake and release of free amino acids by marine invertebrates. *Biol Bull (Woods Hole)* 162:1–17
- ✦ Ferrari MCO, Wisenden BD, Chivers DP (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can J Zool* 88:698–724
- ✦ Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146:265–282
- ✦ Grabowski JH, Brumbaugh RD, Conrad RF, Keeler AG and others (2012) Economic valuation of ecosystem services provided by oyster reefs. *BioScience* 62:900–909
- Hadfield MG, Paul V (2001) Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock JB, Baker BJ (eds) *Marine chemical ecology*. CRC Press, Boca Raton, FL, p 431–461
- ✦ Harvell CD (1990) The ecology and evolution of inducible defenses. *Q Rev Biol* 65:323–340
- ✦ Hidu H (1969) Gregarious setting in the American oyster *Crassostrea virginica* Gmelin. *Chesap Sci* 10:85–92
- ✦ Higgins PJ (1980) Effects of food availability on the valve movements and feeding behavior of juvenile *Crassostrea virginica* (Gmelin). 1. Valve movements and periodic activity. *J Exp Mar Biol Ecol* 45:229–244
- ✦ Hill JM, Weissburg MJ (2013) Predator biomass determines the magnitude of non-consumptive effects (NCEs) in both laboratory and field environments. *Oecologia* 172:79–91
- ✦ Holmes TH, McCormick MI (2010) Smell, learn and live: the role of chemical alarm cues in predator learning during early life history in a marine fish. *Behav Processes* 83: 299–305
- ✦ Johnson KD, Smee DL (2012) Size matters for risk assessment and resource allocation in bivalves. *Mar Ecol Prog Ser* 462:103–110
- ✦ Johnson KD, Grabowski JH, Smee DL (2014) Omnivory dampens trophic cascades in estuarine communities. *Mar Ecol Prog Ser* 507:197–206
- ✦ Johnson LE, Strathmann RR (1989) Settling barnacle larvae avoid substrata previously occupied by a mobile predator. *J Exp Mar Biol Ecol* 128:87–103
- ✦ Kats LB, Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394
- Keck R, Maurer D, Kauer JC, Sheppard WA (1971) Chemical stimulants affecting larval settlement in the American oyster. *Proc Natl Shellfish Assoc* 61:24–28
- ✦ Knauer J, Southgate PC (1999) A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. *Rev Fish Sci* 7:241–280
- ✦ Knights AM, Firth LB, Walters K (2012) Interactions between multiple recruitment drivers: post-settlement predation mortality and flow-mediated recruitment. *PLOS ONE* 7: e35096
- ✦ Koehl MAR, Hadfield MG (2010) Hydrodynamics of larval settlement from a larva's point of view. *Integr Comp Biol* 50:539–551
- ✦ Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. *J Stat Softw* 82:1–26

- Langdon CJ, Newell RIE (1996) Digestion and nutrition in larvae and adults. In: Kennedy VS, Newell RIE, Eble AF (eds) *The eastern oyster Crassostrea virginica*. Maryland Sea Grant College, College Park, MD, p 231–269
- ✦ Lenihan HS, Peterson CH (1998) How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. *Ecol Appl* 8:128–140
- ✦ Lenth RV (2016) Least-squares means: the R package lsmmeans. *J Stat Softw* 69:1–33
- ✦ Leonard GH, Bertness MD, Yund PO (1999) Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. *Ecology* 80:1–14
- ✦ Lima SL (1998) Nonlethal effects in the ecology of predator-prey interactions: What are the ecological effects of anti-predator decision-making? *BioScience* 48:25–34
- ✦ Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640
- ✦ Lively CM (1986a) Competition, comparative life histories, and maintenance of shell dimorphism in a barnacle. *Ecology* 67:858–864
- ✦ Lively CM (1986b) Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. *Evolution* 40:232–242
- ✦ Morello SL, Yund PO (2016) Response of competent blue mussel (*Mytilus edulis*) larvae to positive and negative settlement cues. *J Exp Mar Biol Ecol* 480:8–16
- Newell RIE (2004) Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J Shellfish Res* 23:51–61
- ✦ Newell RIE, Kennedy VS, Shaw KS (2007) Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. *Mar Biol* 152:449–460
- Pawlik JR (1992) Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr Mar Biol Annu Rev* 30:273–335
- ✦ Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning. *BioScience* 48:901–910
- ✦ Pineda J, Reyns NB, Starczak VR (2009) Complexity and simplification in understanding recruitment in benthic populations. *Popul Ecol* 51:17–32
- ✦ Pruett JL, Weissburg MJ (2018) Hydrodynamics affect predator controls through physical and sensory stressors. *Oecologia* 186:1079–1089
- R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- ✦ Robinson EM, Lunt J, Marshall CD, Smee DL (2014) Eastern oysters *Crassostrea virginica* deter crab predators by altering their morphology in response to crab cues. *Aquat Biol* 20:111–118
- ✦ Roughgarden J, Gaines S, Possingham HP (1988) Recruitment dynamics in complex life cycles. *Science* 241:1460–1466
- ✦ Scherer AE, Smee DL (2016) A review of predator diet effects on prey defensive responses. *Chemoecology* 26:83–100
- ✦ Scherer AE, Smee DL (2017) Eastern oysters *Crassostrea virginica* produce plastic morphological defenses in response to crab predators despite resource limitation. *Biol Bull (Woods Hole)* 233:144–150
- ✦ Scherer AE, Lunt J, Draper AM, Smee DL (2016) Phenotypic plasticity in oysters (*Crassostrea virginica*) mediated by chemical signals from predators and injured prey. *Invertebr Biol* 135:97–107
- ✦ Scherer AE, Garcia MM, Smee DL (2017) Predatory blue crabs induce stronger nonconsumptive effects in eastern oysters *Crassostrea virginica* than scavenging blue crabs. *PeerJ* 5:e3042
- ✦ Scherer AE, Bird CE, McCutcheon MR, Hu XP, Smee DL (2018) Two-tiered defense strategy may compensate for predator avoidance costs of an ecosystem engineer. *Mar Biol* 165:131
- ✦ Schmitz OJ, Grabowski JH, Peckarsky BL, Preisser EL, Trussell GC, Vonesh JR (2008) From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology* 89:2436–2445
- ✦ Schoepfner NM, Relyea RA (2009) Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. *Funct Ecol* 23:1114–1121
- ✦ Sherker ZT, Ellrich JA, Scrosati RA (2017) Predator-induced shell plasticity in mussels hinders predation by drilling snails. *Mar Ecol Prog Ser* 573:167–175
- ✦ Smee DL, Overath RD, Johnson KD, Sanchez JA (2013) Intraspecific variation influences natural settlement of eastern oysters. *Oecologia* 173:947–953
- ✦ Tamburri MN, Zimmer-Faust RK (1996) Suspension feeding: basic mechanisms controlling recognition and ingestion of larvae. *Limnol Oceanogr* 41:1188–1197
- ✦ Tamburri MN, Zimmer-Faust RK, Tamplin ML (1992) Natural sources and properties of chemical inducers mediating settlement of oyster larvae: a re-examination. *Biol Bull (Woods Hole)* 183:327–338
- ✦ Tamburri MN, Finelli CM, Wetthey DS, Zimmer-Faust RK (1996) Chemical induction of larval settlement behavior in flow. *Biol Bull (Woods Hole)* 191:367–373
- ✦ Thorson G (1964) Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia* 1:167–208
- ✦ Vail AL, McCormick MI (2011) Metamorphosing reef fishes avoid predator scent when choosing a home. *Biol Lett* 7:921–924
- ✦ von der Meden CEO, Cole VJ, McQuaid CD (2015) Do the threats of predation and competition alter larval behaviour and selectivity at settlement under field conditions? *J Exp Mar Biol Ecol* 471:240–246
- ✦ Ward JE, Targett NM (1989) Influence of marine microalgal metabolites on the feeding behavior of the blue mussel *Mytilus edulis*. *Mar Biol* 101:313–321
- ✦ Weissburg M, Smee DL, Ferner MC (2014) The sensory ecology of nonconsumptive predator effects. *Am Nat* 184:141–157
- ✦ Welch JM, Rittschof D, Bullock TM, Forward RB Jr (1997) Effects of chemical cues on settlement behavior of blue crab *Callinectes sapidus* postlarvae. *Mar Ecol Prog Ser* 154:143–153
- ✦ Wells HW (1961) The fauna of oyster beds, with special reference to the salinity factor. *Ecol Monogr* 31:239–266
- ✦ Wendt DE, Johnson CH (2006) Using latent effects to determine the ecological importance of dissolved organic matter to marine invertebrates. *Integr Comp Biol* 46:634–642
- ✦ Werner EE, Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100
- ✦ Wheeler JD, Luo E, Helfrich KR, Anderson EJ, Starczak VR, Mullineaux LS (2017) Light stimulates swimming behavior of larval eastern oysters *Crassostrea virginica* in turbulent flow. *Mar Ecol Prog Ser* 571:109–120

Williams AB (1984) Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, DC

✦ Wilson ML, Webster DR, Weissburg MJ (2013) Spatial and temporal variation in the hydrodynamic landscape in

intertidal salt marsh systems. *Limnol Oceanogr Fluids Environ* 3:156–172

✦ Zimmer RK, Commins JE, Browne KA (1999) Regulatory effects of environmental chemical signals on search behavior and foraging success. *Ecology* 80:1432–1446

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

*Submitted: January 24, 2019; Accepted: May 20, 2019  
Proofs received from author(s): July 1, 2019*