Chemical sensing of microhabitat by pueruli of the reef-dwelling Caribbean spiny lobster Panulirus argus: testing the importance of red algae, juveniles, and their interactive effect

J Antonio Baeza 1,2,3*
Michael J Childress 1
Louis J Ambrosio 1

ABSTRACT.—Although our knowledge about the early life of the Caribbean spiny lobster, Panulirus argus (Latreille, 1804), has increased considerably during recent decades, little is known about chemical sensing used by pueruli during settlement. Considering previously-reported benefits of inhabiting red algae Laurencia spp., such as increased growth rate and costs of close proximity to already settled benthic juveniles from predators, we predicted that the pueruli of P. argus will be attracted to the metabolites produced by red algae and repulsed by metabolites produced by juvenile conspecifics. We also expected that any preference for Laurencia spp. would cease or decrease if this cue was presented together with metabolites produced by conspecific given the reported costs of associating with them after settlement. Our results were not consistent with the predictions above. Our experiments suggest that pueruli do not display any preference for the metabolites produced by red algae or avoidance of metabolites produced by juvenile conspecifics. Unexpectedly, settling stages were attracted to water with a combination of metabolites produced by red algae and juveniles. We also examined the influence of conspecific and red algae abundance on pueruli settlement in Florida Bay, the most important nursery ground of P. argus in the USA. Partially consistent with our experiments, field data indicated that juvenile lobster density had a positive influence on pueruli settlement, as did a synergistic effect of juvenile lobster density and Laurencia spp. algae cover. Altogether, our field and laboratory data suggest that the interplay of environmental cues drives settlement of the Caribbean spiny lobster in a more complex manner than originally thought. Additional studies on the settling behavior of P. argus pueruli are needed to improve our understanding of the relationship between recruitment and fishery stocks in this heavily exploited species.
microhabitats with particular sets of characteristics might provide greater net benefits than other microhabitats (Hadfield and Paul 2001). Some type of microhabitats might even result in net costs to settlers, such as decreased growth rate in overcrowded microhabitats (Donahue 2006). Therefore, settling stages exhibiting mechanisms that allow them to find and colonize particular types of microhabitats, in which the net benefits are maximized, should be favored by natural selection (Wilson 2000). Overall, settling stages are expected to display strong preferences for and the ability to sense cues from microhabitats that provide the greatest net benefits to them, and to avoid cues from other microhabitats that result in net costs.

Settling stages use a variety of cues to transition from the pelagic to the benthic environment, including, but not limited to, lunar phase (Herrnkind and Butler 1986), chemical odors (Hadfield and Paul 2001), surface rugosity (Prendergast et al. 2008), pressure (Goldstein and Butler 2009), sound (Leis and Carson-Ewart 2003, Jeffs et al. 2005, Hinojosa et al. 2016), or a combination of two or more of the above (Hadfield and Paul 2001). Importantly, the efficiency with which the environment transmits and/or individuals perceive cues depends upon abiotic conditions. In shallow marine environments, for instance, sound and light propagation can be erratic due to sudden shifts in water turbidity and/or density (Astoreca et al. 2006), in turn, impacting the ability of individuals to perceive the direction and/or distance of the signal’s origin (Meager et al. 2005). Similarly, settling stages of many marine organisms exhibit nocturnal habits presumed to be advantageous to avoiding predation (Thorson 1964, Acosta and Butler 1999), and thus, relying on chemical cues, rather than on visual signals, might be more efficient in these environments. Studies examining the sensing ability of both vertebrates and invertebrates are warranted to improve our understanding of communication pathways and signal constraints during settlement and metamorphosis in marine environments.

In the present study, we were particularly interested in exploring whether or not sensing and preference/aversion for particular chemical cues occurs during settlement of the Caribbean spiny lobster, *Panulirus argus* (Latreille, 1804), a keystone species in shallow water coral reefs (Behringer and Butler 2006, Higgs et al. 2016) and target of the most lucrative fishery in the greater Caribbean region (Holthuis 1991, Tavares 2002). The early life cycle of *P. argus* is well known (Holthuis 1991, Booth and Phillips 1994, Herrnkind et al. 1994, Phillips et al. 2013). Adult females can produce 2–4 clutches of eggs per year with larger, older females reproducing earlier and having more clutches per year (Maxwell et al. 2009). Fecundity ranges between 100,000 and 750,000 eggs per female and increases with female body size (Baeza et al. 2016). After completion of embryo development and hatching of larvae, 10 consecutive planktonic stages succeed one another (Goldstein et al. 2008). These planktotrophic “phyllosomata” larvae can spend 4–18 mo suspended in the water column (Phillips et al. 2006, Goldstein et al. 2008). The 10th larval stage undergoes a metamorphosis offshore, turning into a fast-swimming, short-lived (2–4 wks) “puerulus” post-larval stage with morphology similar to that of juvenile and adult benthic lobsters, but almost devoid of coloration (Phillips et al. 2006). By contrast to the planktotrophic phyllosomata, pueruli are secondarily lecithotrophic (non-feeding) relying on intrinsic energy reserves to reach the coast (Cox et al. 2008). Pueruli actively swim from the open ocean to shallow coastal habitats, where they settle in vegetated habitats—seagrass meadows and macroalgal beds—two major structural components in lobster’s nursery grounds (Butler and Herrnkind 2000,
Briones-Fourzán and Lozano-Álvarez 2001). Feeding resumes immediately after molting to the first fully benthic juvenile stage (Lewis et al. 1952, Felder et al. 1985). Juvenile and subadult lobsters are attracted to the odors of conspecifics (Childress and Herrnkind 1996, 2001, Ratchford and Eggleston 1998, Nevitt et al. 2000) and are often found sharing crevice shelters (Berrill 1975, Childress and Herrnkind 1997). Despite the commercial value and ecological importance of *P. argus*, relatively little is known, especially when compared to juveniles, about the sensory capabilities and preference/avoidance for particular microhabitats exhibited by pueruli.

Tethering experiments have demonstrated that early benthic juveniles suffer higher predation rates in the open sand than in structurally complex substrates, including algae clumps, seagrass patches, octocoral branches, sponge crevices, etc. (Herrnkind and Butler 1986, Smith and Herrnkind 1992, Childress and Herrnkind 1994). However, recently-settled lobsters suffer greater mortality by predators in the field when aggregated at spatial scales <0.75 m than when more broadly spaced (Butler et al. 1997). The benefits arising from inhabiting red algae, such as reduced predation and increased feeding, likely explains why pueruli are attracted to water containing metabolites of the red macroalgae *Laurencia* spp. (Goldstein and Butler 2009). Interestingly, a previous study found no support for avoidance of early benthic juveniles by pueruli during settlement (Zito-Livingston and Childress 2009).

A comprehensive set of laboratory experiments has also revealed that pueruli of *P. argus* display behavioral aversion to hypersaline or hyposaline water and prefer coastal over oceanic (collected 30 km offshore from the reef) and artificial seawater (Goldstein and Butler 2009). Lastly, pueruli settle only at pressures equivalent to depths <5 m, which is where most settlement occurs in the field (Goldstein and Butler 2009). Importantly, the possibility that early benthic juveniles can mask pueruli preferences for otherwise beneficial cues, such as red algae, when presented alone remains to be addressed. Studies on sensing and habitat selection by pueruli of *P. argus* are most relevant to improve our understanding of the processes affecting recruitment in this and other heavily exploited marine invertebrates.

The aim of our study was twofold. First, we tested whether pueruli use water-borne chemical cues originating from conspecifics alone and from red algae alone to avoid or locate potential settling microhabitats, respectively. We predicted attraction to red algae, but avoidance of conspecific lobsters, considering previous experimental work (Herrnkind and Butler 1986, Butler et al. 1997). Also, we were particularly interested in exploring whether a preference for a particular “attractant” (i.e., red algae metabolites) either ceased or decreased when such a cue was presented concomitantly with a second metabolite signaling costs after settling (i.e., presence of early benthic juveniles) that potentially indicates increased mortality risk (Butler et al. 1997). We predicted that any preference for *Laurencia* spp. would cease or diminish if this latter cue was presented together with metabolites produced by conspecifics. Lastly, we explored whether our experimental results could be extrapolated to the field by examining the influence of conspecific and red algae abundance on pueruli settlement in Florida Bay, the most important nursery ground for *P. argus* in the USA (Herrnkind and Butler 1994).
Material and Methods

Collection of *Panulirus argus* Pueruli.—Pueruli used in the present study were collected from modified Witham floating postlarvae collectors (Witham et al. 1964) deployed along the ocean side back reef adjacent to Long Key (24.8190°N, 80.8140°W) and Lower Matacumbe Key (24.8562°N, 80.7350°W), Florida Keys, Florida, USA, during June and July of 2015 and 2016. Pueruli were collected 4–7 d after the new moon and transported from collection sites to the laboratory on Long Key, Florida, in plastic 19-L buckets on board of the R/V Soledad. In the laboratory, pueruli were maintained in the same featureless buckets with aerated seawater (salinity 35–37, 27–29.5 °C) collected between 11–12 km offshore prior to use in chemoreception trials.

Chemical Sensing in *Panulirus argus* pueruli.—We tested the preference of pueruli for chemically neutral control seawater [experiment (control) 1] and for water borne chemical cues originating from primary nursery microhabitat, the red algae *Laurencia* spp. (experiment 2), late benthic juvenile conspecific (experiment 3), and *Laurencia* spp. + juvenile conspecific (experiment 4), in a PVC, three-chambered choice apparatus (Fig. 1A). The experiments above allowed us to explore the
potential for an additive or synergistic effect of the two environmental cues on settlement behavior when combined (experiment 4).

The choice apparatus consisted of a central chamber (270 cm$^3$), in which a single test puerulus was positioned at the start of each replicate in each experiment and two satellite chambers (270 cm$^3$) that were connected to the central chamber and received either experimental water (treated with red algae Laurencia spp., conspecifics, red algae + conspecifics, depending on the experiment) or chemically-neutral control seawater. Chemically neutral control seawater (salinity 35–37) collected from the Atlantic Ocean approximately 11 km off the coast of Long Key served as the control water for all experiments in the study. Chemically neutral control seawater was also used as a stock solution to produce specific treatment solutions; red algal treatment solution (used in experiment 2), conspecific treatment solution (used in experiment 3), and red algae + conspecific treatment solution (used in experiments 4). Red algal treatment solutions were produced by maintaining a 1-L volume of Laurencia spp. collected from P. argus settlement habitat and nursery ground in Florida Bay in 18 L of aerated control water for 3 hrs prior to use in any trial. Before employed in the production of treatment water, all algae were intensively but gently cleaned of debris, epiphytes, and macro invertebrates, then rinsed in control water. Similarly, conspecific treatment solutions were produced by holding two healthy (i.e., with no visible signs of PaV1 infection, missing legs, or exoskeleton lesions) late benthic stage P. argus (25–30 mm CL) collected from Florida Bay in 18 L of aerated control water for 3 hrs prior to use in any trial. Mixed stimuli solutions were generated by holding 1 L of cleaned and rinsed Laurencia spp. and two healthy late benthic stage conspecifics (25–30 mm CL) collected from the same local in 18 L of aerated control water for 3 hrs prior to use in trials. Algae were collected no more than 24 hrs prior to the start of each experiment.

All experimental test pueruli used in this experiment were collected within 96 hrs and replicates (see below for details) were conducted during the night (water temperature: 27–29.5 °C; salinity: 35–38), as preliminary observations and previously published data indicated that pueruli are active during the night (but see Goldstein and Butler 2009). For each replicate choice test, the satellite chamber receiving treatment water was selected randomly and the entire choice apparatus was rinsed in tap water and then control water between replicates. All preparations and intermittent observations were made under red light to minimize inadvertent cues that might influence the choice test. In all experiments, water flow of 30 ml min$^{-1}$ dripped from buckets containing treatment or control seawater into each satellite chamber and drained through a central standpipe in the center chamber (Fig. 1A).

At the start of each replicate, all the chambers in the choice apparatus were filled with control water before a single test puerulus was introduced to the central chamber and allowed to acclimate for 10 min while additional control water circulated through the choice apparatus. After the acclimation period, only control (experiment 1) or control and treatment water (experiment 2: red algae, experiment 3: conspecifics, experiment 4: algae + conspecific) were introduced into opposite satellite chambers. As water flowed from satellite chambers to the central chamber, pueruli had the opportunity to respond to control or treatment solution by walking or swimming up current to a satellite chamber of choice (see Fig. 1A for water flow schematic). Data on puerulus chamber choice (control vs experimental) were initially recorded every 15 min for a total of 1 hr immediately after the introduction of the treatment
solution water into the choice apparatus. Preliminary observations indicated that once a choice was made, pueruli did not leave the satellite chamber originally chosen. Thus, final choice by puerulus was determined by the position of the test individual at the end of the 1 hr trial period.

Puerulus behavioral responses were recorded as: (1) preference for chemically-treated water if the test individual relocated to the satellite chamber receiving chemically-treated water, (2) preference for control water if the test individual relocated to the satellite chamber receiving control water, or (3) no response if the test individual failed to move to any of the two satellite chambers within a maximum of 1 hr. Forty-two replicates were conducted for each of the three experimental treatments described herein. Up to seven identically constructed choice chambers were in operation at any one point in time.

We used a binomial test to compare the frequency of test pueruli choosing between chemically-treated water and control water to that predicted by a random (binomial) distribution (1:1) (Wilson and Hardy 2002). Differences between observed and expected frequencies were interpreted as experimental pueruli exhibiting positive chemotaxis to any of the offered odors during the different experiments. We used the formula in Zar (2014) to calculate the standard error of each observed proportion.

We also used the binomial test to compare the frequency of test pueruli choosing one of the experimental chambers in experiments 2–4 with the frequency of test pueruli choosing one of the experimental chambers in experiment 1, in which no odors were presented to pueruli (Wilson and Hardy 2002). Differences between these distributions were interpreted as experimental pueruli exhibiting “initial attraction” (chemically-stimulated random movement), but not chemotaxis (see above) to any of the offered odors during the different experiments.

Lastly, we tested whether or not there was a synergistic (=multiplicative) interaction between red algae and conspecific metabolites when presented concomitantly to pueruli. In other words, we assessed whether the extent to which the effect of red algae and juveniles when presented together exceeded the effect of each considered individually (VanderWeele and Knol 2014). For this purpose, we first fitted a binomial generalized linear model (GLM) using a log-link function to our data (results from experiments 2, 3, and 4) in R (R Core Team 2013) to estimate the different probabilities of pueruli to be attracted to red algae alone, juveniles alone, or red algae and juveniles when presented concomitantly. Based on this binomial GLM, we then performed a test on deviation from additivity using the R package multcomp (Hothorn et al. 2008). The test compares the observed proportion of pueruli attracted to red algae and juveniles when presented concomitantly to the proportion expected under additivity. Significant differences between proportions (observed > expected) denotes a synergistic (=multiplicative) interaction between the two studied stimuli (see Pallmann and Schaarschmidt 2016).

**Settlement by Panulirus argus Pueruli in the Field.—**To estimate the influence of conspecific cues and red algae abundance on pueruli settlement, we surveyed lobsters at 16 sites in Florida Bay, FL, USA, from June to July 2006 and June to July 2008. Survey sites were located in the middle Florida Keys, from immediately west of Lignum Vitae Key (24°54’08.12”N, 80°41’57.07”W) to immediately northwest of Bamboo Key (24°45’15.06”N, 81°00’09.75”W) (Fig. 1B). Each survey included an area examined (25 × 25 m) for all juvenile lobsters and four 25-m line transects to
estimate the percent cover of red algae. The summed linear distance of Laurencia spp. patches under each transect line was compared to the 100-m total transect distance as the percent cover of red algae (Zito-Livingston and Childress 2009). All juvenile lobsters found in each 25 × 25 m quadrant were collected by a scuba diver during a visual census of all crevice shelters at each site. Lobsters were placed in catch bags and taken to a boat for careful inspection. On board the boat, lobsters were sexed (male and female lobsters are distinguished by the presence or absence of gonopores on the coxae of the fifth pereopods, respectively; Anderson et al. 2013), visually checked for disease (PaV1 virus) as in Behringer et al. (2012), and measured to the nearest 0.1 mm of carapace length with a manual caliper. Juvenile lobsters >25.0 mm CL (large benthic juveniles, or LBJs) that occupied the available crevice shelters were presumed to have settled more than 3 mo earlier. A single LBJ produces sufficient odor cue to influence the orientation of a puerulus (Zito-Livingston and Childress 2009). Juvenile lobsters 10.0–25.0 mm CL (early benthic juveniles, or EBJs) found in red algae, seagrass, or octocorals were presumed to have settled within the last 3 mo on the site where they were collected. The abundance of EBJs was thus considered to be an estimate of pueruli settlement for each site.

The influence of red algae cover and conspecific abundance on pueruli settlement was evaluated using a mixed model multiple regression in the software JMP® version 12 (SAS Institute 2007) with site as a random effect, and LBJ density and percent cover Laurencia spp. algae as fixed effects. LBJ density was square-root transformed and percent cover algae arc-sine square–root transformed to meet the assumptions of normality and homogeneity of variances. Disease frequency was low (<5%) and was not found to influence pueruli settlement.

Results

Sensing by Panulirus argus Pueruli.—In the first (control) experiment, 7 out of 42 P. argus test pueruli (16.67%) relocated to a satellite chamber, and, among those 7 individuals responding, 4 pueruli chose one chamber with control water while 3 pueruli chose the opposite chamber with control water (Fig. 2). The proportion of test pueruli choosing between the two chambers did not differ significantly from that expected by chance alone (binomial test: \(P = 0.5\)).

In the second experiment, in which P. argus pueruli were offered a choice between control water and water treated with the red algae Laurencia spp., 34 out of 42 test pueruli (76.19%) relocated to one of the satellite chambers. The proportion of pueruli relocating to one of the satellite chambers in this experiment was greater than the proportion of pueruli relocating to one of the satellite chambers in the first experiment (16.67%), in which no odors were offered to pueruli (binomial test: \(P < 0.0001\)) (Fig. 2). Considering only those test individuals choosing one satellite chamber, 22 pueruli chose the chamber with treated water while 12 pueruli chose the opposite chamber with control water. Although a relatively large number of test individuals relocated to chambers treated with Laurencia spp. (64.7%), the proportion of test pueruli choosing the Laurencia spp. treatment did not differ significantly from that expected by chance alone (\(P = 0.0607\)) (Fig. 3A). Overall, pueruli did exhibit initial attraction, but no positive chemotaxis when exposed to red algae metabolites.

In the third experiment, in which P. argus pueruli were offered a choice between control water and water treated with conspecifics, 19 out of 42 test pueruli (45.24%)
relocated to one of the satellite chambers. The proportion of pueruli relocating to one of the satellite chambers in this experiment was greater than the proportion of pueruli relocating to one of the satellite chambers in the first experiment (binomial test: \( P < 0.0001 \)) (Fig. 2). Considering only those test individuals choosing one satellite chamber, 12 of the 19 test pueruli chose the chamber with treated water while 7 test pueruli chose the opposite chamber with control water. The proportion of test pueruli choosing the conspecifics treatment did not differ significantly from that expected by chance alone (\( P = 0.1796 \)) (Fig. 3B). Overall, pueruli did exhibit initial attraction, but no positive chemotaxis in response to conspecific metabolites.

*Panulirus argus* pueruli did sense and chose experimental chambers with water flowing from conspecifics + *Laurencia* spp. during the fourth experiment. In total, 35 out of 42 test pueruli (83.33%) relocated to one of the satellite chambers in this experiment. The proportion of pueruli relocating to one of the satellite chambers in this experiment was greater than the proportion of pueruli relocating to one of the satellite chambers in the first experiment (binomial test: \( P < 0.0001 \)) (Fig. 2). Considering only those test individuals choosing one satellite chamber, 31 of the 35 test pueruli chose the chamber with treated water while 4 test pueruli chose the opposite chamber with control water. The proportion of test pueruli choosing the conspecifics + *Laurencia* spp. treatment (88.57%) was greater than that expected by chance alone (\( P < 0.0001 \)) (Fig. 3C). Thus, pueruli exhibited both initial attraction and positive chemotaxis when exposed to a mixture of metabolites produced by red algae and conspecifics.
Lastly, we compared the observed proportion of pueruli attracted to red algae and juveniles when presented concomitantly (0.8649) to that expected under additivity (0.8699) and found no significant differences ($z = 0.068$, $P = 0.945$) indicating the absence of synergistic effects between red alga and juvenile metabolites.

**Settlement of *Panulirus argus* pueruli in the Field.**—Pueruli settlement varied by location in Florida Bay with site accounting for 60% of the overall variance of their abundance. Late benthic juvenile lobster density also had a significantly positive influence on pueruli settlement ($F = 14.00$, $P = 0.0004$), as did a synergistic effect of LBJ density and *Laurencia* spp. algae cover ($F = 5.05$, $P = 0.0290$) (Table 1).

**Discussion**

Considering the benefits previously reported for recently settled lobsters of inhabiting red algae *Laurencia* spp. (Herrnkind and Butler 1986, Smith and Herrnkind 1992, Childress and Herrnkind 1994) and costs of living in close proximity to early benthic juveniles (Butler et al. 1997), we predicted that the settling puerulus stage of the Caribbean spiny lobster *P. argus* would be attracted to the metabolites produced by red algae and repulsed by metabolites produced by juvenile conspecifics. We also
expected that any preference for Laurencia spp. would cease or diminish if this latter cue was presented concomitantly with metabolites produced by conspecifics. Our results are in conflict with the expectations above and partially inconsistent with previous empirical studies; pueruli did not display any clear or strong preference for red algae or avoidance of conspecifics. Perhaps more importantly, settling stages were attracted to water containing a mixture of metabolites produced by red algae and conspecifics.

Recently-settled lobsters experience lower mortality rates when inhabiting red algae compared to other less structurally-complex habitats (Herrnkind and Butler 1986, Smith and Herrnkind 1992, Childress and Herrnkind 1994). Other than the benefit above, previous experiments have shown pueruli to be attracted to red algae metabolites (Goldstein and Butler 2009) and to hasten metamorphosis when exposed to Laurencia spp. extracts (but by <1 d; Butler and Herrnkind 1991). Thus, given the reported benefits of inhabiting red algae and previous empirical data, it is difficult to explain why we did not observe pueruli attraction to red algae in our experiments. There are various possible explanations for the disparity between our results and previously published experiments. For instance, such disparity might be explained if the preference of pueruli for algae reported by previous experiments (Herrnkind and Butler 1986, Goldstein and Butler 2009) is an experimental artifact.

Food exudates are known to attract and induce settlement and metamorphosis in other predatory and omnivore marine invertebrates, either sessile or mobile like P. argus (Hayfield and Paul 2001 and references therein). Red algae Laurencia spp. naturally host epifauna (e.g., small gastropods, isopods, amphipods, ostracods, among others) that serve as prey to recently settled juveniles of P. argus (Marx and Herrnkind 1985). The relevance of food-rich red algae for small lobsters is also illustrated by experiments showing that early benthic lobsters disperse more rapidly from algal clumps with reduced food than from clumps containing abundant food (Marx and Herrnkind 1985, but see Butler et al 1997). The first juvenile instar of P. argus exhibits remarkable resistance to starvation, but has a relatively high degree of dependency on food to complete development and molt into the second juvenile stage (Espinosa-Magaña et al. 2017). Thus, algal clumps containing abundant food likely signal benefits (e.g., nutrition) to pueruli after settlement. If algae offered to experimental pueruli in previously published studies contained epifauna, then the reported attraction by pueruli to algae in these studies might actually be explained by the presence of food within algal clumps rather than be elicited by the algae itself.

Table 1. Generalized linear mixed model of early benthic juvenile lobster (EBJ) density. (A) Site is the random effect, with (B) late benthic juvenile lobster (LBJ) density and percent red algae cover as fixed effects.

<table>
<thead>
<tr>
<th>Random effect</th>
<th>Variance component</th>
<th>Variance ratio</th>
<th>Total variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>0.7838</td>
<td>1.531</td>
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<td>Residual</td>
<td>0.5116</td>
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<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>df</th>
<th>F ratio</th>
<th>Probability</th>
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</thead>
<tbody>
<tr>
<td>Large benthic juvenile density</td>
<td>1</td>
<td>14.000</td>
<td>0.0004</td>
</tr>
<tr>
<td>Red algae cover (%)</td>
<td>1</td>
<td>2.072</td>
<td>0.1564</td>
</tr>
<tr>
<td>LBJ density × red algae cover (%)</td>
<td>1</td>
<td>5.051</td>
<td>0.0290</td>
</tr>
</tbody>
</table>
Importantly, at least one previously published experiment did not mention cleansing of algal clumps from epifauna before offered to pueruli during habitat choice experiments (i.e., “experiment A” in Herrnkind and Butler 1986). Thus, we cannot rule out, at least in the case of experiment A in Herrnkind and Butler (1986), that the attraction shown by pueruli to algae might actually be caused by food items within algal clumps. Nonetheless, other studies have shown that the preference of pueruli for red algae remains even after algal clumps are thoroughly cleansed from epifauna (Goldstein and Butler 2009). Also, pueruli do not discriminate between food-supplemented and rinsed (food-deprived) algae (Herrnkind and Butler 1986). Taken altogether, the information above suggests that the preference demonstrated by pueruli for algae reported by the majority of previous studies is likely not an experimental artifact (Herrnkind and Butler 1986, Goldstein and Butler 2009).

For recently settled lobsters, costs of living in close proximity (<0.75 m) to other small conspecifics include augmented mortality by predators (Butler et al. 1997). Considering the above, we expected pueruli to be deterred by metabolites produced by conspecifics. Our results, however, were not consistent with theoretical expectations, but were consistent with laboratory experiments by Zito-Livingston and Childress (2009), which found pueruli had no attraction to conspecific odors. While early benthic juvenile lobsters do not significantly aggregate in clumps of red algae, they also do not significantly avoid clumps of algae with conspecifics (Childress and Herrnkind 1994). These results suggest that the relationship between the costs and benefits provided by conspecifics after settlement in red algae are likely complex and remain to be fully understood. Although recently settled lobsters do suffer costs when in close proximity to small conspecifics in the field (Butler et al. 1997), mortality rate decreases substantially with small increases in distance to the closest conspecific; algae-dwelling lobsters in pairs but separated by 0.5 or 0.75 m are significantly more likely to be killed if an individual is detected by a predator, than are pairs of individuals separated by 2 m (Butler et al. 1997). For pueruli, capable of active and efficient swimming (Kough et al. 2014), close proximity to already settled conspecifics might not be costly (in terms of predation risk) or, at least, as costly as reported for already settled conspecifics that are larger, pigmented and with poor swimming ability (Anderson et al. 2013). If costs to pueruli of settling near conspecifics are indeed low or absent, that could explain why we did not observe aversion to conspecifics by pueruli in our experiment. Additional experiments examining the relationship between costs experienced by pueruli and proximity to already settled conspecifics will improve our understanding about settling decisions by pueruli of *P. argus* when in the presence of conspecifics (Butler et al. 1997).

Proximity to EBJs might also result in benefits to settling pueruli yet to be explored. For larger juveniles of *P. argus*, the presence of conspecifics has been shown to facilitate the choice of crevice shelters (Nevitt et al. 2000) and decrease predation risk by reducing search time to find these shelters (Childress and Herrnkind 2001). However, there is growing evidence that juveniles are less attracted to conspecific odor cues than they once were (Childress et al. 2015). Benefits attained by other marine invertebrates due to gregarious settlement can be more subtle, and include protection against predators, access to sexual partners, increased competitive ability, reduced risk of interacting with competitively superior sympatric species, and decreased exposure to stressful conditions (e.g., temperature, salinity), among others (Donahue 2006 and references therein). Certainly, some of the benefits above (e.g.,
access to mating partners) do not apply to *P. argus* given its longevity and complex reproductive behavior (Booth and Phillips 1994, Herrnkind et al. 1994, Phillips et al. 2013). Still, additional research on the costs and benefits experienced by settling pueruli and recently settled lobsters is warranted.

In our experiments, we did not observe any antagonistic interaction between cues produced by red algae and conspecifics. In conflict with our expectations, pueruli were attracted to water treated with a combination of metabolites produced by red algae and conspecifics. This unexpected result also suggests that settling decisions by pueruli are complex and plastic, varying according to the presence (or absence) of not a single but a set of chemical cues as reported for other marine invertebrates (Harvey 1996 and references therein). Ecological interactions are context-dependent, although poorly explored during settlement in marine invertebrates (Vermeij and Sandin 2008). In *P. argus*, metabolites produced by conspecifics might signal costs when perceived alone, but these costs might decrease or disappear when the same chemical cue is presented concomitantly with, e.g., dense red algae (that might also signal abundant epifauna). Overall, in *P. argus*, the benefits of settling in dense algae might outweigh the costs of settling near conspecifics, especially if algae density and slightly longer distances from conspecifics decrease the risk of attacks by predators.

We explored whether or not our experimental results, albeit unexpected, could be extrapolated to the field and examined the influence of conspecific and red algae abundance on pueruli settlement in Florida Bay. Somewhat consistent with our experimental results, field data indicated that red algae abundance did not affect pueruli settlement, but juvenile lobster density did had a positive influence on pueruli settlement as did a synergistic effect of juvenile lobster density and *Laurencia* spp. algae cover. Importantly, locality (survey area) accounted for approximately 60% of the overall variation in pueruli settlement in Florida Bay. This rather large variation attributable to locality is not totally unexpected taking into account that Florida Bay is a complex basin (Butler et al. 1997, Zito-Livingston and Childress 2009) with considerable spatiotemporal variability in red algae cover, shelter (i.e., crevices) density, predator abundance (e.g., octopi, stone crabs, bone fish, nurse and bonnethead sharks, and southern stingrays, among many others; Smith and Herrnkind 1992), and even pueruli supply, among others (Smith and Herrnkind 1992, Field and Butler 1994, Butler et al. 1997). Furthermore, analysis of the recapture of microwire-marked early benthic juveniles indicate no differences in survival/movement from sites with low or high lobster densities (Zito-Livingston and Childress 2009). That we detected an effect of conspecifics and a synergistic effect of red algae and conspecific abundance on pueruli settlement, even with this remarkably high “environmental background noise,” suggests that red algae, and a mixture of cues produced by red algae and conspecifics, does provide strong settlement cues for pueruli in the field. Although the effect of red algae is not predicted by our results (but see Goldstein and Butler 2009), such an effect might be explained if red algae can provide subtle or unknown benefits to lobsters after settlement. The conflict between experimental and field data, and the greater data variability attributed to sampling site in our study additionally suggest that the distribution of pueruli in the field is not simply a reflection of settlement decisions based on available cues studied herein. Pueruli settle only at pressures equivalent to depths of 5 m and display behavioral aversion to hypersaline or hyposaline water (Goldstein and Butler 2009). Salinity and temperature are also known to vary widely in the Florida Bay (Field and Butler 1994) and likely explained
variance in pueruli settlement during our field survey. Factorial experiments that present different types of cues to settling lobsters are needed to improve our understanding of those biotic and abiotic environmental conditions driving settling decisions by pueruli of *P. argus* in the field.

Lastly, we want to highlight that the disparity between our results and previously published experiments (e.g., Goldstein and Butler, 2009) might be due to dissimilarities between experimental designs among studies. For instance, differences in the water flow rate within the choice chambers [30 ml min⁻¹ in our study vs 150–250 ml min⁻¹ in Goldstein and Butler (2009)] could explain the observed differences in the pueruli attraction to *Laurencia* spp. between the two studies [absence in our study vs presence in Goldstein and Butler (2009)]. Additional differences in experimental designs among studies that might have contributed to a different behavioral response of the pueruli between our results and previously published experiments include the type of choice chamber and the protocol used to prepare stock solutions.

Lastly, during this study, pueruli were collected from inshore artificial collectors on which they settled and not from offshore plankton. Again, it is possible that the behavior of pueruli used during our experiments could have been different from that of pueruli collected from the plankton explaining the observed differences in the pueruli attraction to *Laurencia* spp. between our and previous studies (Goldstein and Butler 2009).

Conclusions.—Overall, our study has advanced our understanding of microhabitat sensing and selection by pueruli of the reef-dwelling Caribbean spiny lobster, *P. argus*. We have tested the importance of red algae, juveniles, and their interactive effect on settling decisions by postlarvae. Our results and previously published studies suggest that a rather complex interplay between chemical cues (and likely others signals that remain to be explored, such as sound; Hinojosa et al. 2016) ultimately dictates decisions by settling stages that are condition-dependant (i.e., phenotypically plastic). Additional experiments examining the role of distance to conspecifics, algal coverage + abundance, and other biotic conditions in modulating the costs and benefits experienced by lobsters while settling are warranted as will improve our understanding about settlement decisions in *P. argus* and other marine organisms. Such experiments will also help understand the relationship between postlarval surplus, settlement, and fishery stocks of this large marine invertebrate that is fully exploited or overexploited over most of its range of distribution. Particular efforts should be directed to understand the effect of diseases (PaV1; Behringer et al. 2012) on settlement decisions by pueruli of *P. argus*. Research on the modulation of costs and benefits of settling on microhabitats with different attributes, including the presence of PaV1-infected EBJs is underway.

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Literature Cited


