# Behavioral enhancement of onshore transport by postlarval Caribbean spiny lobster (*Panulirus argus*)

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### Abstract

We conducted a series of laboratory experiments to examine the orientation, settlement, and metamorphosis of Caribbean spiny lobster (*Panulirus argus*) pueruli (postlarvae) in response to cues characteristic of their nursery in back-reef tropical lagoons. Our results suggest that pueruli were attracted to coastal water sources and the metabolites of red macroalgae (*Laurencia* spp.) when compared with oceanic water and artificial seawater treatments. Pueruli were not attracted to waterborne cues from sea grass, were repelled by hypersaline or hyposaline water, and discerned coastal cues from water collected as far as 30 km offshore from the reef. We also conducted experiments to examine the settlement behavior of pueruli in response to different substrates and hydrostatic pressures. Pueruli only settled at pressures equivalent to depths <5 m, which is where most settlement occurs in the field; settlement behavior was further enhanced by the presence of red macroalgae. Coastal water or artificial seawater containing red macroalgae metabolites hastened puerulus development and molting to the benthic juvenile stage by 20–30% compared with those exposed to artificial seawater. Collectively, our results demonstrate that spiny lobster pueruli use chemical and pressure cues during onshore transport and selection of settlement habitat, highlighting the important role of behavioral responses to physical cues in the recruitment of this species.

Historically, larval dispersal was thought to be primarily directed by advective processes, but recent evidence highlights the importance of behavior in altering larval dispersal, hence population connectivity (Cowen et al. 2006). Both experimental studies (Swearer et al. 1999; Jones et al. 2005) and biophysical modeling (Jones et al. 2002; Cowen et al. 2006; Xue et al. 2008) suggest that local retention of behaviorally adroit larvae can be significant. This is especially true if larvae possess well-developed sensory capabilities (e.g., marine larval fishes) and are competent swimmers (Leis 2007; Lara 2008). Whether the larvae of reef species disperse widely or remain just offshore of their natal source, they must somehow transit from their planktonic pelagic realm to the reef or lagoonal benthic nursery. They do so using tidal fronts, internal wave slicks, turbulence, and Ekman transport (*see* Shanks 1995 for review) among other physical forces, each operating in tandem with larval behavior (e.g., selective tidal stream transport, attraction to surface, depth regulation) to situate larvae in coastal areas where settlement occurs (*see* Young 1995 for review).

For obvious logistical reasons, few studies on larval coastal orientation exist for taxa that possess long pelagic larval durations (PLDs) or for species whose larvae occur at low concentrations. Yet, such characteristics are common, even among species that support economically significant reef fisheries. Spiny lobsters (Decapoda: Palinuridae) are just such an example. As adults, spiny lobsters support valuable fisheries worldwide (reviewed in Phillips et al. 2006) and can control benthic community structure via their foraging activities (reviewed by Butler et al. 2006). Yet the biology of Palinurid larval and postlarval (referred

to as a puerulus) stages is poorly known because their PLDs are among the longest of marine animals (4–18 months), and they occur at low concentrations in nature (Phillips et al. 2006; Goldstein et al. 2008). Coastal orientation by spiny lobster pueruli is largely uninvestigated, despite speculation over its existence and importance (reviewed by Jeffs et al. 2005).

Spiny lobsters infiltrate the coastal zone as fast-swimming but short-lived (2–4 weeks) pueruli after a PLD of several months. Metamorphosis of larvae to the puerulus stage occurs offshore near the shelf break from where the nonfeeding puerulus is advected or swims onshore (Phillips et al. 2006). Physical forcing by wind-driven or tidally driven currents no doubt plays a role in their cross-shelf transport; however, the relationship between the magnitude of these forces and puerulus abundance is typically weak (reviewed in Butler and Herrnkind 2000; Phillips et al. 2006). The pueruli of some species of spiny lobster are capable of swimming from more than 100 km offshore using lipids stored during the prolonged larval period as a source of energy. Although they are capable swimmers, how the pueruli actually orient toward shore is unknown (Jeffs et al. 2005).

Some larval crabs and fishes use sound as a means of navigation to settlement habitats (Jeffs et al. 2003; Leis et al. 2003); although sound has been hypothesized for spiny lobster navigation, there is little evidence for this (Jeffs et al. 2005). Geomagnetic navigation is used by adult spiny lobsters, but it has not been investigated for earlier life stages (Boles and Lohman 2003). Chemical cues are perhaps the most likely signals used by pueruli for orientation, given the importance of chemoreception in so many aspects of juvenile and adult lobster foraging, mating, and den acquisition (*see* Dolan and Butler et al.

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2006 for review). A number of species of spiny lobster preferentially settle within vegetated habitats, including the coral reef–dwelling Caribbean spiny lobster *Panulirus argus* (Latreille 1804), which settles and lives as a juvenile in back-reef lagoonal habitats (Marx and Herrnkind 1985). Secondary plant metabolites associated with marine algae, particularly rhodophytes, are important cues for settlement and metamorphosis in a variety of larval invertebrates, including echinoids, asteroids, polychaetes, and gastropods among others (Boettcher and Targett 1998; Hadfield and Koehl 2004). Of particular importance to settling *P. argus* pueruli is the presence of the bushy red macroalgae *Laurencia* spp., to which pueruli appear to be drawn via chemotaxis (Butler et al. 1997).

Reef-dwelling species whose larvae settle in back-reef habitats close to shore can also be exposed to nursery environments where salinity varies spatially and temporally. Although species that recruit into estuaries are adapted to such conditions, most coral reef–dwelling taxa are not (Ehlinger and Tankersley 2004). Within the broad geographic range of the Caribbean spiny lobster, which stretches from Brazil to Bermuda (Holthuis 1991), lie numerous coastal areas influenced by riverine input (e.g., Amazon and Orinoco) and tidal flow from shallow, hypersaline lagoons. Hypersaline and hyposaline waters are lethal to *P. argus* pueruli (Field and Butler 1994), so it is likely that they would have evolved mechanisms to detect and avoid inappropriate salinities as part of the repertoire of behaviors that control their navigation to coastal nurseries.

Some marine larvae are also sensitive to changes in pressure and use barokinesis for orientation during settlement (Forward 1990). The ability of larvae to control their position in the water column confers significant advantages for avoiding predation, optimizing feeding, and aiding transport (Acosta and Butler 1999; Stake and Sammarco 2003). Indeed, the pueruli of most species of spiny lobster settle in shallow water <10 m depth (Butler and Herrnkind 2000; Butler et al. 2006), so barokinesis may also play a role in controlling their settlement.

Despite their economic importance and hence the wealth of ecological information on spiny lobsters, the environmental cues that govern the shoreward orientation of pueruli are unknown. Therefore, we carried out a series of laboratory studies to investigate whether pueruli could distinguish between coastal vs. offshore water masses and whether they respond to chemical cues (odors) associated with their vegetated lagoonal nursery. We also tested whether they avoid seawater of low or high salinity and determined whether they respond to changes in hydrostatic pressure in ways that might influence their settlement at different depths. Finally, we examined whether exposure to chemical cues derived from their preferred settlement substrate hastened the development of pueruli into the first benthic juvenile instar.

## Methods

*Collection of pueruli*—For all experiments, transparent pueruli were collected at night on rising tides using plankton nets (1 m diameter, 2.5 m length, 750-$\mu$m mesh size) deployed from a bridge connecting Grassy Key and Duck Key, Florida (24°78′N, 80°92′W) during new and first quarter lunar periods between June 2002 and April 2004. Additional pueruli were collected from modified Witham floating surface collectors (Witham et al. 1968) within the same lunar periods as bridge-caught animals. After capture, pueruli were transported and held in featureless 4-liter aerated aquaria filled with artificial seawater (AS; Instant Ocean®) at ambient temperatures. Consistent with other studies of puerulus behavior (Herrnkind and Butler 1986), only transparent, precompetent (Lewis et al. 1952) pueruli were used in our experiments, to avoid developmental bias in swimming and settlement behaviors that may ensue once pueruli begin to pigment. To bolster our sample sizes, we used pueruli collected in nets directly from the plankton and also those obtained from artificial Witham collectors in our experiments. Results of a preliminary mesocosm experiment testing the choice of collector- vs. plankton-caught pueruli for two different settlement habitats indicated that there was no significant difference in habitat selection between pueruli from these two sources (split-plot ANOVA, $F_{1,30} = 14.73$, $p = 0.738$; Goldstein 2007). Also, in none of the experiments that follow did we detect a significant difference in the outcome due to puerulus source (i.e., collector caught vs. plankton caught; *see* Results).

*Chemotactic response of pueruli*—We tested puerulus preference for several different water masses in the lab including (1) inshore vs. offshore water sources, (2) macroalgal vs. sea grass settlement habitats, and (3) seawater of different salinities. In brief, we tested the "choice" of pueruli for different water sources at night in four custom-made acrylic choice chambers (95 cm long × 20 cm diameter, Fig. 1) in which pueruli were given the opportunity to swim up-current toward one of two cues or remain in the central chamber where the waterborne cues mixed.

First, we tested whether pueruli are attracted to cues present in the back-reef environment by permitting them to choose between water from the Florida Bay nursery (bay) vs. oceanic water collected beyond the reef ~10 km from shore. Then, we tested their response to water collected from increasing distances offshore (bay, reef, 15 km offshore, 30 km offshore) compared with water collected from 50 km offshore, our control. Next, we tested the preference of pueruli for water containing cues derived from the red alga *Laurencia* spp. and from a mixture of sea grasses (*Thalassia*, *Syringodium*, *Halodule*) vs. artificial seawater (AS). Finally, we tested puerulus choice in AS for differing salinities (25, 30, 45, 50) vs. AS of 35 as a control. For each experiment, two-way log-linear contingency table analyses (*G*-test with Williams adjustment, Zar 1999) were used to test whether the choice of water source by pueruli was nonrandom.

*Test solutions*—AS (salinity = 33–35 and pH = 7.8–8.0) served as the control water treatment in all of the experiments and was the stock solution in which other
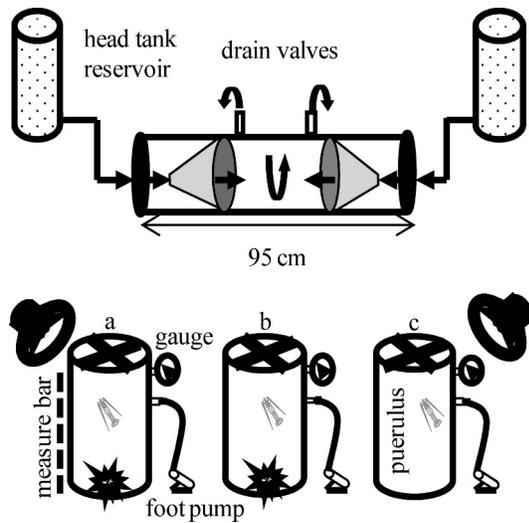
Fig. 1. (Top) Schematic drawing of choice tube chamber apparatus for which *P. argus* pueruli were tested. Arrows indicate the flow of water. (Bottom) Schematic drawing of pressure chamber apparatus. Treatments are denoted as follows: (a) *Laurencia* spp. habitat, (b) artificial habitat, and (c) no habitat (*see* Methods for details).

factors of interest were mixed and tested. Preliminary trials established that pueruli showed no preference for AS compared with oceanic water ($G = 0.12$, df $= 1$, $p > 0.05$, $n = 30$), nor for AS mixed from two separate batches ($G = 0.25$, df $= 1$, $p > 0.05$, $n = 15$) (Goldstein 2007). For plant metabolite treatments, live material (algae or sea grass) ($\sim$500–700 g L$^{-1}$) was added to a 6-liter perforated bucket with a lid, that in turn was placed within a 50-liter tank filled with AS for 12–15 h. Fresh samples of all marine plants were collected 1–2 d prior to the start of each experiment. Plant samples were washed and cleaned lightly with AS to rinse them of epiphytes and debris prior to incubation.

Oceanic water was obtained by boat on flood tides 1–2 d before the monthly experimental trials were to begin and at five different distances offshore, beginning at the reef tract ($\sim$10–12 km offshore) and then approximately 10, 15, 30, and 50 km beyond the reef. Seawater was pumped from $<$1 m depth and filtered through a 0.5-$\mu$m inline cartridge filter into large storage barrels then transported to the laboratory. Bay water was obtained in Florida Bay on ebb tides and transported similarly. All treatment stocks were aerated and continuously mixed prior to use in the experiment.

*Chemotactic experimental procedures*—Chemotactic responses of pueruli in the laboratory were tested at night between the new and first quarter lunar periods when pueruli are normally arriving onshore each month (Acosta and Butler 1999). All preparation work and initial observations were made under red light (600–700 nm), which decapod crustaceans do not detect; then the lights were turned off for the duration of the trials. To initiate a trial, we filled each choice chamber with 7.5 liters of AS, filled the two 11-liter head tanks that supplied each

chamber with the appropriate test solution, and measured the ambient water temperature (mean $= 26^\circ$C; range $= 20^\circ$C–28$^\circ$C). Seawater test solutions flowed into the opposite ends of each choice chamber through a 50-cm segment of 6-mm diameter airline tubing from the two head tanks where it mixed, then flowed out of the chamber through valves at the chamber top (Fig. 1). Water flowed (range $= 0.15$–0.25 L min$^{-1}$) into each choice chamber for $\sim$2.0 $\pm$ 0.5 h after the start of the trial. We conducted and videotaped chemical dye tests prior to some trials to confirm this flow regime.

A clear segment of 2.5 cm diameter $\times$ 30 cm long PVC was placed into the center of the chamber into which a single puerulus was added. After 5 min, the PVC tube was slowly lifted out of the tube, and the puerulus was at liberty to move. The test animal could remain in that part of the chamber or swim into the current toward a chemical cue through funnels leading into either end of the chamber (Fig. 1). Once in an end section, it was unlikely that a puerulus would swim back out through the inverted funnel. Following each trial, water was drained from each chamber and the position of the puerulus noted. A total of four choice chambers (replicates) were run each night simultaneously.

*Effect of odor on puerulus development*—Individual pueruli were assigned to one of three water treatments: (1) AS, (2) AS conditioned with *Laurencia* spp., and (3) bay water to determine whether development (i.e., time to ecdysis to the early benthic juvenile stage [EBJ]) is fixed or is a plastic response to waterborne cues associated with their settlement habitat. Pueruli ($n = 45$ per trial) were collected from the plankton (as described previously) on the same night and were randomly assigned to 400-mL plastic Nalgene® beakers (one puerulus per beaker), each containing the assigned seawater treatment and a small 9-cm$^2$ clump of plastic window mesh screening to serve as a refugia ($n = 15$ for each of three water treatments). Treatment water, kept at ambient seawater temperature, was exchanged in each container and replenished with fresh treatment water daily. Pueruli were checked for visual signs of the time to pigmentation (TTP) (i.e., the start of transformation to EBJ), the primary indicator being development of coloration at the base of the third basal segment of the antennae (Lewis et al. 1952), once in each 24-h period. Assays were replicated over 5 months (Aug, Nov, and Dec 2002 and Jan–Feb 2003). These data were analyzed using a one-factor model I randomized block ANOVA where the factor of interest was water treatment (three levels), the block was month (to control for the effect of temperature differences on development), and the response variable measured as the mean number of days to pigmentation. The raw data were $\ln(x + 1)$ transformed to meet parametric assumptions.

*Effect of hydrostatic pressure on puerulus settlement*—We observed in laboratory experiments the swimming behavior of pueruli and their benthic settlement when exposed to different hydrostatic pressures indicative of depth changes of approximately 0–14 m using three habitat structure

types: no structure, artificial structure, and macroalgal structure. The pressure test chambers were 4.5-liter commercially manufactured clear, cylindrical canisters (dimensions, outer diameter = 24 cm, inner diameter = 23 cm, height = 21 cm, Red Sea Pharm, Fig. 1). An external pressure gauge was threaded into one side of the canister and provided pressure readings in increments of 0.145 kPa (1 psi). Pressure was gradually increased by injecting air from a foot pump into the chamber through a valve; it was decreased by releasing air slowly from the chamber.

Prior to a set of trials, each chamber was filled with AS and outfitted with the appropriate habitat treatment: either one 9-cm$^2$ tuft of *Laurencia* spp., window screening (artificial habitat), or no habitat. A single puerulus was introduced into each chamber via a PVC pipe and, after 5 min, the pipe was removed to free the puerulus and the chamber lid was secured. Pressures of 0, 35, 69, 103, and 137 kPa corresponding to depths of 0, 3.4, 6.8, 10.2, and 13.5 m (respectively) were tested to mimic the gradient of depths that pueruli are likely to encounter once they cross the reef and move onshore toward vegetated back-reef nursery areas (Acosta and Butler 1999). Higher pressures corresponding to deeper depths were not tested because of the technical limitations of our experimental apparatus. However, few if any juvenile *P. argus* are ever observed in deep water (i.e., >10 m). All trials were conducted at night under red light.

The position of the puerulus in the water column or bottom of container was noted 1 min after acclimation at ambient pressure (0 kPa). One minute later, the pressure was slowly increased to 35 kPa and the position of the puerulus again noted after 1 min of exposure to that pressure (2 min total). This procedure was repeated for each of five designated pressures (at ~34 kPa increments) reaching a maximum pressure of 137 kPa, at which time we incrementally decreased the pressure and repeated our observations back to 0 kPa. We analyzed our data using a two-factor repeated measures ANOVA. Habitat (three levels) was treated as a model I factor, pressure (10 levels total, five increasing and five decreasing) was the repeated measures factor, and the position of each puerulus at the end of each 2-min period was the response variable. The data from this experiment were ln(x + 1) transformed prior to analysis to meet parametric assumptions.

## Results

*Experiment 1: chemotactic response of pueruli—P. argus* pueruli preferred seawater containing chemicals leached from red macroalgae, and they preferred water of moderate salinity (Fig. 2b,c). Pueruli were attracted to odors from *Laurencia* spp. (62%) but not sea grass (47%) as compared with seawater controls. Low (25) and high (50) salinities were avoided by pueruli (8% and 0%, respectively) as compared with seawater at 35. However, pueruli showed no preference for seawater of more moderate salinity (46% selected 30 and 49% chose 45) as compared with seawater at 35 (Fig. 2c).

With respect to the response of pueruli to water masses from different distances offshore, pueruli were attracted to
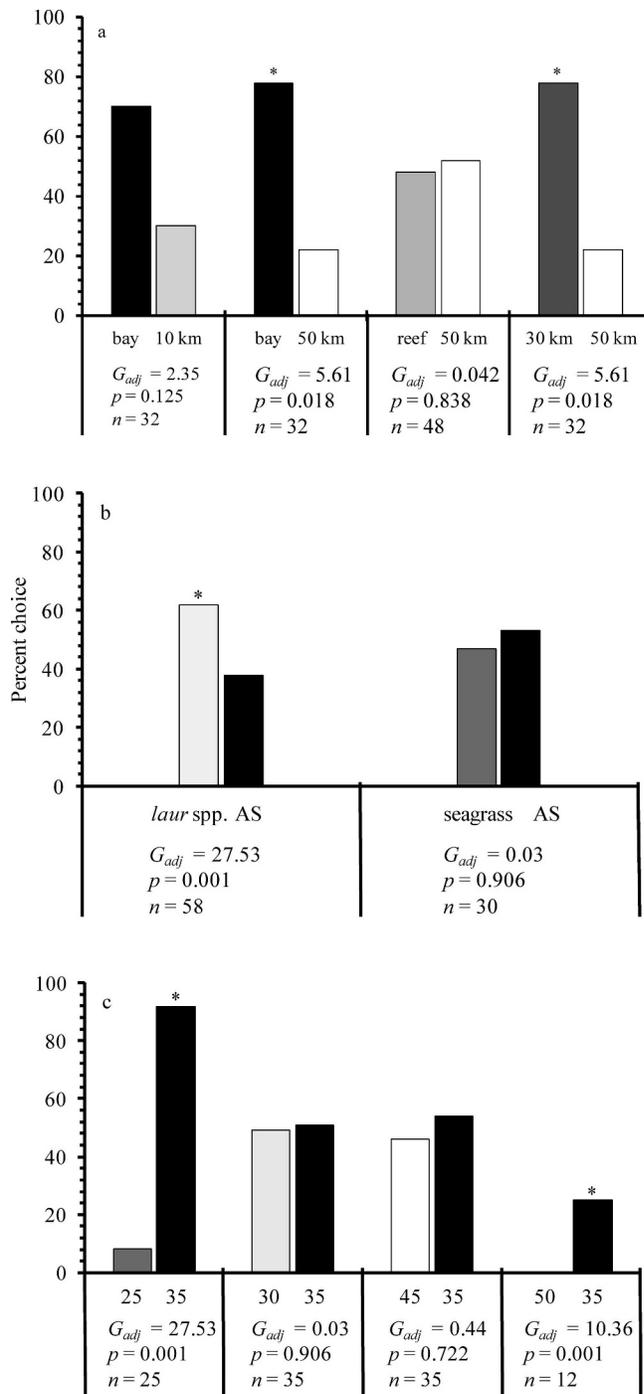


Fig. 2. Summary of choice chamber trials testing *P. argus* puerulus selection of water masses depending on (a) water source, (b) presence of chemical cues from plants, and (c) salinity. Results are pooled for pueruli obtained from the plankton and from collectors. AS = artificial seawater (salinity = 35). Degrees of freedom = 1 for each two-way *G*-test.

bay water (70–78%) more often than oceanic water, whether collected 10 km offshore or 50 km offshore (Fig. 2a). Similarly, when given a choice between water collected from 30 km offshore vs. 50 km offshore, the pueruli were again attracted to the inshore water (78%
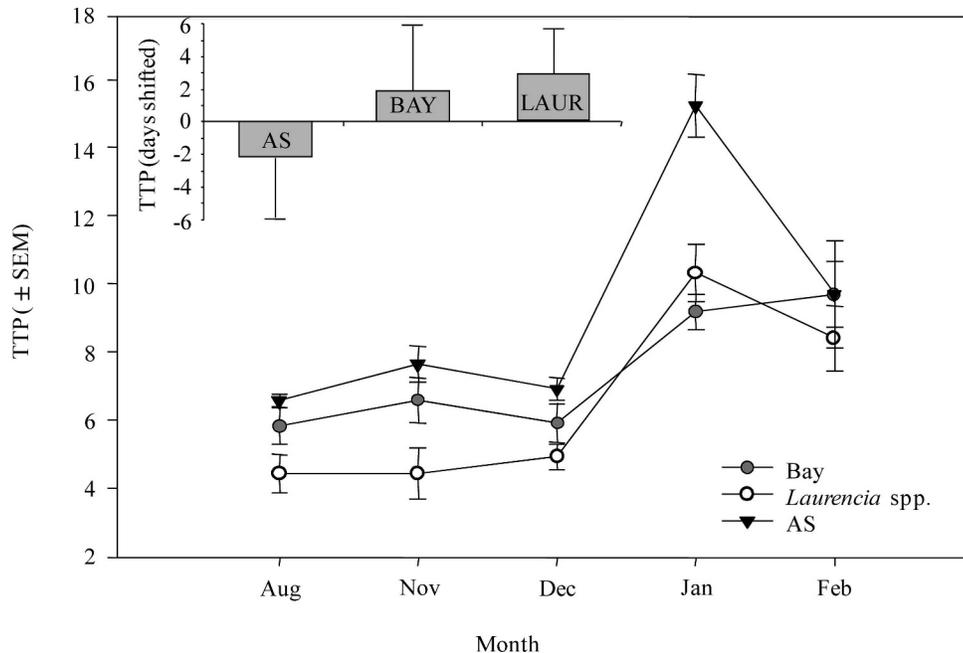
Fig. 3.    Time to pigmentation (TTP—mean days to pigmentation $\pm 1$ SEM), an indication of the onset of metamorphosis, for *P. argus* pueruli as a function of month and exposure to artificial seawater, bay water, and artificial seawater containing chemical cues from the red macroalgae, *Laurencia* ($n = 10$–15 pueruli per treatment per month). (Inset) Average deviation ($\pm$SE) from the mean (in days) that each water treatment condition shifted time to pigmentation (TTP) of *P. argus* pueruli ($n \sim 45$ per treatment). Data from all monthly trials combined. Both bay and *Laurencia* spp. (LAUR) treatments accelerated TTP by 1.9–2.9 d (average = 2.4) compared with TTP in artificial seawater (AS).

chose water from 30 km). However, pueruli showed no preference for water collected near the reef (10–15 km from the bay nursery) where predation on pueruli is known to be extraordinarily high (Acosta and Butler 1999), as compared with water collected 50 km offshore.

The choice of a water mass by individual pueruli was consistent in repeated trials; 17 of the 21 pueruli (81%) that were retested made the same choice of water mass in the subsequent trial. *P. argus* pueruli also showed no preference for two identical AS controls (53% vs. 47%), nor could they distinguish between oceanic water from greater than 10 km offshore (57%) and the AS control (43%) (Goldstein 2007).

*Experiment 2: effect of odor on puerulus development*—Pueruli incubated in bay water and water containing chemical cues from *Laurencia* spp. pigmented significantly faster compared with those in AS ($F_{2,180} = 26.86$, $p < 0.001$, Ryan-Einot-Gabriel-Welsch [REGWF] post hoc comparisons; Fig. 3). As expected, TTP varied among months because of differences in seawater temperatures (block effect in analysis; $F_{4,180} = 37.54$, $p < 0.001$, Fig. 3); monthly temperature averages were 26.5°C (Aug), 25.3°C (Nov), 24.1°C (Dec), 21.3°C (Jan), and 23.8°C (Feb). TTP for all treatments in August averaged 5.6 d compared with 11.6 d in January. Despite these temperature differences, water from the bay nursery where *Laurencia* spp. naturally occurs and from artificial seawater containing metabolites from *Laurencia* spp. consistently lowered puerulus TTP by

about 2 d (mean = 2.4 d) compared with AS (Fig. 3), a decrease of approximately 25%.

*Experiment 3: effect of hydrostatic pressure on puerulus settlement*—Pueruli swam upward into the water column at higher pressures indicative of depths >7 m and settled down to the bottom when pressure was lower (Table 1; Fig. 4). The response of pueruli also differed between treatments containing structure (artificial structure or *Laurencia* spp.) compared with the featureless control, but their behaviors in these two treatments were similar (Table 1; Fig. 4). As pressure increased, pueruli in the no-structure control swam rapidly and higher into the water column (4.6 cm) compared with their response in the *Laurencia* (0.43 cm mean position) and artificial shelter treatments (0.67 cm mean position). Pueruli swam off the tank bottom in the no-structure control when pressures were as low as 0–1 kPa. In contrast, pueruli in the *Laurencia* and artificial structure treatments remained within structure on the bottom until pressures increased to ~69 kPa and then swam to heights comparable with animals in the control treatment (9.5–13.7 cm over all three treatments, Fig. 4). Under conditions of decreasing pressure, pueruli in the *Laurencia* and artificial structure treatments responded similarly by swimming downward from their maximum mean heights of 14.2 cm and 16.6 cm (respectively) at 137 kPa, whereas pueruli in the control treatment remained within the water column significantly longer over a range of decreasing pressures. Regardless of

Table 1.    Results of a two-factor repeated measures ANOVA testing the effect of benthic structure (three levels, fixed) and changing pressure treatments (10 levels) on the vertical position in the water column of *P. argus* pueruli. Results of a post hoc REGWF test are below the ANOVA table and show that the results for the no-structure control treatment differ significantly from the artificial structure and *Laurencia* treatments, which were similar.

| Source | df | SS (Type III) | MS | *F* | *p* |
|---|---|---|---|---|---|
| Height | 2 | 83.21 | 41.605 | 38.92 | 0.001 |
| Pressure (repeated) | 9 | 510.228 | 56.692 | 52.303 | 0.001 |
| Height × pressure | 18 | 153.01 | 8.500 | 21.73 | 0.023 |
| Error | 87 | | 1.084 | | |
| Total | 116 | | | | |

Post hoc REGWF results: Treatment group: control <u>artificial *Laurencia*</u>

the pressure, pueruli remained (on average) closer to the bottom (6.32 cm) when in the presence of *Laurencia* compared with those in the artificial structure treatment (8.73 cm) and no-structure control (11.59 cm).

Once pueruli made contact with structure on the bottom of the tank (i.e., either *Laurencia* or artificial structure) they spent more time on the bottom and thus less time in the water column (Fig. 4). Pueruli maintained in featureless chambers (control) often swam erratically, and although they made more excursions to the bottom, those excursions were shorter in duration. In contrast, animals in both the *Laurencia* and artificial structure treatment chambers often swam toward the tufts of *Laurencia* or artificial structure and conducted short (1–2 s) tactile interactions ("touch-downs" and "lift-offs") with these structures.

## Discussion

Our results demonstrate for the first time that the postlarvae of palinurid lobsters are attracted from far offshore to coastal chemical cues and, once inshore, to physical cues that signal the presence of conditions suitable for settlement. Specifically, we have shown that *P. argus* pueruli (1) are repulsed by salinities much different than 35; (2) are attracted to coastal seawater, particularly seawater containing chemical cues derived from the red macroalgae in which they settle; and (3) settle when pressure cues are similar to those found in shallow (<7 m) lagoonal habitats in which most settlement occurs in nature. We also show that macroalgal odors hasten the development of pueruli to the benthic juvenile stage, a transition that significantly reduces their risk of predation (Acosta and Butler 1999).

Given the wealth of cues available to them, it is not surprising that spiny lobster larvae and postlarvae use multiple cues for shoreward navigation and settlement, as do other species. For example, the megalopae of the blue crab (*Callinectes sapidus*) respond to a suite of scalar (e.g., pressure, salinity, temperature, chemical odors) and vector (e.g., gravity, light, currents) cues in addition to endogenous swimming rhythms that transport them shoreward to estuarine nurseries (Forward et al. 2003). Similarly, postlarval clawed lobsters (*Homarus americanus*) use a variety of cues (e.g., thermal gradients, chemical odors from predators) to orient to coastal nurseries and select settlement substrates (Boudreau et al. 1993).

Pueruli are strong swimmers with documented average sustained swimming speeds of 15 cm s$^{-1}$ and burst speeds of 30 cm s$^{-1}$ or more (Calinski and Lyons 1983; Jeffs et al. 1999). Yet pueruli are incapable of feeding and maintain these metabolically costly swimming speeds for weeks through the catabolism of phosolipid reserves (Jeffs et al. 1999). Numerous studies of the spatial distribution of planktonic palinurid larvae (Yeung and McGowen 1991; Sekiguchi 1997; Chiswell and Booth 1999; Rudorff et al. 2009) have shown that late-stage phyllosomas (larval stage) and pueruli are routinely caught near or beyond the continental shelf break where metamorphosis to the puerulus stage presumably occurs. Jeffs and colleagues (1999) estimate that rock lobster (*Jasus edwardsii*) pueruli swim as far as 200 km to shore and up to 17% arrive onshore in New Zealand metabolically exhausted. Thus, mechanisms that promote the arrival and early metamorphosis of pueruli in coastal nurseries, such as those demonstrated in this study, would be of obvious adaptive benefit in maximizing available metabolic reserves.

*Chemotaxis and metamorphosis*—Chemotaxis is the most common cue used by decapod crustaceans for orientation (Forward et al. 2003), although geomagnetic navigation is known in adult *P. argus* (Boles and Lohman 2003). Until now, there was no published evidence that spiny lobster pueruli use chemotaxis, although such evidence exists for larval fishes (Sweatman 1988). We found that *P. argus* pueruli are attracted to seawater collected bayside of the Florida Keys and its reef tract—an area of mixed macroalgae, sea grasses, and hard bottom typical of lobster nursery habitat in the region. Moreover, *P. argus* pueruli were attracted to chemical substances associated with red macroalgae (*Laurencia* spp.) but not to chemical cues associated with sea grasses.

The bushy red algae *Laurencia* spp. is a ubiquitous component of the shallow hard-bottom back-reef habitat in the Florida Keys (Chiappone 1996). Although this habitat is not as abundant elsewhere in the Caribbean, *Laurencia* can be found in other habitats intermixed with sea grass, mangroves, and rocky reefs. *Laurencia* is the preferred settlement substrate for *P. argus* pueruli (Marx and Herrnkind 1985; Herrnkind and Butler 1986), a settlement substrate in which food is plentiful and the mortality of pueruli and juveniles is lower than elsewhere (Acosta and
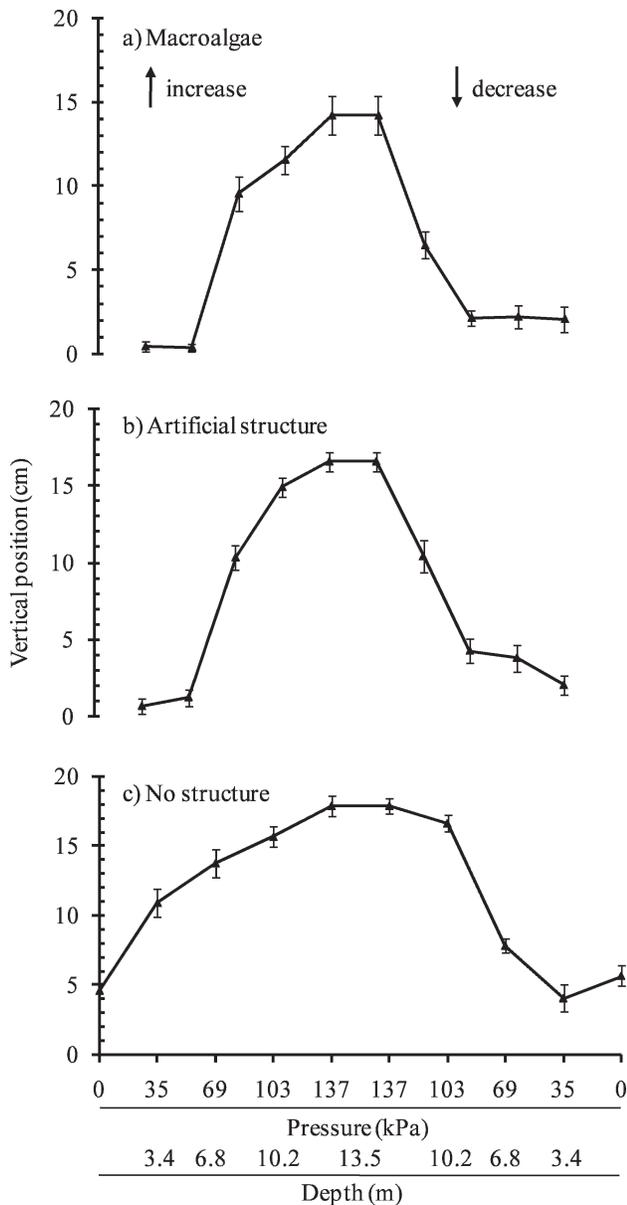
Fig. 4.    The vertical position in the test chamber (i.e., swimming response) of *P. argus* pueruli in response to increasing and decreasing pressures indicative of potential settlement depths. Puerulus response ($n = 30$ per treatment) was tested in chambers containing either (a) macroalgae (*Laurencia*), (b) artificial structure, or (c) no structure (control), to settle.

Butler 1999; Sharp et al. 2000). Selection would thus favor preference for this nursery habitat, which not so coincidentally produces chemical metabolites that are water soluble and stable (Boettcher and Targett 1998). Not only does *Laurencia* serve as an architecturally complex refuge for the vulnerable puerulus and EBJ stages, it also hastens the transition of pueruli to the EBJ (this study) and induces metamorphosis of other marine invertebrate larvae including mollusks and echinoderms (Boettcher and Targett 1998). We did not investigate which compounds in the *Laurencia* metabolites are attractive to pueruli; however, a number of compounds are known to induce metamorpho-

sis in marine larvae, including free amino acids (Boettcher and Targett 1998). Having demonstrated that pueruli respond strongly to macroalgal cues, tests of their response to a gradient of declining metabolite concentrations indicative of onshore–offshore dilution are a logical next step.

Perhaps an equally important finding was the lack of response by pueruli to chemical cues from locally abundant sea grasses, which is consistent with studies of their habitat preference (Sharp et al. 2000; Behringer et al. 2009). Despite the abundance of sea grass in the shallow waters of the Florida Keys and the Caribbean, there is no evidence that *P. argus* pueruli preferentially settle in sea grass, where mortality rates are higher than in algae (Herrnkind and Butler 1986; Behringer et al. 2009). Some pueruli undoubtedly settle in sea grass and in other architecturally complex substrates (e.g., mangrove roots), but these are not preferred habitats (Acosta and Butler 1997).

*Enhancement of onshore transport*—Lagoons and bays produce chemical "fingerprints" characteristic of their ecological constituents (Atema et al. 2002). Thus, the presence and influence of a large, heavily vegetated coastal shelf off the southern tip of the Florida creates a scenario that presumably provides a potent offshore chemical plume by which pueruli can orient. Studies of coastal hydrodynamics in the Florida Keys indicate that the net tidal flow of water moves north to south from the Gulf of Mexico through the bays and lagoons surrounding the Florida Keys and out over the reef tract to the Straits of Florida (Smith 1994). Although the oscillation of tides, incursions of oceanic water, coastal countercurrents, local gyres and eddies, and significant wind-driven currents complicate this flow, the net flow is nonetheless offshore (Smith 1994; Lee and Smith 2002; Smith 2009). The concentration and distance that the coastal chemical signal travels offshore is undoubtedly variable, but if water quality conditions along offshore–inshore coastal boundaries are sufficiently different they may still offer a large-scale "coastal odor gradient" through which fast-swimming larvae can navigate.

The diminished attraction of *P. argus* pueruli to water collected near coral reefs 10–15 km from their bayside back-reef nursery also suggests that pueruli may use chemical cues to avoid contact with predator-rich coral reefs. We did not directly test this hypothesis, but our results are consistent with studies showing that predation on planktonic *P. argus* pueruli is higher than on the reef in the bay or back-reef lagoon (Acosta and Butler 1999). Juvenile *P. argus* use chemical cues to detect and avoid octopus predators (Berger and Butler 2001) and diseased conspecifics (Behringer et al. 2006), so it is not inconceivable that pueruli avoid coral reefs where planktivorous fishes pose a challenge to their continued inshore transport to bayside nurseries.

*P. argus* pueruli were also adept at discriminating salinity and avoided hypersaline and hyposaline conditions. These findings corroborate work previously conducted by Field and Butler (1994), who reported that combinations of extreme salinity and temperatures (particularly high temperatures and salinity) reduced survivorship and

recruitment of *P. argus* pueruli. Not surprisingly, salinity stress also effects growth, metamorphosis, survival, and other biotic processes in a variety of marine larvae, particularly when coupled with the effects of suboptimal temperatures (Richmond and Woodin 1996). These findings are perhaps most relevant to coastal nursery areas for *P. argus* adjacent to large landmasses or rivers and thus subject to periodic freshwater input, or nurseries in shallow lagoons where evaporation is high and tidal exchange restricted.

Among the areas subject to both hypersaline and hyposaline conditions is the only Caribbean spiny lobster nursery area in the continental United States—the shallow, coastal waters of the Florida Keys and Florida Bay. Looming in the near future is one of the largest anthropogenic changes to affect this region in decades: the massive multibillion dollar effort to restore the historical flow of freshwater in the Everglades, a major source of freshwater to Florida Bay. Estimates suggest that local decimation of spiny lobster nursery habitat from drastic changes in temperature, salinity, and water quality is likely, along with a projected 5–10% loss of recruitment for the south Florida region (Butler 2005). However, those estimates did not include the possible avoidance of low salinity areas by pueruli, which would presumably magnify further the effects on lobster recruitment.

*Response to hydrostatic pressure*—Our results indicate that pueruli respond to changes in hydrostatic pressure in a way that would favor settlement in shallow habitats <5 m deep. Negative barokinesis has been demonstrated in several marine larvae (Forward 1990), but this is the first report of barokinesis in the postlarvae of any species of spiny lobster. With few exceptions (e.g., *Palinurus elephas* in the Mediterranean), most palinurid pueruli settle in shallow water (<5 m; Butler et al. 2006). Although there are anecdotal reports and at least one published report (Heatwole et al. 1991) of newly settled pueruli and juveniles of *P. argus* found on artificial structures at depths up to 30 m, these observations are atypical and may be instances of ''temporary settlement'' where emigration from these locations is imminent or mortality likely. Nowhere in the Caribbean are juvenile *P. argus* common at depths over 5 m. Although higher predation-driven postsettlement mortality in deeper habitats is one possible explanation for this pattern, differences in puerulus settlement in response to depth is another.

Many marine invertebrate larvae respond to small changes in hydrostatic pressure at the micropressure level of 20 kPa or less, mostly in response to tidally driven changes in water depth that aid, for example, in selective tidal stream transport (reviewed in Macdonald and Fraser 1999). In such instances, larvae are typically responding to the *rate of change* in pressure associated with changes in tidal amplitude (Forward 1990). Other larvae react to changes in pressure *magnitude* (e.g., zoeae of the crab *Neopanopae sayi*; Forward 1990), which indicates how far a larva has descended from its original depth. This type of behavior is more suited to discrimination among habitats differing in depth, which was our objective in this study.

The incremental pressure changes that we tested ($\sim$34 kPa per increment) were based on the depths that pueruli would experience during their onshore transport over the fore reef (10–12 m), across the reef lagoon (5–8 m), and into the vegetated back-reef settlement habitat (1–3 m). Caribbean spiny lobster postlarvae are clearly capable of rapidly moving from the surface to the bottom to test settlement habitat suitability (e.g., pressure magnitude) in all of these environments, given their average swimming speeds of 15 cm s$^{-1}$ (or 9 m min$^{-1}$) in coastal waters (Calinski and Lyons 1983). Yet pressure alone does not control their settlement.

Our results and those of others suggest that at least three mechanisms promote settlement of *P. argus* pueruli in shallow coastal areas: chemotaxis, barotaxis, and thigmotaxis. The settlement scenario that we propose is as follows. *P. argus* pueruli are drawn to coastal habitats by waterborne odors, where they are especially attracted to cues produced by red macroalgae, while avoiding hypersaline or hyposaline waters and coral reefs. Although they seek depths of <5 m in which to settle, they may temporarily stop in deeper habitats during the day during their onshore migration. As they enter the back-reef lagoon, pueruli may periodically swim to the bottom to ''test'' the suitability of the benthos for settlement, responding to changes in pressure to gauge their arrival in a shallow nursery. Finally, the presence of a physically (and perhaps chemically) appropriate substrate promotes more exploratory behavior and then selection of particular settlement sites.

In conclusion, our results provide the first demonstration that postlarval spiny lobsters use chemotaxis along with barotaxis when orienting toward nursery habitat and, in the case of chemotaxis, can detect those cues in water at least 30 km offshore. They may also use odors during their onshore transport to vegetated, back-reef nursery habitat. Our results are consistent with the emerging notion that meroplanktonic larvae are not passive particles whose transport is driven solely by ocean currents, a hypothesis that has been discounted for many species in light of growing proof of their remarkable behaviors (Sale and Kritzer 2003; Cowen et al. 2006). Understanding how pelagic marine larvae detect suitable coastal settlement habitat is crucial for predicting patterns of recruitment under different local environmental conditions, which can be dynamic and altered by human activities. This is especially relevant in modern times in which widespread, anthropogenically driven changes in coastal water quality and nursery habitat structure are adversely affecting ecosystem biodiversity and stability.

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