Ecological determinants and sensory mechanisms in habitat selection of crustacean postlarvae

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Animals use sensory stimuli to assess and select habitats, mates, and food as well as to communicate with other individuals. One of the great mysteries of crustacean ecology is how postlarvae locate the relatively rare patches of coral reef habitat on which they settle. The present study aimed to estimate, by experiments in aquaria and biochemical analysis, the sensory modalities of crustacean postlarvae for senses used in searching for their species' settlement habitat. The study was carried out on 9 crustacean species (*Calappa calappa, Pachygrapsus planifrons, Xanthidae* sp., *Lysiosquillina maculata, L. sulcata, Raoulserenea* sp., *Stenopus hispidus, Palaemonidae* sp., and *Panulirus penicillatus*). For each species, a cohort of 30 postlarvae was captured on the same night on the reef crest of Rangiroa Atoll (French Polynesia). Among the 9 crustacean species studied, 6 made active habitat choices among the 4 habitats tested (live coral, dead coral, macroalgae, and sand) at the postlarval stage, but the presence or absence of conspecifics on the habitat did not influence their selective choice. Sensory experiments found that 4 species differentiated between their preferred habitat versus another habitat and 2 species differentiated between conspecifics and heterospecifics, using visual and/or olfactory cues. Lastly, the high performance liquid chromatography experiments showed that the 4 habitats and conspecifics (except *L. maculata* and *S. hispidus*) tested have different and unique chemical odors. Overall, our study is the first to highlight the sensory modalities for a broad range of crustacean species to detect and move toward settlement habitats and/or conspecifics. *Key words:* coral reefs, HPLC, settlement cues. *[Behav Ecol 21:599–607 (2010)]*

Behavioral ecologists view decision making in animals as the outcome of the costs and benefits of alternative behaviors (Blumstein and Bouskila 1996). Animals constantly make decisions: They choose mates, select a place to live or forage, and they decide whether to engage in activities, which expose them to predation risks (Cassier et al. 2000). On coral reefs, one of the greatest challenges facing the majority of marine organisms with larval stages that potentially disperse and develop in offshore waters is how to relocate patchily distributed reef environments in a vast oceanic matrix (for review, see Myrberg and Fuiman 2002).

Most coral reef organisms (i.e., molluscs, fish, and crustaceans) have stage-structured life histories with 2 distinct stages: a relatively sedentary benthic stage (usually juveniles and adults) and a pelagic larval stage capable of long-distance dispersal that can last for weeks or months (Werner 1988). The replenishment and persistence of most reef marine species depend on larvae finding suitable adult habitat at the end of the offshore dispersive stage (for review, see Doherty 2002). Thus, some fish and crustacean larvae have swimming abilities sufficient to control their pattern of oceanic disper-

sion and their return to an adult habitat (for review, see Kingsford et al. 2002; Myrberg and Fuiman 2002; Montgomery et al. 2006). However, larvae not only need to detect orientation cues to locate a suitable settlement habitat but they also need to detect environmental cues that trigger settlement, and it is unlikely that successful settlement is solely a matter of chance (Doherty 2002).

Successful settlement of pelagic larvae to the reef environment depends on processes that function at multiple spatial scales (Myrberg and Fuiman 2002; Montgomery et al. 2006). At the broadest scale (from hundreds of meters to tens of kilometers), hydrodynamic forces may disperse larvae over long distances, potentially delivering them to suitable settlement reefs far from the source population. Alternatively, complex, 3D secondary flows resulting from barriers, such as headlands and islands, can retain larvae within estuaries or around islands, resulting in more settlement to native populations (e.g., Armsworth 2000; Codling et al. 2004). In addition, researchers continue to be surprised by the sensory and swimming capabilities of marine larvae (e.g., Jeffs and Holland 2000; Leis and McCormick 2002; Montgomery et al. 2006). The majority of fish and crustaceans can swim either at hatching or soon thereafter, irrespective of the presence of chemical or sounds cues. However, they also have the potential to use these chemical and sound cues to control their position within the water column, increasing the probability that they will be transported to suitable settlement reefs (e.g., Shanks 1995; Kingsford et al. 2002). Once a larva of any taxon

has located a reef, successful settlement shifts to a smaller scale (from a centimeter to tens of meters) and larvae must locate a suitable microhabitat on which to settle. Marine larvae often show marked selectivity between the microhabitats that they choose for settlement based on a variety of environmental factors, such as the presence of specific benthic substrata or the presence of conspecifics or other species (for review, see Pechenik 1990; Doherty 2002). Overall, although evidence is mounting that larval reef fish and crustaceans are active participants in the process of dispersal and settlement, the sensory and behavioral mechanisms by which larvae disperse and return from their oceanic phase to the reefs remain relatively unknown (for review, see Kingsford et al. 2002; Myrberg and Fuiman 2002; Montgomery et al. 2006).

In the present study, laboratory experiments were conducted on 9 postlarval crustacean species to determine: 1) behavioral preferences for microhabitats (live coral, dead coral, macroalgae, and sand), 2) behavioral preferences for microhabitat versus conspecifics, 3) sensory modalities underlying behavioral preferences (i.e., visual or olfactory cues), and 4) chemical fingerprints of microhabitats and conspecifics. We used the term "postlarvae" (or presettlement larvae) for the organisms tested, as they were morphologically different from the stages straight after hatching, which are considered larvae (Leis and McCormick 2002).

MATERIALS AND METHODS

Study site and crustacean capture

This study was conducted on the North coast of Rangiroa lagoon, French Polynesia (lat 14°57′48S, long 147°38′79W). The list of target species was not predetermined but depended on daily catches from crest nets. Crest nets were placed on the reef crest of Rangiroa in order to capture crustacean postlarvae just before they entered the lagoon to settle (Dufour and Galzin 1993). Thus, our studies used postlarvae with no prior experience of settlement habitats (i.e., naïve postlarvae). Postlarvae were captured daily in July 2007 and March 2008. Each net had a rectangular mouth (1.8-m wide and 1-m high) positioned across the water flow and was made of 2-mm mesh that was fine enough to retain all incoming postlarvae. Cod ends were attached to the nets in the afternoon to minimize catching debris during earlier daylight hours when few marine postlarvae are normally captured (Dufour and Galzin 1993) and detached in the early morning to remove the captured postlarvae. Crustaceans captured during the night were collected at dawn, transferred to the laboratory, and subsequently maintained in aquaria (0.3 × 0.2 × 0.2 m) until 1800 h when experiments were begun. Prior to the initial experiments and between all subsequent experiments, crustaceans were maintained individually in aquaria supplied with flow through seawater from the adjacent lagoon and without any added artificial or natural habitats.

Postlarval identification was undertaken using the meristics and morphology characteristics of the crustacean (for review, see Poupin 2007). Moreover, some postlarvae of each species tested were kept alive in aquaria for several days to confirm their identification.

The conspecifics (individuals of the same species than that of the postlarvae tested), used as cue transmitters, were juveniles caught with crest nets and maintained in aquaria over 21 days. The 4 different microhabitats tested were spherical living coral colonies (6-cm radius) of *Pocillopora sp.*, *Porites sp.*, and *Acropora sp.*; spherical dead coral colonies (6-cm radius) of the same 3 species; spherical piles of living macroalgae (equal parts *Turbinaria ornata* and *Sargassum mangarevasae*) with a radius of 6 cm; and spherical piles of sand with a radius

of 6 cm. These specific habitats were chosen because they generally correspond to the main settlement habitats for crustacean species in French Polynesia (Poupin 1996, 1998).

Experiment 1: habitat preferences

To examine the preferences by postlarval crustacean species for different habitats, 3 identical aquaria were built. Each aquarium (0.6 × 0.4 × 0.2 m; Figure 1) contained the 4 habitats (sand, macroalgae, living coral, and dead coral). The 3 aquaria were located in a laboratory room isolated from outside noises, and indoor light was provided by florescent lights that were regularly distributed throughout the room in an attempt to minimize postlarval responses to variable levels and direction of light and noise. All trials were conducted between 1800 and 2100 h. The aquaria were divided into 4 quadrants (0.2 × 0.2 m) in size and separated into 2 pairs by a central area (Figure 1).
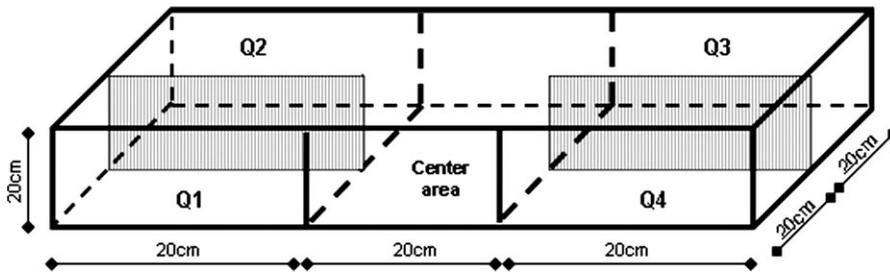
For each trial (1 crustacean per trial), each habitat was randomly assigned to one of the 4 quadrants. Experimental subjects (crustacean) were introduced one at a time at the center of an aquarium, by way of a cylindrical clear acrylic tube (0.1 m in diameter) that was placed equidistant from the 4 habitats (although the habitats were not equidistant from one another). Crustacean postlarvae were introduced into the tube via a small net and allowed to acclimate for 1 min. After the removal of the tube, crustacean postlarvae were free to choose among the 4 available habitats. The observer was 3 m from the tank and always in the same fixed position (treatments were randomly placed relative to the observer). A "choice" was scored as the first habitat selected by an experimental subject. Individuals were continuously observed, and the choice was recorded as the habitat on which the individual first settled (after any initial exploration) and subsequently remained for at least 1 min. If the postlarvae were still in the central part of aquaria after 5 min, the trial was stopped (scored as "no choice"). After each trial, seawater was replaced and habitats reassigned to quadrants. The distribution of choices for 15 postlarvae of each species (captured during the same night with crest nets) was recorded and analyzed with chi-square tests.

Experiment 2: effect of conspecifics

The objective was to evaluate whether the presence of conspecifics affected habitat choice. One postlarvae (different from the previous ones but captured during the same night) of each species was introduced into an aquarium used for the habitat experiment (Experiment 1) but with the plexiglass partition removed resulting in only 2 quadrants. Both quadrants contained standardized quantities of the most preferred habitat (determined from Experiment 1), and 10 older conspecifics were added to 1 quadrant. The conspecifics remained associated with the habitat patch and thus the quadrant to which they were assigned. Choices were recorded for 15 postlarvae of each species. All trials were conducted between 2100 and 2300 h. As with Experiment 1, water was replaced, and the positions of the treatments were randomized for each trial. The distribution of choices exhibited by postlarvae was analyzed with chi-square tests.

Experiment 3: sensory modes

To explore the sensory modalities of crustacean postlarvae in habitat selection, experiments were conducted between midnight and 0500 h using a "choice chamber," designed to isolate the sensory mode (visual or olfactory) used by crustacean postlarvae (Figure 2; for more detail, see Lecchini, Planes, et al. 2005; Lecchini, Shima, et al. 2005). Two types of sensory modes were tested on 2 types of cues. The 2 sensory
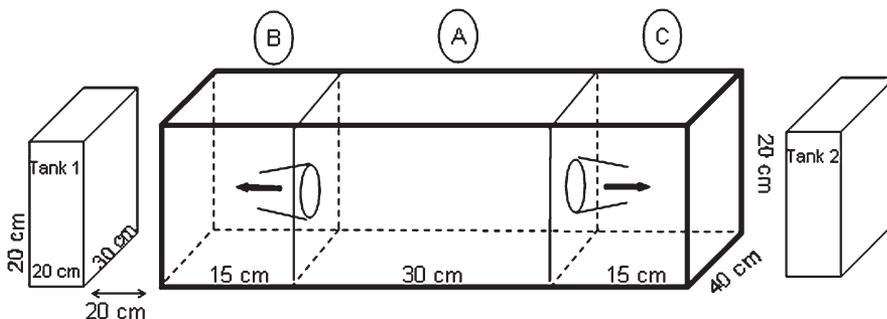
modes were vision and olfaction. The 2 cues were heterospecific versus conspecific crustaceans and preferred habitat versus second preferred habitat (according to the results in Experiment 1). To evaluate the potential role of vision on choice, competing stimuli (conspecific vs. heterospecific crustacean and habitat 1 vs. habitat 2) were randomly assigned to tanks 1 and 2 (Figure 2). To evaluate the potential role of olfaction, 10 l of seawater in which conspecific versus heterospecific crustaceans and the 2 habitat types had been immersed for 6 h was randomly assigned to compartment B or C (Figure 2). Experimental test subjects (crustacean postlarvae) were introduced into compartment A (Figure 2), and subsequent movement of the test subject into the adjoining compartment (B or C) within 5 min was scored as a "choice," whereas retention in the central compartment was scored as "no choice." Prior to each sensory experiment, a null distribution of choices was first derived by introducing crustacean postlarvae to the chamber without cues (a "control" for aquaria artifacts).

In Experiment 3A, the response of crustacean larvae to conspecifics versus heterospecifics used the 15 postlarvae of each species that had been used in Experiment 1. Ten conspecific juveniles and 10 heterospecific juveniles (individuals belonging to another crustacean species that were readily available at the time of the experiment) were used. The groups of stimulus crustaceans were changed after each sensory test (visual and chemical cues). A chi-square test for each species was carried out to compare the observed distribution with a baseline distribution (number of postlarvae in compartments A, B, and C in the absence of external effects on the aquarium system). As we conducted 2 successive chi-square tests for each species (visual and chemical cues), we performed a correction of Bonferroni test for multiple tests (Holm 1979). To obtain a significant difference, the P value of the chi-square test must be lower than the P_k value ($P_k = 0.05/2 = 0.025$, 0.05 is the probability threshold at 5% and 2 is the number of multiple tests performed with the same data).

In Experiment 3B, the response of crustacean postlarvae to the preferred versus the second most preferred habitat (according to the results in Experiment 1) used the same 15 postlarvae that had been used in Experiment 2. A chi-square test for each species was carried out to compare the observed distribution with a baseline distribution. As we conducted 2 successive chi-square tests for each species (visual and chemical cues), the P value of the chi-square test must be lower than the P_k value ($P_k = 0.05/2 = 0.025$).

Figure 3
The proportion of crustacean postlarvae (15 individuals tested for each species) that chose each habitat (live coral, dead coral, macroalgae, and sand). If crustacean postlarvae were still in the central part of aquaria after 5 min, the trial was stopped (scored as "no choice"). The 9 species were pooled into 3 taxonomic groups: Brachyura (*C. calappa*, *P. planifrons*, and *Xanthidae* sp.), Stomatopod (*L. maculata*, *L. sulcata*, and *Raoulserenea* sp.), and Caridae + Palinuridae (*S. hispidus* and *Palaemonidae* sp. + *Panulirus penicillatus*). Lines above bars refer to 1 standard error (computed between species).
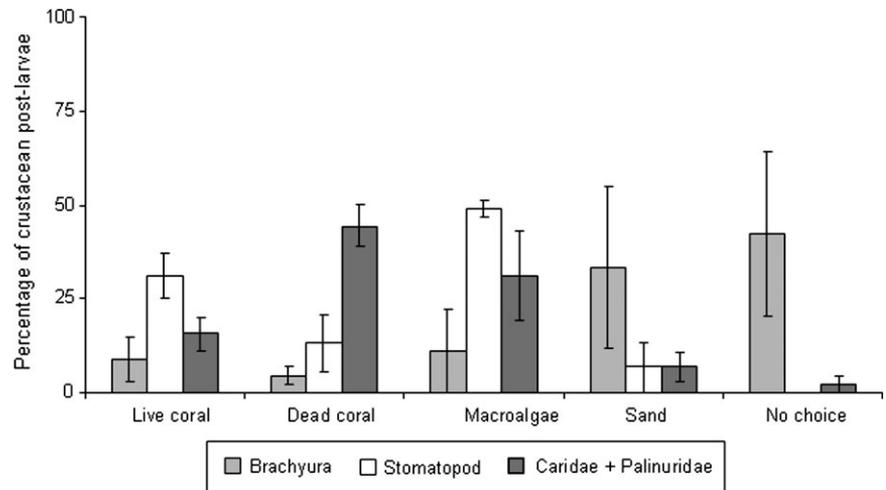
Experiment 4: biochemical analyses to compare the chemical fingerprints of different water samples

High performance liquid chromatography (HPLC) was used as a powerful tool to determine the profiles of seawater metabolites. Subsequently, the chemical fingerprints of the different water samples used in Experiments 3: conspecifics (only 4 crustacean species could be analyzed due to logistical problems) and the 4 habitats (live coral, dead coral, sand, and macroalgae), were compared. We collected 10 l of water from tanks containing 1) each species of conspecifics (10 individuals) immersed for 6 h, 2) each type of habitat (live coral, dead coral, macroalgae, or sand) immersed for 6 h, and 3) 10 l of water from tanks unoccupied (i.e., "ambient") filtered seawater.

The 9 different seawater collections were filtered under vacuum through solid phase extraction cartridges containing a C_{18} silica gel–based bonded phase sorbent, then washed with 50 ml of distilled water, and then desorbed with 50 ml of methanol. The organic phase of each collection was then freeze dried leaving a powdery organic residue. The organic extracts from the 9 water samples were dissolved in 1 ml of methanol before analysis. HPLC was performed with a system from Waters (Bellefonte, PA), including the Alliance separations module 2695, the column heater, and the 2998

photodiode array detector. The liquid chromatograph was equipped with a 5-μm Phenomenex GeminiC18 column (150 × 3.00 mm). The equipment was controlled, and the data were handled using Empower Chromatography Data software (Waters). The following analytical solvent and gradient systems were used: A, 0.1% aqueous trifluoroaceticacid; B, acetonitril:trifluoroaceticacid (99.9:0.1, v:v); linear gradient from 25% B to 100% B within 15 min and then 100% B in additional 5 min; and the flow rate was 0.5 ml min^{-1}, and injection volume was 20 μl. Column temperature was set at 30 °C, and the data collection across the 210- to 500-nm wavelength range was performed as follows: sampling 1 point per second, resolution 1.2 nm, and no smoothing. To maximize sensitivity, the data were processed to create a Max Plot chromatogram, which plots the maximum spectral absorbance measured at each time point. The Max Plot enables the detection of all detectable UV-absorbing components in the sample.

Overall, the study was carried out on 9 crustacean species with a cohort of 30 postlarvae captured on the same night for each species. A total of 15 postlarvae of each species were tested in Experiments 1 and 3A, and the 15 other postlarvae were tested in Experiments 2 and 3B. For the clarity of figures,

Figure 4
The proportion of crustacean postlarvae (15 individuals tested for each species) that chose between the presence or absence of 10 older conspecifics in standardized quantities of the habitat that was preferred in Experiment 1. Lines above bars refer to 1 standard error (computed between species).
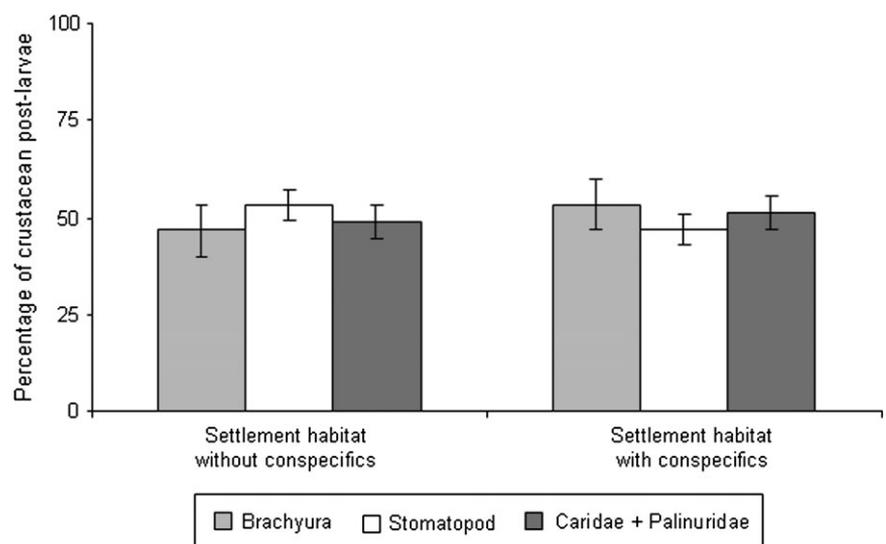
the 9 species were pooled into 3 taxonomic groups: Brachyura (*Calappa calappa*, *Pachygrapsus planifrons*, and *Xanthidae* sp.), Stomatopod (*Lysiosquillina maculata*, *L. sulcata*, and *Raoulserenea* sp.), and Caridae + Palinuridae (*Stenopus hispidus* and *Palaemonidae* sp. + *Panulirus penicillatus*). However, the statistical analyses were conducted at species level.

RESULTS

Experiment 1: habitat preferences

Sixty-seven percent of crustacean species made active habitat choices at the postlarval stage (Figure 3). For Brachyura taxa, postlarvae of *C. calappa* and *P. planifrons* remained mainly in the central part of aquaria (no choice—74% and 54% of postlarvae, respectively). In contrast, *Xanthidae* postlarvae made active habitat choices (distribution differed significantly from the null distribution: equal repartition of postlarvae among the 4 habitats—chi-square test: $\chi_3^2 = 19.9$, $P < 0.0001$) with a preference for the sand habitat. For Stomatopod taxa, postlarvae made active habitat choices with a preference for macroalgae: 54% of *L. maculata* postlarvae ($\chi_3^2 = 8.7$, $P = 0.03$), 47% of *L. sulcata* postlarvae ($\chi_3^2 = 8.3$, $P = 0.04$), and 47% of *Raoulserenea* postlarvae ($\chi_3^2 = 8.3$, $P = 0.04$). For Caridae and Palinuridae taxa, *Palaemonidae* sp. postlarvae made active hab-

itat choices ($\chi_3^2 = 9.3$, $P = 0.02$) with a preference for macroalgae. *P. penicillatus* postlarvae preferred dead coral ($\chi_3^2 = 8.7$, $P = 0.03$), whereas this preference was not significant for *S. hispidus* ($\chi_3^2 = 4.6$, $P = 0.10$).

Experiment 2: effect of conspecifics

All crustacean postlarvae responded identically to conspecifics (Figure 4). Postlarvae did not discriminate between the presence or absence of conspecifics on their preferred habitat (chi-square test: all χ_1^2 values < 3.84, $P > 0.05$). Postlarvae were both attracted by their preferred habitat with or without conspecifics.

Experiment 3: sensory modes

In the absence of added stimuli, most crustacean postlarvae remained in the central compartment (exhibiting "no choice") and the remaining crustaceans (which did display a "choice") showed no preference between compartment B versus C (all χ_1^2 values < 3.84, $P > 0.05$, Figures 5 and 6).

In Experiment 3A (Figure 5), the distributions of crustacean postlarvae were often similar to the null distribution when presented with visual or olfactory cues separately (chi-square test: χ_2^2 values < 5.99, $P > P_k$). Thus, postlarvae of

a) **Brachyura**

b) **Stomatopod**

c) **Caridae + Palinuridae**

Percentage of crustacean post-larvae

☐ No cue ▨ Visual cues ▮ Chemical cues

Figure 5
The distribution of choices by crustacean postlarvae presented with no cue (unshaded bars) or 2 competing cues (visual or chemical cues—gray and black bars). Choices offered in compartments B and C are indicated by "conspecifics" or "heterospecifics," whereas "no choice" refers to crustacean postlarvae that remained in compartment A (see Figure 2). Lines above bars refer to 1 standard error (computed between species).
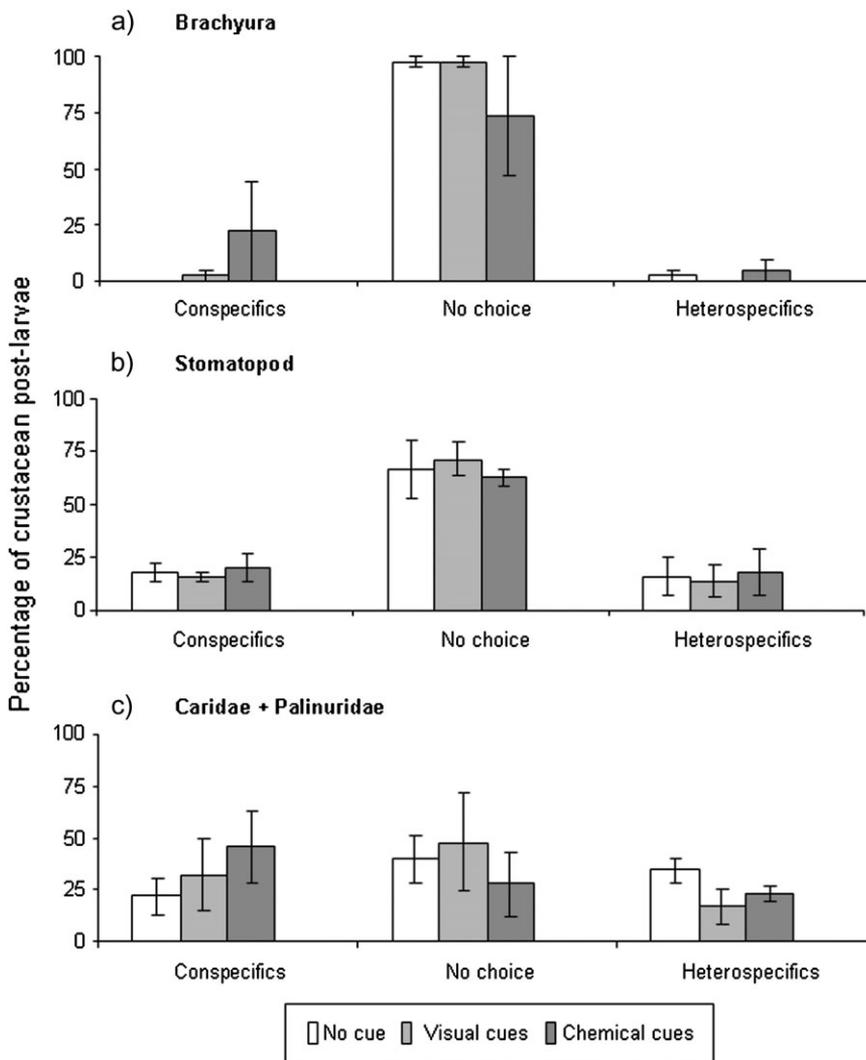
Figure 6
The distribution of choices by crustacean postlarvae presented with no cue (unshaded bars) or 2 competing cues (visual or chemical cues—gray and black bars). Choices offered in compartments B and C are indicated by "preferred habitat" (the most preferred habitat in Experiment 1) or "other habitat" (the second preferred habitat in Experiment 1), whereas "no choice" refers to crustacean postlarvae that remained in compartment A (see Figure 2). Lines above bars refer to 1 standard error (computed between species).
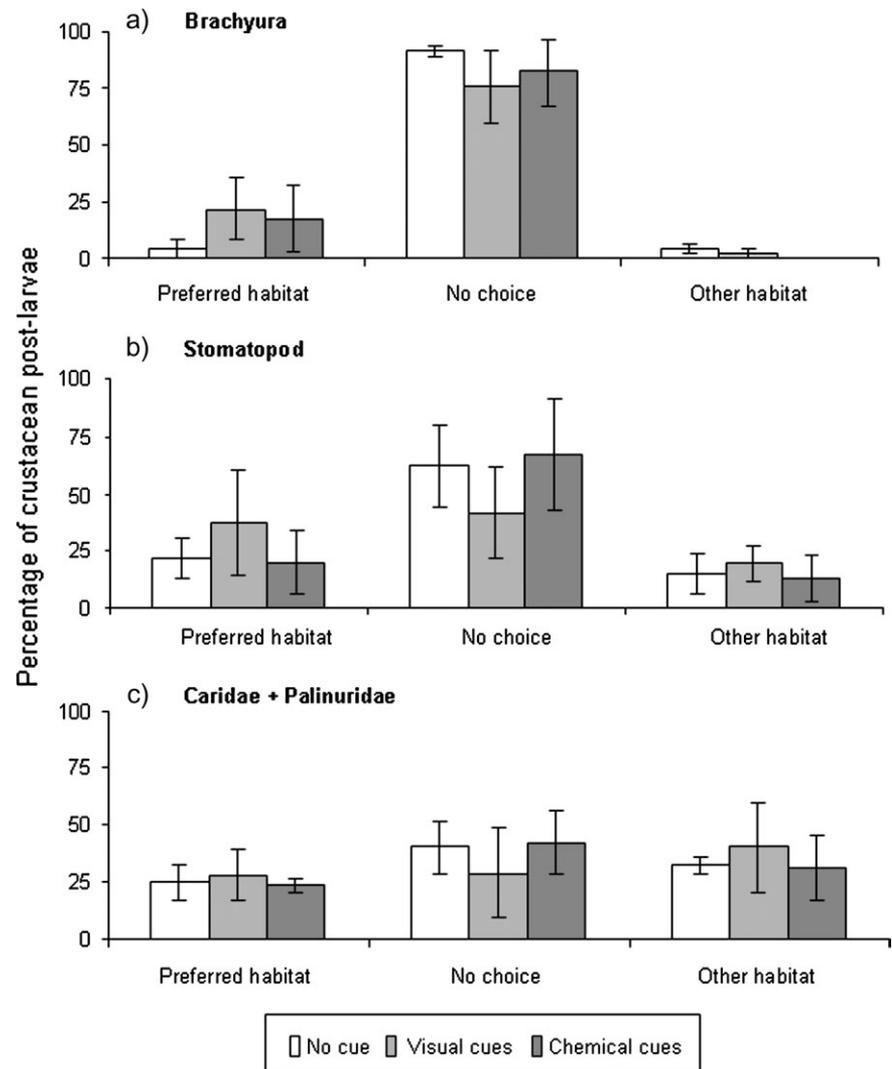
7 species (*C. calappa, P. planifrons, L. maculata, L. sulcata, Raoulserenea* sp., *S. hispidus,* and *P. penicillatus*) were not attracted to and did not differentiate between conspecifics versus heterospecifics. In contrast, *Xanthidae* postlarvae significantly preferred conspecifics over heterospecifics when using olfactory cues ($\chi_2^2 = 91.6$, $P < 0.001$), but no preference was found with visual cues ($\chi_2^2 = 0$, $P > P_k$). Similarly, *Palaemonidae* postlarvae preferred conspecifics over heterospecifics with both visual ($\chi_2^2 = 38.5$, $P < 0.001$) and olfactory cues ($\chi_2^2 = 58.3$, $P < 0.001$).

In Experiment 3B (Figure 6), 4 species could differentiate between their preferred habitat versus their second preferred habitat from Experiment 1. *Xanthidae* postlarvae preferred sand over macroalgae using visual ($\chi_2^2 = 10.3$, $P = 0.005$) and olfactory cues ($\chi_2^2 = 39.6$, $P < 0.001$). *P. planifrons* postlarvae preferred macroalgae over sand using visual cues ($\chi_2^2 = 15.3$, $P < 0.001$), but no preferences were found with olfactory cues ($\chi_2^2 = 3.3$, $P = 0.20$). Similarly, the 2 *Lysiosquillina* species preferred macroalgae over dead coral with visual cues (*L. maculata*: $\chi_2^2 = 10.2$, $P = 0.005$ and *L. sulcata*: $\chi_2^2 = 24.6$, $P < 0.001$), but no differences were found when olfactory cues were used (*L. maculata*: $\chi_2^2 = 0.4$, $P > P_k$ and *L. sulcata*: $\chi_2^2 = 0$, $P > P_k$). Lastly, postlarvae of the 5 other species (*C. calappa, Raoulserenea* sp., *S. hispidus, Palaemonidae sp.,* and *P. penicilla-*

tus) were not attracted to and did not differentiate between their preferred habitat versus their second preferred habitat (chi-square test: χ_2^2 values < 5.99, $P > P_k$).

Experiment 4: biochemical analyses to compare the chemical fingerprints of different water samples

As a first result, we obtained HPLC traces with well observable (0.001–0.6 AU) and resolved peaks corresponding to chemical fingerprints of each seawater sample (Figure 7). Seawater collected from tanks containing conspecifics or habitats and unoccupied tanks showed 1 major peak in common at the retention time of 7 h and 30 min (offset slightly) as well as 2 minor peaks (in polar compounds' zone—shown by arrows in Figure 7). We interpreted these shared patterns as background signatures in seawater.

Chromatograms from tanks containing conspecifics and habitats showed a higher molecular diversity than that from unoccupied tank water (especially in medium polar and nonpolar compounds' zones) except for *L. maculata* and *S. hispidus*, indicative of specific odors produced by each conspecific and habitat. Moreover, the chromatograms from each habitat type and conspecific species showed differences in chemical fingerprints with differences in the absorption quantity of
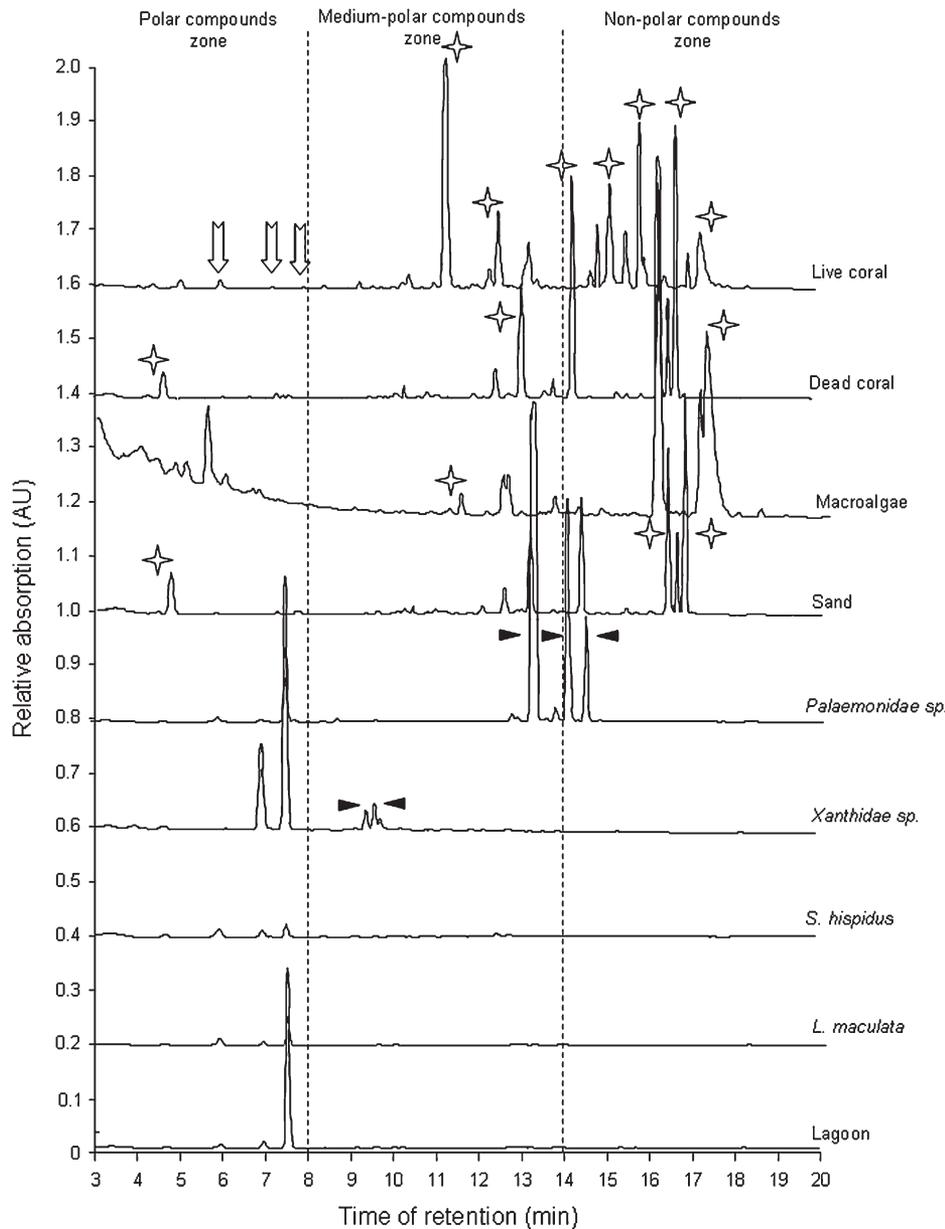
Figure 7
HPLC chromatograms of seawater collected from tanks containing each conspecific species or each habitat and unoccupied tank (filtered lagoon water). We deleted the first 3 min of chromatograms as it correspond to dead volume of the column. The chromatograms were divided in 3 zones (Mant and Hodges 1991): one zone where polar compounds were eluted (3–8 min), one zone where medium polar compounds were eluted (8–14 min), and one zone where nonpolar compounds were eluted (14–20 min). To maximize sensitivity, the data were processed to create Max Plot chromatograms, which plots the maximum spectral absorbance measured at each time point. Arrows represent the major and minor peaks in common with all water samples but with varying degrees of absorption quantity. Triangles represent the major peaks specific to one or more conspecific chromatograms. Crosses represent the major peaks specific to one or more habitat chromatograms.

major peaks and in the apparition of some other major peaks (shown by stars and triangles in Figure 7). The diversity of major peaks was more pronounced from habitat than species chromatograms. We interpreted these different patterns as each habitat type and each conspecific species (except *L. maculata* and *S. hispidus*) having a specific and unique chemical fingerprint.

DISCUSSION

Among the 9 crustacean species studied, 6 made active habitat choices at the postlarval stage, but the presence or absence of conspecifics did not influence their selective choice. Our sensory experiments found that 4 species differentiated between their preferred habitat versus another habitat and 2 species differentiated between conspecifics and heterospecifics, using visual and/or olfactory cues. Lastly, the HPLC experiments showed that the 4 habitats and conspecifics (except *L. macu-*

lata and *S. hispidus*) tested have different and unique chemical fingerprint.

The settlement phase has been widely studied for coral reef crustaceans, and it has often been demonstrated that postlarvae choose a suitable habitat according to the presence of conspecifics and/or based on habitat characteristics (for review, see Pechenik 1990; Forward et al. 2001). Our results confirmed active selection according to habitat characteristics (Experiment 1) but not to the presence of conspecifics (Experiment 2). Our results further suggest that crustacean postlarvae may use a range of sensory modalities for effective habitat selection at settlement (Experiment 3). Indeed, 55% of crustacean species tested could discriminate between species and habitats using chemical and/or visual cues. Several studies have highlighted the chemical or visual cues emitted by habitats and conspecifics that induce the metamorphosis of crustacean postlarvae (e.g., Gebauer et al. 2003; Diele and Simith 2007; O'Connor 2007; Krimsby and Epifanio 2008). In the absence of such cues, crab megalopae or spiny lobster

pueruli are capable of postponing metamorphosis until a suitable signal is detected, subsequently increasing their time spent in the plankton and the chance of finding a suitable habitat (for review, see Pechenik 1990; Pawlik 1992). This metamorphic plasticity may increase postlarval dispersal and be beneficial for those species that do not settle in areas in which conspecifics reside. Compared with the numerous studies on metamorphosis-stimulating cues, few studies have demonstrated that crustacean postlarvae are attracted by chemical and visual cues emitted by conspecifics and habitat (see for exceptions Shanks 1995; Harvey 1996; O'Connor and Judge 1997; Jeffs et al. 2005; Zito-Livingston and Childress 2009). For example, Forward et al. (2003) demonstrated that megalopae of the blue crab (*Callinectes sapidus*) orientated themselves toward nursery areas with sea grass beds using specific habitat odors. In our study, crustacean postlarvae were more attracted by habitats than conspecifics, and they used visual cues preferentially over chemical cues (Experiment 3). However, the HPLC experiments showed that the 4 habitats and conspecifics tested having different and unique chemical fingerprint (Experiment 4). Interestingly, *Xanthidae* and *Palaemonidae* conspecifics with 2 and 3 specific chemical peaks, respectively, and *S. hispidus* and *L. maculata* conspecifics having none (Figure 7), only *Xanthidae* and *Palaemonidae* postlarvae responded positively to the chemical cues of conspecifics (Figure 5). However, our HPLC sampling protocol needs to be improved. Future studies will have to highlight the link between the odor choice results and patterns of water chemistry. HPLC chromatograms showed subtly different chemical fingerprints from conspecifics and settlement habitats (Figure 7). But, the present study did not highlight a clear link between the variation in these chemical signals (Fig. 4) and the preferences exhibited by postlarvae (Figures 5 and 6).

The use of environmental cues (from habitats and/or conspecifics) by crustacean postlarvae would allow them to orientate themselves toward different available habitats in a coral lagoon and to find a suitable habitat more easily. A multitude of other animals also use public information to find a suitable habitat, but generally they use conspecific more often than habitat information (for review, see Stamps and Krishnan 2005). For example, territorial lizards and grasshoppers use the presence of conspecifics to evaluate the potential quality of particular habitat (Cassier et al. 2000). In previous experiments, Lecchini, Planes, et al. (2005) and Lecchini, Shima, et al. (2005) showed that postlarvae of 25 fish species were more attracted to cues emitted by conspecifics than to habitat itself (coral or algal habitat). For coral reef fish, the social aggregation of postlarvae with older conspecifics may be the result of individuals using conspecific "guides" to find potentially beneficial resources (Childress and Herrnkind 2001; Leis et al. 2002). It will be a fruitful avenue for future research to understand why coral reef fish postlarvae prefer to use conspecific information, whereas coral reef crustacean postlarvae prefer to use habitat information for settlement. One possible explanation could be the specific rates of metamorphosis between crustacean and fish postlarvae at settlement. At settlement, fish postlarvae are almost metamorphosed, having morphological and ecological characteristics relatively close to juveniles (McCormick et al. 2002). In contrast, crustacean postlarvae are often not metamorphosed at settlement; their metamorphosis occurs once on the settlement habitat (for review, see Forward et al. 2001; Pechenik 1990). Thus, fish postlarvae may potentially "search" conspecifics in order to settle on the same habitat, as postlarvae and juveniles have relatively close morphological and ecological characteristics. In contrast, crustacean postlarvae may potentially search an adequate habitat for settlement to accomplish their metamorphosis, and once meta-

morphosed, crustacean individuals could then change habitat to live in groups with older conspecific juveniles (i.e., asocial to social ontogenetic shift in conspecific attraction, as found, e.g., in the spiny lobster; Butler et al. 1999).

To conclude, the ability to find a suitable habitat after a period in open water represents a significant challenge to marine postlarvae, and the mechanisms by which this is achieved are poorly understood (Dixson et al. 2008). Our study is the first to highlight the sensory modalities for a broad range of crustacean species (9 species belonging to Brachyura, Stomatopod, Caridae, and Palinuridae) to detect and move toward settlement habitats and/or conspecifics. However, our experiments were conducted in aquaria, and the conclusions of the study must be taken with some caution. Indeed, negative results (no movement toward conspecifics or habitats) with the experimental protocol used do not allow us to conclude that postlarvae lack the sensory modalities to recognize their settlement habitat or other crustaceans. They could remain in the central compartment even after recognizing cues from conspecifics or habitats. Overall, increasing knowledge of how postlarvae navigate to suitable adult habitats could contribute to management actions that aim to sustain viable marine populations. Our work could thus have fundamental implications for restoration ecology: How chemical cues that shape behavior patterns of crustacean postlarvae may be isolated (by HPLC technique) and harnessed to facilitate 1) colonization of empty patches of otherwise suitable habitat or 2) recolonization of patches driven extinct locally by past environmental impacts (e.g., human-mediated habitat degradation or overharvesting).

REFERENCES

Armsworth PR. 2000. Modelling the swimming response of late stage larval reef fish to different stimuli. Mar Ecol Prog Ser. 195:231–247.

Blumstein DT, Bouskila A. 1996. Assessment and decision making in animals: a mechanistic model underlying behavioral flexibility can prevent ambiguity. Oikos. 77:569–576.

Butler MJ, Macdiarmid AB, Booth J. 1999. The cause and consequence of ontogenetic changes in social aggregation in New Zealand spiny lobsters. Mar Ecol Prog Ser. 188:179–191.

Cassier P, Bohatier J, Descoins C, Nagnan Le Meillour P. 2000. Communication chimique et environnement. Paris: Belin.

Childress MJ, Herrnkind WF. 2001. The guide effect influence on the gregariousness of juvenile Caribbean spiny lobsters. Anim Behav. 62:465–472.

Codling EA, Hill NA, Pitchford JW, Simpson SD. 2004. Random walk models for the movement and recruitment of reef fish larvae. Mar Ecol Prog Ser. 279:215–224.

Diele K, Simith DJB. 2007. Effects of substrata and conspecific odour on the metamorphosis of mangrove crab megalopae, *Ucides cordatus* (Ocypodidae). J Exp Mar Biol Ecol. 348:174–182.

Dixson DL, Jones GP, Munday PL, Planes S, Pratchett MS, Srinivasan M, Syms C, Thorrold SR. 2008. Coral reef fish smell leaves to find island homes. Proc R Soc Lond B Biol Sci. 275:2831–2839.

Doherty PJ. 2002. Variable replenishment and the dynamics of reef fish populations. In: Sale PF, editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. San Diego (CA): Academic Press. p. 327–358.

Dufour V, Galzin R. 1993. Colonization patterns of reef fish larvae to the lagoon at Moorea Island, French Polynesia. Mar Ecol Prog Ser. 102:143–152.

Forward RB, Tankersley RA, Rittschof D. 2001. Cues for metamorphosis of brachyuran crabs: an overview. Am Zool. 41:1108–1122.

Forward RB, Tankersley RA, Smith KA, Welch JM. 2003. Effect of chemical cues on orientation of blue crab, *Callinectes sapidus*, megalopae in flow: implications for location of nursery areas. Mar Biol. 142:747–756.

Gebauer P, Paschke K, Anger K. 2003. Delayed metamorphosis in Decapod crustaceans: evidence and consequences. Rev Chil Hist. 76:169–175.

Harvey AW. 1996. Delayed metamorphosis in Florida hermit crabs: multiple cues and constraints (Decapoda: Paguridae and Diogenidae). Mar Ecol Prog Ser. 141:27–36.

Holm S. 1979. A simple sequentially rejective multiple test procedure. Scand J Stat. 6:65–70.

Jeffs A, Holland RC. 2000. Swimming behaviour of the puerulus of the spiny lobster, *Jasus edwardsii* (Decapoda, Palinuridae). Crustaceana. 73:847–856.

Jeffs A, Montgomery JC, Tindle C. 2005. How do spiny lobster post-larvae find the coast? NZ J Mar Freshw Res. 39:605–617.

Kingsford MJ, Leis JM, Shanks A, Lindeman K, Morgan S, Pineda J. 2002. Sensory environments, larval abilities and local self-recruitment. Bull Mar Sci. 70:309–340.

Krimsby LS, Epifanio CE. 2008. Multiple cues from multiple habitats: effect on metamorphosis of the Florida stone crab, *Menippe mercenaria*. J Exp Mar Biol Ecol. 358:178–184.

Lecchini D, Planes S, Galzin R. 2005. Experimental assessment of sensory modalities of coral reef fish larvae in the recognition of settlement habitat. Behav Ecol Sociobiol. 56:18–26.

Lecchini D, Shima J, Banaigs B, Galzin R. 2005. Larval sensory abilities and mechanisms of habitat selection of a coral reef fish during settlement. Oecologia. 143:326–334.

Leis JM, Carson-Ewart BM, Webley J. 2002. Settlement behaviour of coral reef fish larvae at subsurfae artificial reef moorings. Mar Freshw Res. 53:319–327.

Leis JM, McCormick MI. 2002. The biology, behavior, and ecology of the pelagic, larval stage of coral reef fishes. In: Sale PF, editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. San Diego (CA): Academic Press. p. 171–199.

Mant CT, Hodges RS. 1991. High-performance liquid chromatography of peptides and proteins: separations, analysis, and conformation. Boston: CRC Press.

McCormick MI, Makey LJ, Dufour V. 2002. Comparative study of metamorphosis in tropical reef fishes. Mar Biol. 141:841–853.

Montgomery JC, Jeffs A, Simpson SD, Meekan M, Tindle C. 2006. Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. Adv Mar Biol. 51:143–196.

Myrberg AA, Fuiman LA. 2002. The sensory world of coral reef fishes. In: Sale PF, editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. San Diego (CA): Academic press. p. 123–148.

O'Connor NJ. 2007. Stimulation of molting in megalopae of the Asian shore crab *Hemigrapsus sanguineus*: physical and chemical cues. Mar Ecol Prog Ser. 352:1–8.

O'Connor NJ, Judge ML. 1997. Flexibility in timing of molting of fidler crab megalopae: evidence from in situ manipulation of cues. Mar Ecol Prog Ser. 146:55–60.

Pawlik JR. 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr Mar Biol. 30:273–335.

Pechenik JA. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? Ophelia. 32:63–94.

Poupin J. 1996. Crustacea Decapoda of French Polynesia (Astacidea, Palinuridea, Anomura, Brachyura). Atoll Res Bull. 442: 1–114.

Poupin J. 1998. Crustacea Decapoda and Stomatopoda of French Polynesia (Dendrobranchiata, Stenopodidea, Caridea, Thalassinidea, and Stomatopoda, with additions to Astacidea, Palinuridea, Anomura, and Brachyura). Atoll Res Bull. 451:1–62.

Poupin J. 2007. Database of Crustacea (Decapoda and Stomatopoda) from central Pacific Islands. [Internet]. Brest (France): Marine Nationale. Available from: http://decapoda.free.fr; http://decapoda .ecole-navale.fr/.

Shanks A. 1995. Orientated swimming by megalopae of several eastern North Pacific crab species and its potential role in their onshore migration. J Exp Biol Ecol. 186:1–16.

Stamps J, Krishnan VV. 2005. Non intuitive cue use in habitat selection. Ecology. 86:2860–2867.

Werner EE. 1988. Size, scaling and the evolution of complex life cycles. In: Ebenman B, Perssons L, editors. Size-structured populations. Berlin (Germany): Springer-Verlag. p. 61–81.

Zito-Livingston AN, Childress MJ. 2009. Does conspecific density influence the settlement of Caribbean spiny lobster *Panulirus argus* postlarvae? NZ J Mar Freshw Res. 43:313–325.