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Larval sensory abilities and mechanisms of habitat selection of a coral reef fish during settlement

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Abstract Sensory abilities and preferences exhibited by mobile larvae during their transition to juvenile habitats can establish spatial heterogeneity that drives subsequent species interactions and dynamics of populations. We conducted a series of laboratory and field experiments using coral reef fish larvae (*Chromis viridis*) to determine: ecological determinants of settlement choice (conspecifics vs. heterospecifics vs. coral substrates); sensory mechanisms (visual, acoustic/vibratory, olfactory) underlying settlement choice; and sensory abilities (effective detection distances of habitat) under field conditions. *C. viridis* larvae responded positively to visual, acoustic/vibratory, and olfactory cues expressed by conspecifics. Overall, larvae chose compartments of experimental arenas containing conspecifics in 75% of trials, and failed to show any significant directional responses to heterospecifics or coral substrates. In field trials, *C. viridis* larvae detected reefs containing con-

specifics using visual and/or acoustic/vibratory cues at distances <75 cm; detection distances increased to <375 cm when olfactory capacity was present (particularly for reefs located up-current). We conducted high performance liquid chromatography (HPLC) analyses of seawater containing *C. viridis* juveniles and isolated high concentrations of several organic compounds. Subsequent laboratory trials demonstrated that *C. viridis* larvae responded positively to only one of these organic compounds. This compound was characterized by a weak polarity and was detected at 230 nm with a 31-min retention time in HPLC. Overall, our results suggest that fishes may use a range of sensory mechanisms effective over different spatial scales to detect and choose settlement sites, and species-specific cues may play a vital role in establishment of spatial patterns at settlement.

Keywords Coral reef fish larvae · Sensory mechanisms · Habitat selection · Settlement cues · High performance liquid chromatography

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Introduction

Perhaps one of the greatest challenges facing the majority of marine reef organisms in larval stages that potentially disperse and develop in offshore waters is how to relocate patchily distributed reef environments in a vast oceanic matrix (Doherty 2002; Myrberg and Fuiman 2002). Much recent work has focused on the sensory capabilities of larval stages of reef organisms that allow them to detect distant reefs (e.g., Tolimieri et al. 2000; Kingsford et al. 2002; Leis et al. 2002), and the swimming abilities (e.g., Leis and Carson-Ewart 1997; Stobutzki and Bellwood 1997; Dudley et al. 2000) and/or hydrographic features (e.g., Natunewicz et al. 2001; Poulin et al. 2002; Sponaugle et al. 2002) that allow these larvae to ultimately reach and settle to

suitable post-larval habitats. Relatively few studies have examined the role of larval sensory mechanisms in patch identification and patch selection at smaller scales (e.g., among patches separated by centimeters to meters within a single reef or lagoon). Sensory cues useful for locating patches at these smaller scales may differ from those employed at large scales; visual cues may be of limited use to many reef fishes which settle disproportionately during moonless nights (Dufour and Galzin 1993).

Although a number of studies have examined patterns of habitat use of settling reef fish larvae at small spatial scales (e.g., Booth 1992; Sweatman 1983; Shima 2001a), we continue to lack understanding of the underlying sensory mechanisms and specific cues that trigger individuals to choose patches within a reef matrix (but see Sweatman 1988, Booth 1992, and Elliot et al. 1995 for three partial investigations). Such small-scale behavioral responses resulting in patchy distributions can have strong effects on subsequent growth and survival of individuals (e.g., Connell and Jones 1991; Tupper and Boutilier 1997; Shima and Osenberg 2003), and can also reinforce or ameliorate spatial heterogeneity in environmental features (e.g., Tupper and Hunte 1994; Schmitt and Holbrook 1996; Tolimieri et al. 1998).

Here we examine patterns and mechanisms underlying habitat selection by larvae of the coral reef fish *Chromis viridis* (Cuvier 1830) at settlement. *C. viridis* is a small planktivorous damselfish found commonly on coral reefs throughout much of the Indo-Pacific region (Allen 1991). At many sites, *C. viridis* probably serves an important ecological role as a patchily distributed, highly aggregated planktivore (i.e., a resource competitor with other planktivorous species, including some corals), and as an important prey species for many reef-based predators. *C. viridis* juveniles and adults shelter in a relatively narrow range of branching coral habitats (Lecchini 2003). Consequently, *C. viridis* likely respond to and contribute to environmental heterogeneity in reef ecosystems.

Materials and methods

We used a series of laboratory and field experiments conducted during March–April 2002 on the island of Moorea, French Polynesia (17°30'S, 149°50'W) to explore sensory abilities (effective detection distances of settlement habitat), sensory mechanisms (visual, acoustic/vibratory, olfactory), and ecological determinants of settlement choices (conspecifics vs. heterospecific fishes vs. coral substrates) of *C. viridis* larvae. Our studies used only larvae with no prior experience on settlement habitats (i.e., naïve larvae), obtained from nets fixed to the reef crest (Dufour and Galzin 1993). Fish captured in crest nets during the night were collected at dawn, transferred and subsequently maintained in aquaria containing UV-sterilized seawater filtered through a 50- μ m filter. All experiments described below were conducted during the evening after larval capture (i.e., within 24 h of collection). Lab experiments were conducted in an indoor facility (insulated from external sources of light and sound), under artificial lighting conditions provided by evenly distributed neon light sources. Field experiments were conducted under ambient moonlight between 9 and 11 pm.

Laboratory experiments to determine ecological factors and sensory mechanisms underlying settlement choice

We explored the patterns and mechanisms of settlement behavior of *C. viridis* using pairwise choice experiments conducted in 16 purpose-built experimental arenas. Each arena (Fig. 1) consisted of a large rectangular aquarium subdivided into five compartments along the long-axis by transparent panels, and with two additional smaller aquaria at each end.

Trials consisted of individual test subjects (i.e., one *C. viridis* larva per arena) that were released into the central compartment (A). Subsequent movements of test

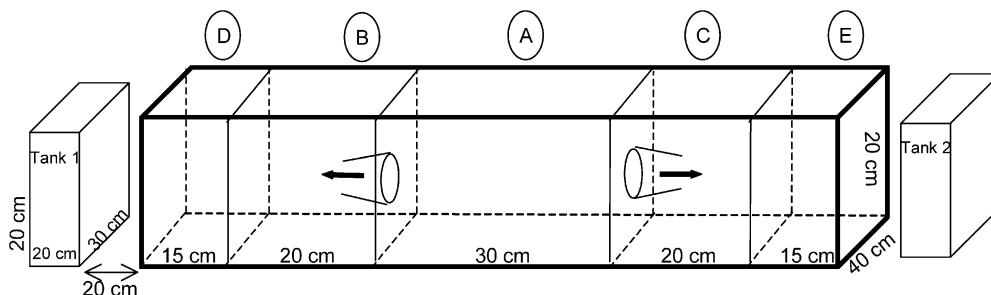


Fig. 1 Diagram of experimental arena used to evaluate patterns and mechanisms underlying settlement choice. The arena consists of an aquarium with five compartments (A–E), with A, B and C interconnected via funnels and D and E isolated from central compartments via plastic panels affixed with removable opaque barriers. Additional tanks on either side of arena (labeled no. 1 and 2) are isolated from experimental arenas and mounted upon

separate platforms to prevent transfer of vibratory signals. Experimental test subjects (*C. viridis* larvae) are introduced into compartment A (one larva per arena), cues are presented in compartments B, C, D, E or tanks 1 or 2 (to test sensory mechanisms separately), and settlement choice is determined as the pattern of movement of test subjects to compartments B or C over 2-minute observation periods

subjects into adjoining compartments (B or C) and towards specific stimuli were scored as “choices”. Each trial was terminated after 2 min (preliminary behavioral observations and results presented subsequently suggest this duration was sufficient for *C. viridis* larvae to respond to cues). Openings to compartments B and C were fitted with funnels that impeded return movements to compartment A. Consequently, our scored choices primarily reflect initial decisions of individual larvae (i.e., individuals had limited opportunity sample multiple compartments prior to making a choice).

Sensory mechanisms underlying settlement choice

To evaluate the potential role of visual cues on choice, competing stimuli (e.g., conspecifics vs. heterospecific fishes vs. coral substrates) were randomly assigned to the separate smaller aquaria at either end of the arena. These smaller aquaria were disconnected from the main arena and mounted on a separate foam-insulated platform to ensure that only visual cues (and not olfactory or acoustic/vibratory cues) were available to test subjects.

To evaluate the potential role of acoustic/vibratory cues on choice, conspecifics or heterospecific fishes were randomly assigned and transferred to compartments D or E. The compartments D and E were isolated from compartments A, B, and C via sealed, watertight plastic panels, and we added additional opaque barriers to separate these compartments visually from A, B, and C. In this configuration, we presume that only acoustic/vibratory cues were available to test subjects (by way of sounds and/or vibrations transmitted through the sealed and opaque plexiglass tank dividers). Note that this experiment did not include substrates as treatments, as we had no expectation for corals producing audible cues above ambient laboratory noise.

To evaluate potential olfactory cues in the absence of visual or acoustic/vibratory cues, 2 l of seawater in which conspecifics, heterospecific fishes or coral substrates had been immersed for 6 h (i.e., containing olfactory signatures of competing stimuli) were randomly assigned and transferred to compartments B or C. Openings to compartment A were temporally sealed with inserted plastic panels to facilitate seawater transfer. These were removed immediately prior to release of test subject in compartment A.

Ecological determinants of settlement choice

Using the protocol described above, we conducted three separate experiments to evaluate the relative importance of several probable ecological determinants of settlement choice for *C. viridis* (e.g., conspecifics, heterospecific fishes, coral substrates). For each experiment, we examined relevant sensory mechanisms underlying choice. Each sensory evaluation comprised

16 replicate trials conducted using a single cohort of 16 fish (i.e., collected on the same day; individual test subjects were not recycled across sensory evaluations; a total of 48 larvae were used to evaluate three sensory mechanisms). In each sensory evaluation (i.e., for each cohort), we first constructed an expected distribution of directional movement in the absence of manipulated cues (a “control” for tank artifacts). In all cases, test subjects predominately remained in the central compartment (A) in the absence of external cues. Chi-square tests were used to evaluate significant ($P < 0.05$) responses to stimuli in subsequent trials as deviations from these expected distributions.

Experiment 1: conspecifics versus heterospecific fishes as settlement stimuli In addition to evaluating directional movement in the absence of stimuli, we determined the distribution of choices exhibited by *C. viridis* larvae presented with (1) “no stimuli” versus “heterospecific fishes”, and (2) “heterospecific fishes” versus “conspecifics”. We used groups of ten juvenile *Acanthurus triostegus* as our representative heterospecific fish treatments, as these were readily available to us at the time of our experiments. We used groups of ten juvenile *C. viridis* for our conspecific treatments. This design allowed us to determine separately for each sensory cue whether test subjects were responding to the sight, smell or activity of fish in general, versus specifically to conspecifics.

Experiment 2: living coral versus dead coral as settlement stimuli After again evaluating directional movement in the absence of stimuli, we determined the distribution of choices exhibited by *C. viridis* larvae presented with (1) “no stimuli” versus “dead coral”, and (2) “dead coral” versus “live coral”. We used pieces of coral rubble to construct the “dead coral” treatment, and similar-sized colonies (10 cm diameter) of living *Porites rus* as the “live coral” treatment. Both substrates were collected from the lagoon of Moorea, where *C. viridis* juveniles are commonly found associated with *P. rus* and rarely found on dead coral rubble (Lecchini 2003). This design allowed us to determine whether test subjects were responding to the sight or smell of lagoon hard substrates in general, versus specifically to living colonies of *P. rus*; acoustic/vibratory sensory mechanisms were not tested in this experiment.

Experiment 3: living coral versus conspecifics as settlement stimuli After evaluating directional movement in the absence of stimuli, we determined the distribution of choices exhibited by *C. viridis* larvae presented with “living coral” (colony of *P. rus* of 10 cm diameter) versus “conspecifics” (ten juvenile *C. viridis*). This design allowed us to determine whether test subjects were responding to the sight or smell of *P. rus* or conspecifics; as in experiment 2, acoustic/vibratory sensory mechanisms were not tested.

Field experiment to estimate sensory abilities and detection of settlement habitat

To estimate sensory abilities of *C. viridis* larvae (i.e., effective detection distances) under field conditions, we constructed a circular test arena in a section of the lagoon comprised of barren sand substrate, and with a water depth of ~60 cm. We used a series of stakes to divide our circular arena into 12 pie-shaped wedges, where the outer boundary of each wedge was an arc measuring 1/12 the circumference of the circular arena. At the center of each alternating arc (i.e., at intervals along circle perimeter), we placed a colony of living *P. rus* (~50 cm diameter) stocked with 40–60 juvenile *C. viridis*; intervening arcs contained only barren sand substrate and no conspecifics. At the circle's origin, test subjects were introduced into the test arena from a transparent box affixed mid-water to a stake, via a remote release mechanism, and after a 1-min period of acclimation.

We manipulated the size of the circular arena (and hence, the spacing between patches of *P. rus*/conspecifics) to test detection distances of test subjects. We repeated the experiment using arena sizes of (1) 600 cm circumference (larvae were released 95 cm from arena's perimeter), (2) 1,200 cm circumference (larvae released 191 cm from perimeter), (3) 2,400 cm circumference (larvae released 382 cm from perimeter), and (4) 4,800 cm circumference (larvae released 764 cm from perimeter). Consequently, for the smallest arena, 12 circle arcs each measuring 50 cm, settlement habitats (coral colonies ~50 cm diameter, plus conspecifics) occupied the whole of alternating circle arcs, and these were separated from one another by ~50 cm. We assumed that random movement of test subjects coupled with no effective detection of settlement habitats would produce an expected distribution of 50% of fish succeeding in locating settlement habitats (e.g., where 50% of circular arena perimeter was occupied by settlement substrate). If the settlement on coral colonies was significant (chi-square test), then the detection distance of settlement habitat was at least 25 cm. For the arena of 1,200 cm circumference (12 circle arcs each measuring 100 cm), the coral colonies (plus conspecifics) still occupied a length of 50 cm and the spacing between two patches of *P. rus*/conspecifics was at 150 cm. The random movement of test subjects coupled with no effective detection of settlement habitats would produce an expected distribution of 25% of fish succeeding in locating settlement habitats (e.g., where 25% of circular arena perimeter was occupied by settlement substrate). If the settlement on coral colonies was significant (chi-square test), then the detection distance of settlement habitat was at least 75 cm. The experiment was then conducted with the arena of 2,400 cm circumference (spacing between two patches of *P. rus*/conspecifics: 350 cm, expected distribution of 12.5% of fish succeeding in locating settlement habitats, detection distance was at least 175 cm) and of 4,800 cm circumference (spacing between two patches of *P. rus*/conspecifics: 750 cm, expected distribution of

6.25% of fish succeeding in locating settlement habitats, detection distance was at least 375 cm).

To test the relative importance of specific sensory mechanisms in situ, we manipulated the physiological states of test subjects. Performance of unmanipulated test subjects (i.e., individuals with their full complement of sensory capabilities) was compared to that of (1) larvae with olfactory capabilities impaired by the temporary addition of petroleum jelly (Vaseline) to the nostrils (after Liang et al. 1998), and (2) larvae previously immersed for 12 h in a streptomycin bath (44 mg/l seawater) to temporarily inhibit sensory cells of the lateral line (Kaus 1987; Janssen et al. 1995; Liang et al. 1998). Physiological manipulations were conducted under carefully scrutinized animal ethics protocols, and had only temporary effects on test subjects. Test subjects were re-collected following field trials, and were allowed to recover completely in laboratory aquaria prior to final release back into the lagoon. We note the absence of “disturbance controls” for physiological manipulations, and therefore interpretations based upon our results from this study must be interpreted with some caution. Overall, for each arena size, 16 *C. viridis* larvae of each physiological state were tested individually during the same night. Two runs (two successive sampling nights) were conducted to reduce the variability from environmental conditions and the physiological states of larvae. We used a total of 384 larvae for this field study.

Biochemical analyses to detect, identify, isolate, and test specific chemical cues

We used high performance liquid chromatography (HPLC) to detect and isolate chemical compounds emitted by conspecifics and heterospecific fishes, and subsequently tested the attractive properties of these compounds on settling *C. viridis* in experimental laboratory arenas (Fig. 1).

We collected 2 l of water from tanks containing (1) ten juvenile *C. viridis* immersed for 6 h, (2) ten juvenile *A. triostegus* immersed for 6 h, or (3) unoccupied (i.e., “ambient”) filtered seawater (for all treatments, tanks and seawater collection/filtration/UV-sterilization were as described above). The three different seawater collections (*C. viridis* seawater, *A. triostegus* seawater and ambient seawater) were extracted three times with ethylacetate and then the combined extracts were concentrated under vacuum leaving a powdery organic residue. The three different organic extracts were analyzed and compared using standard HPLC procedures (Mant and Hodges 1991), with a photodiode array detector (Waters PDA 996) using Jasco 880-PU pumps with the following conditions: solvent mixture H₂O:acetonitrile:trifluoroacetic acid (gradient of elution from 90:10:0.01 to 0:100:0.01 in 30 min + 5 min 0:100:0.01) with a flow rate of 1 ml/min; column Uptisphere UP5ODB-25 K. We collected dominant “peaks” of chromatograms (i.e., discrete biochemical

constituents of water) in separate eppendorf tubes. These compounds (or sets of compounds of similar chromatographic properties) were then lyophilized and dissolved in 100 μ l of distilled water.

We identified three major peaks indicating compounds uniquely associated with *C. viridis*, four major peaks uniquely associated with *A. triostegus*, and we constructed three additional treatments comprised of (i) pooled minor peaks of *C. viridis*, (ii) pooled minor peaks of *A. triostegus*, and (iii) pooled major and minor peaks of ambient seawater. The five treatments of *C. viridis* (three major peaks and pooled minor peaks of *C. viridis* + pooled peaks of ambient seawater) were introduced singly into a compartment (either B or C) of the experimental arenas (filled with pure artificial water), and the responses of six individual test subjects (*C. viridis* larvae)

were evaluated for each treatment (as described above). The same experiment was conducted with six treatments of *A. triostegus* (four major peaks and pooled minor peaks of *A. triostegus* + pooled peaks of ambient seawater) and the responses of six individual test subjects (*A. triostegus* larvae) were evaluated for each treatment.

Results

Ecological factors and sensory mechanisms underlying settlement choice

In the absence of externally imposed settlement stimuli, the majority of *C. viridis* larvae remained in the central compartment (<68% in every trial) and exhibited no

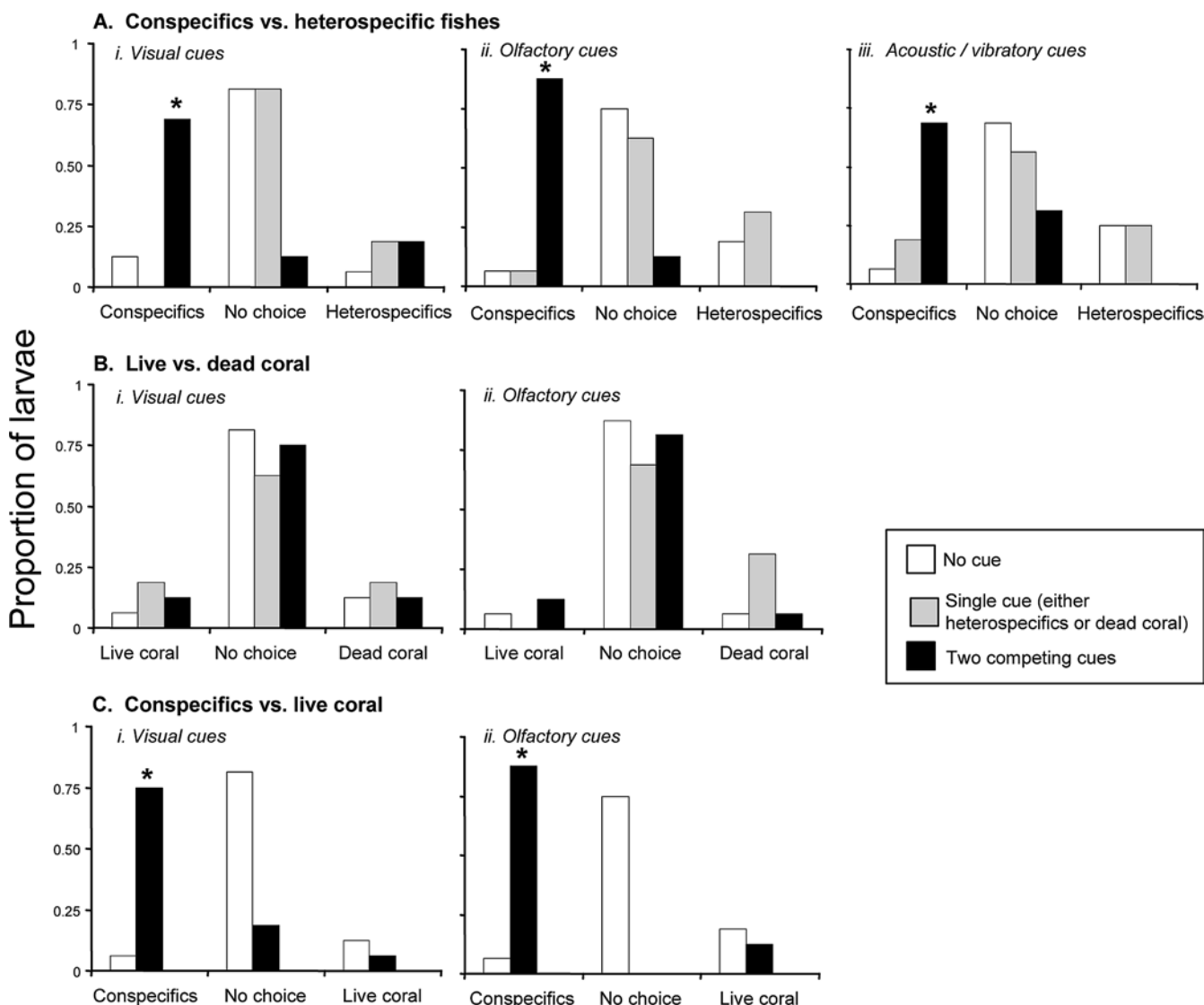


Fig. 2 Distributions of settlement choices of larval *C. viridis* presented with no cues (unshaded bars), a single cue (gray bars), or two competing cues (black bars). Effects of **a** conspecifics (juveniles of *C. viridis*) versus a heterospecific reef fish (juveniles of *A. triostegus*), **b** live coral (*P. rus*) versus dead coral rubble, and

c conspecifics versus live coral, when cues were (i) visual, (ii) olfactory, and (iii) acoustic/vibratory. Significant deviations from null distributions ($P < 0.05$) are indicated by '*'. The term "No choice" from the figure means that *C. viridis* larvae remained in compartment A (see Fig. 1)

clear patterns of preference for either compartments B or C (Fig. 2). The distribution of choices exhibited by *C. viridis* larvae presented with no stimuli versus heterospecific fishes or dead coral was similar to baseline movements (i.e., statistically indistinguishable from distribution of choices in the absence of manipulated cues, Fig. 2).

In contrast, larvae showed significant affinities for conspecifics, and used visual ($\chi^2_{0.05,2}=53.80$, $P<0.0001$), olfactory ($\chi^2_{0.05,2}=180.33$, $P<0.0001$) and acoustic/vibratory cues ($\chi^2_{0.05,2}=107.27$, $P<0.0001$) to actively choose compartments containing *C. viridis* juveniles over *A. triostegus* juveniles in 75% of trials. Larvae did not respond to visual or olfactory cues of live coral (*P. rus*, a settlement habitat commonly occupied by *C. viridis* at Moorea Island), and chose compartments containing live or dead coral in similar proportions to baseline movements (visual cues: $\chi^2_{0.05,2}=1.07$, $P=0.61$; olfactory cues: $\chi^2_{0.05,2}=1.08$, $P=0.61$). Larvae presented with a choice between conspecifics and live coral (*P. rus*) used both visual ($\chi^2_{0.05,2}=129.19$, $P<0.0001$) and olfactory cues ($\chi^2_{0.05,2}=181.33$, $P<0.0001$) to actively select and settle amongst conspecifics over empty live coral habitat (Fig. 2).

Sensory abilities and detection of settlement habitat in situ

For the arena size of 600 cm circumference, the settlement of *C. viridis* larvae was significant as soon as 75% test subjects (12 of 16 larvae tested) were present on the coral colonies ($\chi^2_{0.05,1}=4.00$, $P<0.05$). For the arena size of 1,200, 2,400 and 4800 cm, the settlement was significant respectively as soon as 50% test subjects (8 of 16 larvae tested), 37.5% test subjects (6 of 16 larvae tested) and 25% test subjects (4 of 16 larvae tested) were present on the coral colonies. Thus, larval *C. viridis* possessing all functional senses were able to significantly detect and settle on colonies of *P. rus* containing juvenile conspecifics in 84% of trials. Performance remained relatively constant regardless of arena size (one-factor ANOVA: four arena sizes with fish possessing all functional senses, $df=3$, $F=0.44$, $P=0.73$).

Patterns suggest larvae used combined sensory capabilities to detect settlement habitat (Fig. 3). Although larvae with impaired olfactory capabilities were able to significantly detect and settle on coral colonies regardless of arena size, detection/settlement rates appeared to decrease with arena size relative to fish possessing all functional senses (Fisher's PLSD tests,

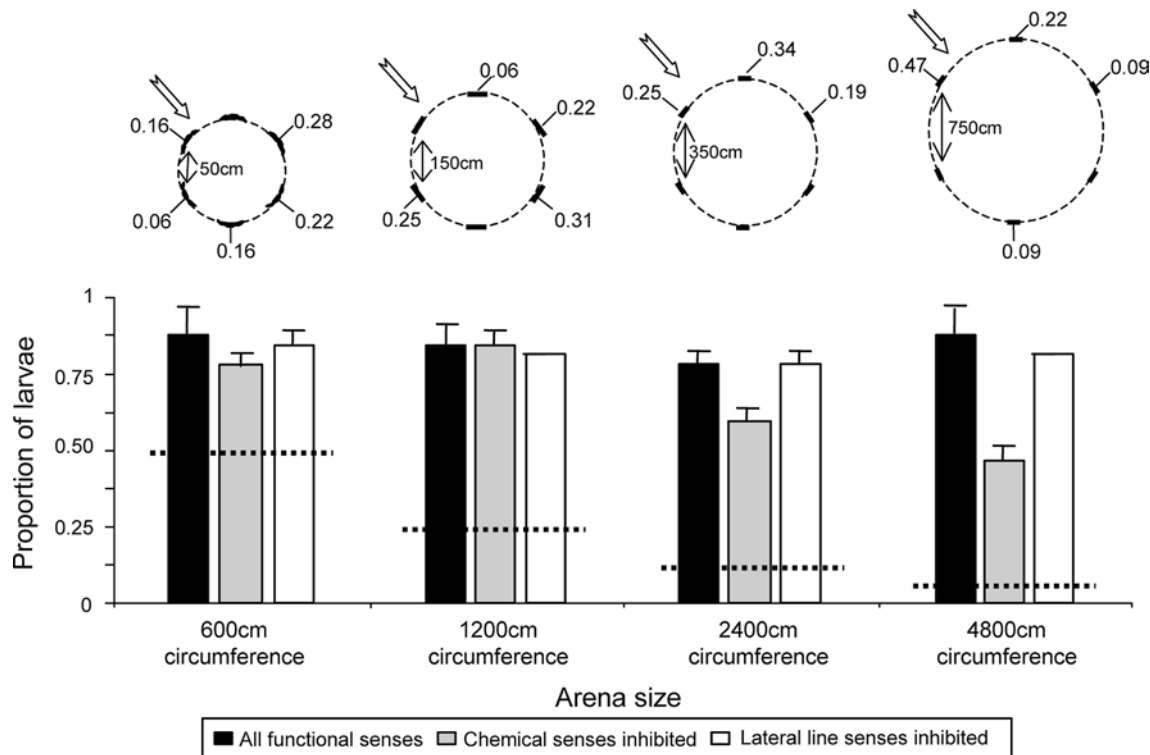


Fig. 3 Proportion of larval *C. viridis* (mean over two successive nights, + 1 SE) that successfully located and settled upon settlement habitat units (i.e., colonies of *P. rus* with resident *C. viridis* juveniles) arrayed in circular arenas and spaced at varying distances (arena size of 600, 1,200, 2,400 and 4,800 cm circumference). Performance of *C. viridis* was tested for subjects with (1) all functional senses (black bars), (2) chemical senses inhibited (gray bars), or (3) lateral line senses inhibited (unshaded bars). Dotted lines indicate null hypothesis (i.e., random movement of larval

C. viridis coupled with no effective detection of settlement habitat units would produce an expected distribution of 50, 25, 12.5 or 6.25% successful settlement according to the arena size, see Materials and methods). Diagrammed circular arenas (with shaded arcs depicting locations of settlement habitats—coral colonies ~50 cm diameter, plus conspecifics—within a sand habitat; arrows denote prevailing current direction) show proportions of larvae with all functional senses that successfully settled on specific patches of settlement habitat relative to current direction

$P < 0.001$). Impairment of the lateral line system (i.e., mechanical sensory capabilities) appeared to have little effect on habitat detection/settlement success (Fisher's PLSD tests, $P = 0.33$).

Spatial distributions of successfully settled larvae (possessing all functional senses) suggest that larvae in close proximity to settlement habitats (arena size $\leq 1,200$ cm circumference) identified, selected, and settled in habitats irrespective of prevailing current direction (i.e., consistent with visual orientation, Fig. 3, top panels). As arena sizes increased (2,400 cm and 4800 cm circumference), distributions of successful settlers suggest increasingly directional patterns of detection/settlement, with greater settlement in up-current patches of habitat (i.e., consistent with olfactory orientation).

Biochemical cues affecting settlement choice

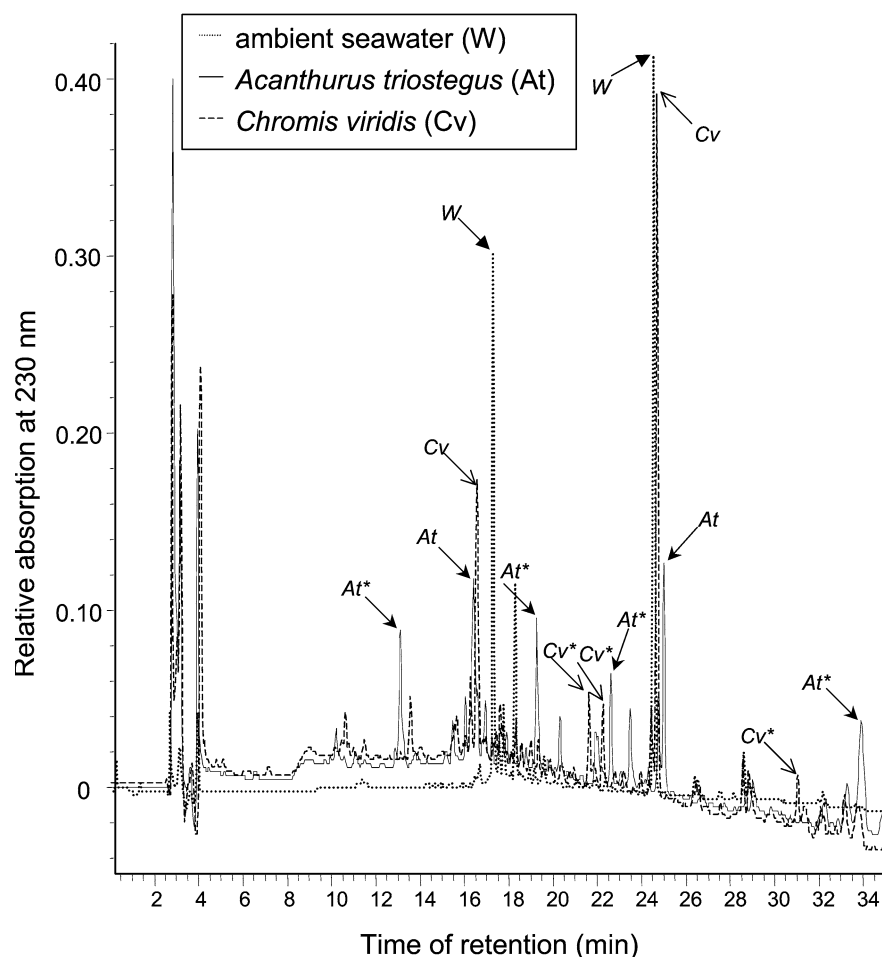
We identified at least two major peaks in HPLC chromatographs (at 230 nm, with retention times of 17 min and 24 min) for ambient seawater collected from Moorea lagoon (Fig. 4). Water collected from aquaria housing *C. viridis* and *A. triostegus* showed the same major peaks (offset slightly) as well as several minor

peaks in common with ambient seawater. We interpreted these shared patterns as background signatures in the *C. viridis*/*A. triostegus* water, indicative of the biochemical composition Moorea lagoon seawater.

Analyses of water collected from aquaria housing *C. viridis* showed a higher molecular diversity than the ambient water, with three unique major peaks in HPLC chromatographs (retention times: 22, 23, and 31 min). Similarly, water from *A. triostegus* aquaria showed a higher molecular diversity with four unique major peaks (retention times: 13, 19, 23 and 34 min). A comparative analysis of chromatograms of two species showed two different chemical signatures with some differences in the apparition and in absorption quantity of major and minor peaks. Overall, the juveniles of *C. viridis* and *A. triostegus* secreted some organic molecules specific for each species.

When larval *C. viridis* or *A. triostegus* were presented with purified constituents of ambient seawater (pooled major and minor peaks), none of the individuals showed a directional response in the experimental arenas. In contrast, 83% of *C. viridis* test subjects selected compartments containing the biochemical constituents comprising the *C. viridis* major peak with a 31-min retention time, but failed to show any directional

Fig. 4 Chromatograms (HPLC) of ambient water collected from Moorea lagoon (stippled line), water associated with ten juvenile *A. triostegus* for 6 h (solid line), and water associated with ten juvenile *C. viridis* for 6 h (dashed line). Arrows denote dominant organic compounds ("W" for ambient seawater, "At" for *A. triostegus*, and "Cv" for *C. viridis*) and those marked with the symbol "*" denote compounds used in subsequent choice experiments



movement toward other purified constituents of *C. viridis* water. Hundred percent of *A. triostegus* larvae selected compartments containing the pooled minor biochemical constituents (i.e., “minor peaks”) of *A. triostegus* water, but failed to respond directionally to any of the major constituents of *A. triostegus* water.

Discussion

Our results indicate that *C. viridis* larvae use visual, olfactory and vibratory/auditory cues, effective over different distances, to detect and settle preferentially on patches of habitat containing conspecifics. Field experiments suggest that olfactory cues are particularly important for the location of settlement habitat at larger distances. However, we note that *C. viridis* larvae were also able to discriminate vibratory and/or auditory cues of conspecifics from those produced by another species (*A. triostegus*). Using HPLC techniques, we isolated a specific biochemical constituent of seawater affiliated with juvenile *C. viridis* that serves as a settlement cue for larvae.

Our observations of settling larvae responding strongly and positively to cues produced by conspecifics are consistent with field observations of aggregations of recently settled juveniles and adult *C. viridis* (D. Lecchini and J. Shima, personal observations; Allen 1991; Ohman et al. 1997). Furthermore, such preferences for conspecifics have interesting implications for density-dependent interactions occurring at the time of settlement (e.g., Schmitt and Holbrook 1996) and in subsequent stages (e.g., Schmitt and Holbrook 1999; Shima 2001b). From our findings, we would predict that settlement rates of *C. viridis* are facilitated by resident conspecific densities (i.e., “inverse” or “positive” density dependence at settlement). Given the strong preference for conspecifics at settlement (and assuming this pattern of behavior confers some selective advantage), we would predict that (1) density-dependence is either weak or positive (i.e., facilitative) in older age classes, and/or (2) the relative “costs” of failing to locate suitable settlement habitat (signaled by past success/presence of conspecifics) greatly exceed any negative effects attributable to density-dependent interactions experienced during subsequent juvenile stages.

Our experiments suggest olfactory cues play a particularly important role in patch selection by the settling larvae. The distribution of successful settlers in our experimental arenas at the end of the field experiment (i.e., predominantly at up-current sites) highlights the potential for such mechanisms interacting with hydrodynamic features (e.g., unidirectional flow) to produce and reinforce nonrandom patterns of settlement. Dispersal/diffusion patterns of olfactory signals important to settling *C. viridis* are likely influenced by prevailing water movements, such that potential cues from suitable sites “down-current” may be effectively undetectable to larvae. On Moorea, patterns of water flow within the lagoon systems are predominately unidirectional (Galzin

and Pointier 1985); consequently, we predict spatial variation in *C. viridis* densities should be non-random across the lagoon, and relatively consistent through time (because *C. viridis* have multiple overlapping generations within a site, providing cues to successive cohorts of larvae). Given the high relative abundance of *C. viridis* on Moorea (Lecchini 2003), this species likely plays a significant ecological role as a planktivore, and as a prey species for many reef-based predators. Temporally consistent spatial heterogeneity of an important resource consumer and/or prey species (with distributions potentially independent of substrate type) has important and largely unexplored consequences for the dynamics of other species, and for the structure of the greater reef community (e.g., potentially resulting in patchy planktonic food resources and/or differential survival/settlement of planktonic larvae other species, spatially consistent prey sources for predators, etc.).

Our study is among the first to provide a comparative evaluation of multiple sensory capabilities and their relative effectiveness as mechanisms enabling larvae to detect and select settlement habitats. Other workers have examined subsets of sensory mechanisms and found that responses to visual and/or olfactory cues produced by conspecifics and/or substrates vary among species and developmental states (c.f. Sweatman 1988; Pawlik 1992; Elliott et al. 1995; Gebauer et al. 2002; Myrberg and Fuiman 2002). Our results indicate that *A. triostegus* larvae and *C. viridis* larvae each responded with positive taxis to specific chemical compounds produced by conspecifics. These organic compounds are characterized by a weak polarity, extractable in organic solvent and detectable at 230 nm for both species. For *C. viridis*, the compound corresponds to one major peak with a 31-min retention time in HPLC, whereas for *A. triostegus*, the compound corresponds to a pool of minor peaks. Although we did not attempt to identify the structural composition of these organic compounds providing cues, workers studying other marine species suggested that such substances may reflect neurotransmitter-like substances produced specifically for communication (e.g., Painter et al. 1998; Browne and Zimmer 2001), or metabolic byproducts such as fish mucus, urine or feces (Fontaine et al. 1982; Sola et al. 1993; Baker and Montgomery 2001). At present, little is known of how specific biochemical compounds produced by conspecifics, substrates, and potential predators and/or competitors potentially interact to shape the preferences, and hence settlement patterns of marine larvae; we suggest this may be a fruitful avenue for future research.

Overall, our work has fundamental implications for the potential role of “biotechnology” as a tool for restoration ecology. Specifically, we demonstrate how biochemical cues that shape behavior patterns of larvae may be isolated and harnessed to facilitate (1) colonization of empty patches of otherwise suitable habitat, or (2) recolonization of patches driven locally extinct by past environmental impacts (e.g., human-mediated habitat degradation or over-harvesting).

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