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REPORT

Ontogenetic changes in habitat selection during settlement in a coral reef fish: ecological determinants and sensory mechanisms

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Abstract The behavior of marine larvae during and after settlement can help shape the distribution and abundance of benthic juveniles and therefore the intensity of ecological interactions on reefs. Several laboratory choice-chamber experiments were conducted to explore sensory capabilities and behavioral responses to ecological stimuli to better understand habitat selection by "pre-metamorphic" (larval) and "post-metamorphic" (juvenile) stages of a coral reef fish (*Thalassoma hardwicke*). *T. hardwicke* larvae were attracted to benthic macroalgae (*Turbinaria ornata* and *Sargassum mangarevasae*), while slightly older post-metamorphosed juveniles chose to occupy live coral colonies (*Pocillopora damicornis*). Habitat choices of larvae were primarily based upon visual cues and were not influenced by the presence of older conspecifics. In contrast, juveniles selected live coral colonies and preferred those occupied by older conspecifics; choices made by juveniles were based upon both visual and olfactory cues from conspecifics. Overall, the laboratory experiments suggest that early life-history stages of *T. hardwicke* use a range of sensory modalities that vary through ontogeny, to effectively detect and possibly discriminate among different microhabitats for settlement and later occupation. Habitat selection, based upon cues provided by environmental features and/or by conspecifics, might have important consequences for subsequent competitive interactions.

Keywords Habitat selection · Settlement cues · Sensory mechanisms · Ontogenetic shifts · *Thalassoma hardwicke*

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Introduction

Most marine organisms have a stage-structured life history consisting of a relatively sedentary benthic stage (usually juveniles and adults) and a pelagic larval stage capable of long-distance dispersal (for review, see Werner 1988). A commonly held view among marine ecologists is that these two stages are coupled by a fairly discrete process called "settlement". During this important transition, larvae of marine invertebrates and fish often show marked selectivity in the habitats they choose based on a variety of environmental factors, including the presence of specific benthic substrata, or the presence of conspecifics or other species (e.g., Ohman et al. 1998;

Gebauer et al. 2002; Holbrook et al. 2002). Although a number of studies have examined patterns of habitat use of settling marine larvae (for reviews, see Pechenik 1990; Doherty 2002), a lack of understanding exists about the sensory capabilities and behavioral preferences that lead to habitat selection (although see Sweatman 1988; Gebauer et al. 2002; Lecchini et al. 2005a, b; Wright et al. 2005). The proximate mechanisms (e.g., detection of cues and associated behavioral responses) underlying the settlement process will partly shape post-settlement growth and survival and the intensity of interactions among marine organisms.

Successful settlement may require detection of cues and appropriate responses that operate over a range of spatial and temporal scales (for reviews, see Pawlik 1992; Myrberg and Fuiman 2002). Sound and/or olfactory cues may be used by late-stage larvae of marine organisms to detect the presence of nearby reefs over scales of 10 s of km (e.g., Cato 1992; Kingsford et al. 2002; Leis et al. 2002). Once within the vicinity of a reef (e.g., hundreds of meters), larvae may switch to other cues and behavior patterns to locate appropriate regions within a reef ecosystem (e.g., fore-reef, lagoon, back-reef, etc) (e.g., Qian 1999; Gebauer et al. 2002; Lecchini et al. 2005a, b). Thereafter (on the scale of meters), marine organisms may respond to still other cues to discriminate and select settlement sites (e.g., coral vs. algae).

After its initial choice of a settlement site, a fish may readjust its location and move into other habitats. This "settlement transition" (sensu McCormick and Makey 1997) is likely common in reef fishes. For example, some labrids first settle into the sand and then move onto reefs after several days (Victor 1982). Sequential habitat shifts also have been observed in other fishes, including apogonids, mullids, microdesmids, muraenids and scorpaenids (Finn and Kingsford 1996; McCormick and Makey 1997; Lecchini 2005). Thus, we might better understand settlement by quantifying habitat selection, sensory capabilities, and behavioral responses to environmental cues at several points in the early life history of marine organisms (i.e., as larvae transition to the juvenile stage during the settlement transition).

In the present study, laboratory experiments were conducted using pre- versus post-metamorphic stages of a coral reef fish (the six-barred wrasse, *Thalassoma hardwicke*) to determine: (1) whether behavioral preferences for microhabitats vary through ontogeny (i.e., "pre-metamorphosis" larvae versus "post-metamorphosis" juveniles); (2) the relative importance of settlement cues from microhabitat versus conspecifics; and (3) the sensory modalities underlying behavioral preferences (i.e., visual, acoustic/vibratory, olfactory cues from microhabitat features or conspecifics).

Materials and methods

Study species and system

Thalassoma hardwicke is an omnivorous wrasse found commonly on coral reefs in the Indo–Pacific. Adults spawn pelagic eggs yielding larvae with a pelagic larval duration of ~47 days (Victor 1986). Larvae are transparent and ~12–13 mm total length at settlement (Lecchini 2003). In contrast to some species of labrid that shelter in sand prior to emerging onto reefs (Victor 1982), previous observations of *T. hardwicke* suggest that larvae remain relatively inactive within clumps of macroalgae or within interstices of branching corals for ~1–3 days while they complete their metamorphosis and develop juvenile pigmentation patterns (Shima 1999a, b; Lecchini 2003). Older juveniles range over larger areas and regularly shelter within branching coral habitats (e.g., *Pocillopora* spp.) (Shima 1999a, b; Lecchini 2003).

Experiments were conducted in April–May 2004 on Moorea (French Polynesia, 17°30′S, 149°50′W). Crest nets were used to capture fish larvae as they first entered the island's lagoon (i.e., larvae enter the lagoon via a unidirectional flow of water over the reef crest and can therefore be collected prior to metamorphosis with nets placed on the reef crest (Dufour and Galzin 1993; Doherty et al. 2004). Larvae captured in crest nets during the night were collected at dawn (~0800 hours), transferred to the laboratory (an indoor space with neon lights regularly distributed throughout the room), and subsequently maintained in aquaria (1.0 × 0.6 × 0.8m) until 1900 hours when experiments were begun. Prior to the initial experiments and between all subsequent experiments, fish were maintained in aquaria supplied with flow-through sea water from the adjacent lagoon, and without any added artificial or natural habitats or substrates. Due to logistic constraints, fish were housed as small groups of conspecifics within aquaria (ten fish per aquarium). During the first 11 h (prior to the initial set of experiments), larvae remained in a pelagic state as evidenced by the absence of appearance of juvenile color pattern, which would usually develop within 48 h of capture with a substratum present.

To describe habitat preference of "pre-metamorphic" (larval) stage and "post-metamorphic" (juvenile) stage fish, two sets of three experiments each (one set for each stage) were conducted. A total of 20 larvae were captured on each of two consecutive nights and used in the first set of trials (a total of 40 fish); 36 of these fish survived to the juvenile stage and were used in the second set of trials 3 weeks later. Between the two sets of experiments, fish were maintained in flow-through aquaria in groups without added habitat, and fed live *Artemia*. The "post-metamorphic" fish were housed without substrate to eliminate bias in later choice

experiments, but with conspecifics because they naturally occur in groups, and housing individuals separately in the lab was not feasible. All fish achieved a juvenile color pattern within 10 days following the completion of Experiment 1.

For each collection of larvae, the first set of three experiments was conducted the first evening after capture (11 h after collection). Overall, the three sets of experiments were designed to: (1) determine patterns of habitat selection; (2) determine whether habitat selection was affected by the presence of conspecifics; and (3) explore the cues used to choose habitats. Each individual tested at the juvenile stage had been previously tested as a larva; however, individual identities were not tracked (because they were unmarked and housed in groups). Each fish had similar experiences at each test point with the exception of Experiment 3 in which subsets of fish were used in different tests. The assignment of fish to groups was random so this difference in experience should introduce no bias in the results.

Experiment 1: microhabitat preferences

The objective of Experiment 1 was to examine how preferences for different substrates varied through the early juvenile stage for *T. hardwicke* (larvae vs. juveniles). Each of six shallow aquaria (1.0 × 0.4 × 0.2 m, Fig 1) contained four microhabitats: sand, coral rubble, living coral (colonies of *Pocillopora damicornis*), and macroalgae (equal parts *Turbinaria ornata* and *Sargassum mangarevasae*). These specific microhabitats were chosen because other wrasse species have been observed to complete metamorphosis while buried in sand (Victor 1982), and because young *T. hardwicke* have frequently been observed on macroalgae and *Pocillopora damicornis* during the monitoring of natural settlement on Moorea (Shima 1999a, b; Lecchini 2003).

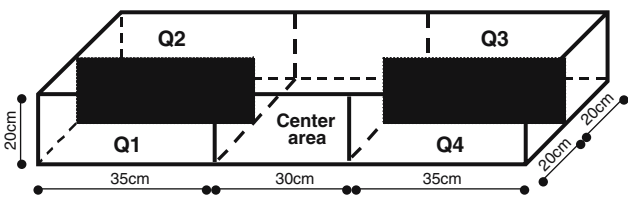

Fig. 1 Diagram of the aquarium used to examine how preferences for different substrates varied through the early juvenile stage for *Thalassoma hardwicke* (larvae vs. juveniles). The aquarium (measuring 1.0 m × 0.4 m × 0.2 m deep) was divided into four quadrants (*Q1*, *Q2*, *Q3* and *Q4*) separated by a center area. Each pair of adjacent quadrants was separated from the other by a small plexiglass partition, which prevented direct movement from one quadrant to the other, although fish could migrate via the center area. For each trial (one fish per trial), each microhabitat (sand, coral rubble, living coral and macroalgae) was randomly re-assigned to one of the four quadrants. Fish were introduced to the central area in a clear plexiglass tube, which was lifted to initiate a trial

The six shallow aquaria were located in a laboratory room isolated from outside noises, and indoor light was provided by neon lights that were regularly distributed throughout the room in an attempt to minimize larval responses to variable light and noise levels and direction. All trials were conducted between 1900 and 2200 hours. The aquaria were divided into four quadrants (0.2 × 0.35 m in size, and separated in two pairs by a central area: see Fig 1). Aquaria were supplied with flow-through sea water from the lagoon and introduced at the center of each aquarium at a rate of 5 l min^{-1}.

For each trial (one fish per trial), each microhabitat was randomly assigned to one of the four quadrants. Experimental subjects (fish) 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 four microhabitats (although the microhabitats were not equidistant from one another). Fish were introduced to the tube via a small net and allowed to acclimate for 1 min. Following the removal of the tube, fish were free to choose among the four available microhabitats. The observer was ~4 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 microhabitat selected by an experimental subject. Individuals were continuously observed, and the choice was recorded as the habitat upon which the individual first settled (after any initial exploration) and subsequently remained for at least 3 min. Seawater was replaced and habitats reassigned to quadrants after each trial. The distribution of choices and the times-to-choice for a sample of 40 larvae were recorded. The same individuals ($n = 36$, due to some mortality) were re-tested after 3 weeks (at the juvenile stage) using identical methods. The distributions of choices exhibited by larvae and juveniles were analyzed with chi-square tests.

Experiment 2: effect of conspecifics

The presence of conspecifics can be a reliable indicator of suitable habitat but may also reflect a more competitive environment. The objective of Experiment 2 was to evaluate whether the presence of conspecifics affected habitat choice. Following a 2 h recovery period after Experiment 1, individual fish were re-introduced to one of the six experimental aquaria, now reconfigured to give each fish two choices: standardized quantities of the microhabitat most often preferred in Experiment 1 (macroalgae for larvae; live coral for juveniles) with or without five older conspecifics (the conspecifics remained associated with the microhabitat patch to which they were assigned). As with Experiment 1, water was replaced and positions of treatments were randomized for each trial. Choices were recorded for each of the 40 larvae; 3 weeks later the experiment was repeated

with the 36 surviving juveniles. All trials were conducted between midnight and 0200 hours. The distributions of choices exhibited by larvae and juveniles were analyzed with chi-square tests.

Experiment 3: sensory cues

Fishes can use a diversity of cues (sensory systems) to find or discriminate between microhabitats. To explore these cues, three separate experiments (each using ten different fish) were conducted between 0300 and 0600 hours (following a 1 h recovery period after Experiment 2). Each experiment used a "choice chamber" and was designed to isolate the sensory mode (visual, acoustic/vibratory or olfactory) used by *T. hardwicke* as a proximate mechanism to discriminate between two microhabitat options. Each experiment focused on two different options (Experiment 3A: heterospecific vs. conspecific fish; Experiment 3B: coral vs. algae; Experiment 3C: conspecifics vs. settlement habitat). Olfaction was assessed by introducing conditioned water (vs. unconditioned water) into the sides of the chamber (into chambers B vs. C, Fig. 2); visual cues were assessed by placing the factor (e.g., conspecific fish) in an adjacent aquarium (but on a different table so that auditory or vibratory cues could be isolated) (e.g., in Tank 1 vs. 2, Fig. 2); auditory or vibratory cues were assessed by placing the factor in the same aquarium but behind an opaque partition (into chambers D vs. E, Fig. 2) (for more detail on experimental protocol to isolate the sensory mode, see Lecchini et al. 2005a, b).

For each trial, one *T. hardwicke* larva or juvenile was released into the central compartment (Fig. 2, chamber A). Subsequent movement of the test subject into the adjoining compartments (B or C) within 3 min (the maximum time required to choose a microhabitat in Experiment 1) was scored as a "choice"; 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 *T. hardwicke* to the chamber without cues (a "control" for tank artifacts). Chi-square tests were used to evaluate significant ($P < 0.05$) responses to stimuli in subsequent trials as deviations from these expected distributions. When significant differences occurred (comparing the numbers of fish in chambers A, B and C), it was necessary to test whether the pattern in compartments B and C differed from the null distribution (i.e., the preference given that a "choice" was made). A different set of ten fish was used in each of the three experiments (3A, 3B, 3C).

Experiment 3A: conspecifics versus heterospecific fishes The response of *T. hardwicke* to "conspecifics" versus "heterospecifics" was compared when only visual, olfactory, or acoustic/vibratory cues were available. Five juveniles of *T. hardwicke* were used for the conspecifics treatment. Five juveniles of *Acanthurus triostegus* were used as representative heterospecifics, as these were readily available at the time of the experiments. The groups of stimulus fish were changed after each sensory test (visual, chemical and acoustic/vibratory cues).

Experiment 3B: macroalgae versus live coral The response of *T. hardwicke* to "macroalgae" versus "live coral" was compared when only visual or olfactory cues were available. Acoustic/vibratory sensory mechanisms were not tested in this experiment as we had no expectation that corals or algae would produce audible cues above ambient laboratory noise.

Experiment 3C: conspecifics versus settlement habitat The response of *T. hardwicke* to "conspecifics" versus "settlement habitat" (the microhabitat most often preferred in Experiment 1: macroalgae for larvae and live coral for

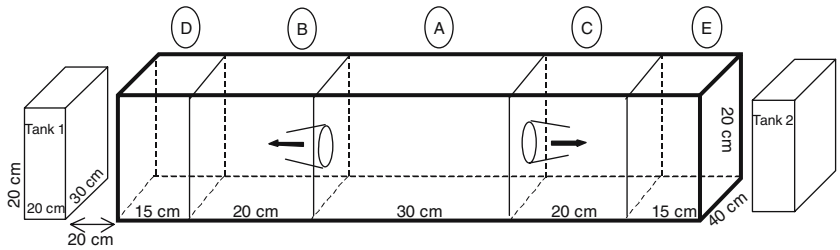

Fig. 2 Diagram of choice chamber used to evaluate sensory cues underlying microhabitat choice. The chamber consists of an aquarium with five compartments, with *A*, *B* and *C* interconnected via funnels and *D* and *E* isolated from central compartments via plastic panels. Additional aquaria on either side of the choice chamber (labeled *tank no. 1* and *2*) are isolated from the choice chamber and mounted upon separate platforms to prevent transfer of vibratory signals. Experimental test subjects (*Thalassoma hardwicke* larvae or juvenile) are introduced into compartment *A*, cues are presented in compartments *B*, *C*, *D*, *E* or *tanks 1* or *2* (to test sensory mechanisms separately). To evaluate the potential role of visual cues on choice, competing stimuli (conspecifics vs. heterospecific fishes vs. benthic substrates) were randomly assigned to *tanks 1* and *2*. To evaluate the potential role of acoustic/vibratory cues, conspecifics or heterospecific fishes were randomly assigned to compartments *D* or *E* (opaque barriers were added to separate these compartments visually from *A*, *B*, and *C*). To evaluate potential olfactory cues, two liters of seawater in which conspecifics, heterospecific fishes or benthic substrates had been immersed for 6 h were randomly assigned to compartments *B* or *C* (for more details, see Lecchini et al. 2005a, b)

juveniles) was compared when only visual or olfactory cues were available.

Overall, three separate experiments were conducted (Experiment 3A: heterospecific vs. conspecific fish; Experiment 3B: coral vs. algae; Experiment 3C: conspecifics vs. settlement habitat). A different set of ten fish was used in each of the three experiments (3A, 3B, 3C). An individual fish was used in six trials in Experiment 3A (three trials with cues and three as controls), and four trials in each of Experiments 3B and 3C (because only two sensory cues were examined).

Results

Experiment 1: Microhabitat preferences

Results were obtained from 40 pre-metamorphic larvae and 36 post-metamorphic juveniles. Both larval and juvenile *T. hardwicke* made active substrate choices in Experiment 1 (Fig. 3). Larvae preferred macroalgae and avoided live coral; 21 larvae (52%) chose macroalgae, 13 (32%) chose sand, 4 (10%) chose coral rubble, and 2 (6%) chose live coral, which differed significantly from the null distribution of 10:10:10:10 (chi-square test: $X_3^2 = 23$, $P < 0.0001$). Larvae also took less time to choose the more preferred habitats (Fig. 3): times-to-choice mirrored the choice rankings of microhabitats, with larvae taking just 21 (±2) seconds (mean ± SE, throughout) to move into the most preferred microhabitat (macroalgae) but >80 s to move into the least preferred microhabitats (coral rubble and live coral).

In contrast, juveniles preferred live coral and avoided macroalgae (Fig. 3); 22 juveniles (55%) chose live coral, with the remainder closely split among the remaining three microhabitats ($X_3^2 = 10$, $P = 0.02$). Times-to-choice varied among the substrates, but in contrast to larvae, these patterns were not concordant with the rankings of substrates (Fig. 3): e.g., the average time taken to select the preferred substrate, live coral, was intermediate to other substrates, with an average of 69 (±6) seconds. Patterns of habitat selection exhibited by larvae and juveniles differed significantly from one another (Fig. 3: $X_3^2 = 68$, $P < 0.0001$).

Experiment 2: Effect of conspecifics

Larval and juvenile *T. hardwicke* responded differently to conspecifics (Fig. 4: $X_1^2 = 30$, $P < 0.0001$). Larvae did not discriminate between the presence or absence of conspecifics on their preferred substrate (macroalgae). In contrast, juveniles chose microhabitats (in this case, live coral) occupied by conspecifics over microhabitats without conspecifics ($X_1^2 = 7.2$, $P = 0.007$).

Experiment 3: Sensory cues

In the absence of added stimuli, most *T. hardwicke* larvae remained in the center chamber (exhibiting "no choice") and the remaining fish (which did display a "choice") showed no preference for compartment B versus C (Fig. 5). In Experiment 3A (Fig. 5a), the distributions of *T. hardwicke* larvae were similar to the null distribution when presented separately with visual, olfactory, or acoustic/vibratory cues. Thus, larvae were not attracted to, and did not differentiate between, conspecifics versus heterospecifics. In Experiment 3B (Fig. 5b), the distribution of larvae did not differ from the null distribution when larvae were reliant upon only olfactory cues but did differ when visual cues were available ($X_2^2 = 40$, $P = 0.0001$); macroalgae was preferred over live coral ($X_1^2 = 4.5$, $P = 0.03$). In Experiment 3C (Fig. 5c), larval distributions differed from the null distribution when given only visual cues ($X_2^2 = 23$, $P < 0.0001$) or olfactory cues ($X_2^2 = 35$, $P < 0.0001$).

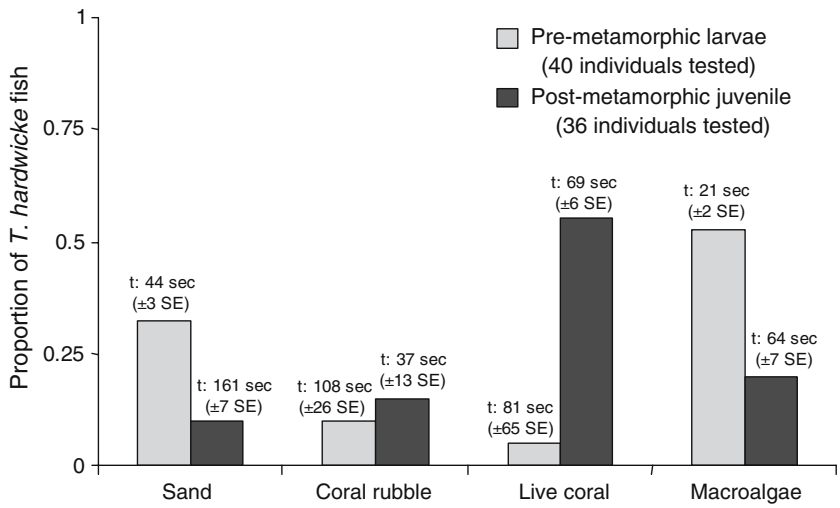

Fig. 3 Proportion of *Thalassoma hardwicke* larvae (40 individuals tested) and juveniles (36 individuals tested) that chose each microhabitat (*sand*, *coral rubbles*, *live coral* or *macro-algae*). Time-to-choice (mean ± 1 SE) is given above each bar

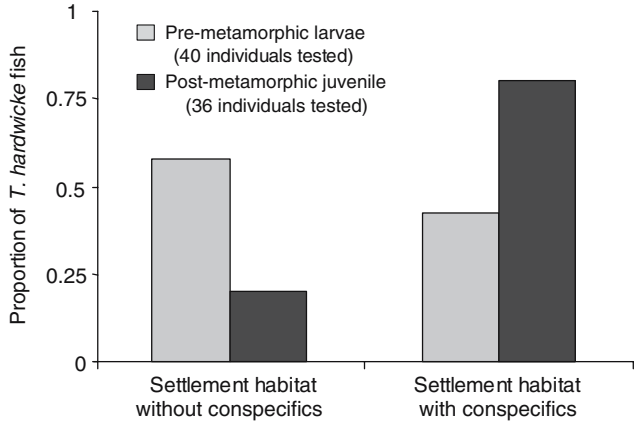

Fig. 4 Proportion of *Thalassoma hardwicke* larvae (40 individuals tested) and juveniles (36 individuals tested) that chose between standardized quantities of the microhabitat preferred in Experiment 1 (macro-algae for larvae; live coral for juveniles), with five older conspecifics either present or absent

Macroalgae was chosen more often than conspecifics in the presence of visual cues ($X_1^2 = 4.4$, $P = 0.04$), but not in the presence of olfactory cues.

In the absence of added stimuli, *T. hardwicke* juveniles also exhibited no significant patterns of preference for either compartments B or C (Fig. 6), although juveniles (relative to larvae) were more likely to choose B or C than to remain in the center compartment (compare Fig. 6 with 5). In Experiment 3A (Fig. 6a), *T. hardwicke* juveniles used both visual ($X_2^2 = 12$, $P = 0.002$) and olfactory ($X_2^2 = 7.2$, $P = 0.03$) cues, but not acoustic/vibratory cues to successfully differentiate between conspecifics and heterospecifics ($X_1^2 = 12.5$, $P = 0.0004$ for visual cues and $X_1^2 = 7.1$, $P = 0.008$ for olfactory cues). In Experiment 3B (Fig. 6b), they did not discriminate between macroalgae and live coral using visual or olfactory cues. In Experiment 3C (Fig. 6c), the use of compartments differed from the null expectation ($X_2^2 = 17$, $P = 0.0002$), with juveniles choosing

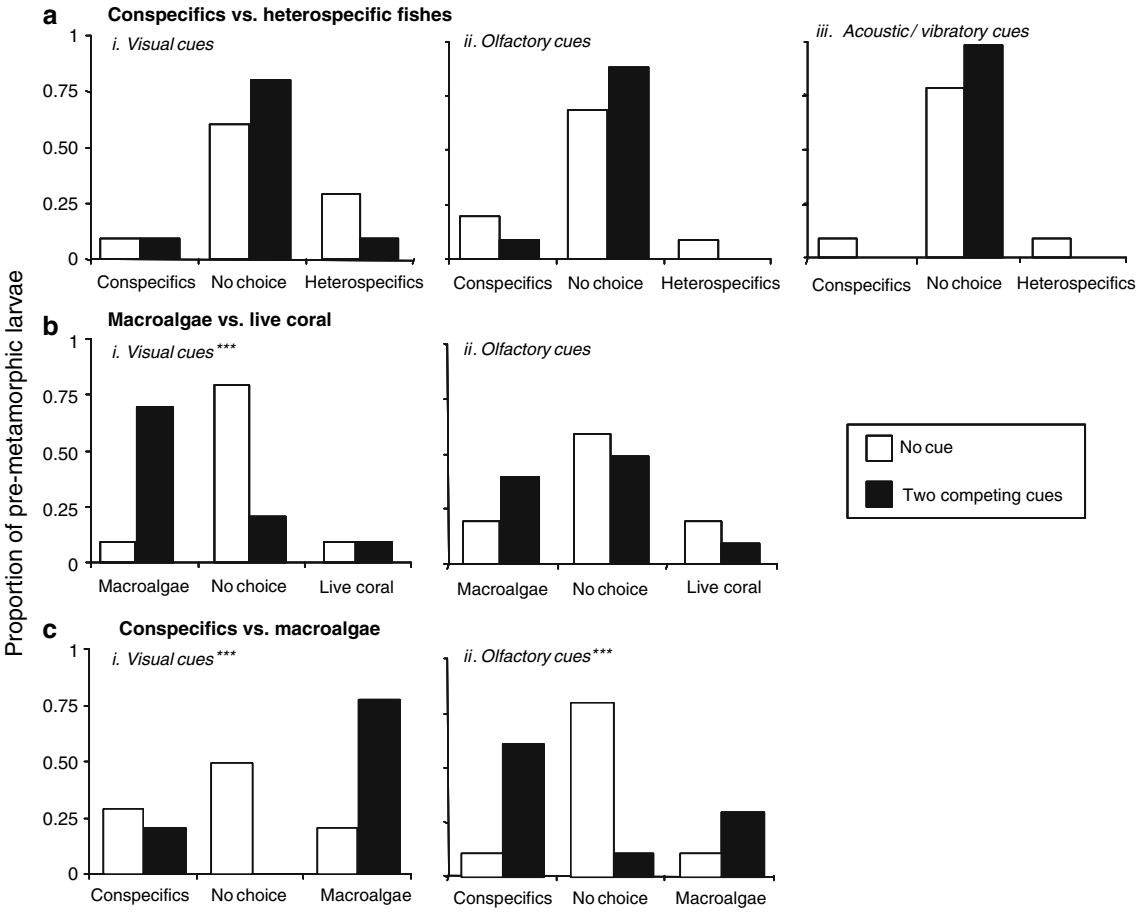

Fig. 5 Distributions of choices of larval *Thalassoma hardwicke* presented with no cues (*unshaded bars*), or two competing cues (*black bars*), in Experiment 3. Effects of **a** conspecifics (juveniles of *T. hardwicke*) vs. heterospecifics (juveniles of *Acanthurus triostegus*), **b** macroalgae vs. live coral, and **c** conspecifics vs. macroalgae, when cues were (*i*) visual, (*ii*) olfactory, and (*iii*) acoustic/vibratory. Ten larvae were tested in each type of trial. Significant deviations (chi-square test; $P < 0.05$) between distribution with no cues (null distribution) vs. distribution with two competing cues (observed distribution) are indicated by "***". Choices offered in chambers **b** and **c** are indicated by "macroalgae", "live coral", "conspecifics" or "heterospecifics", whereas "No choice" refers to *T. harwicke* larvae that remained in compartment *A* (see Fig. 2)

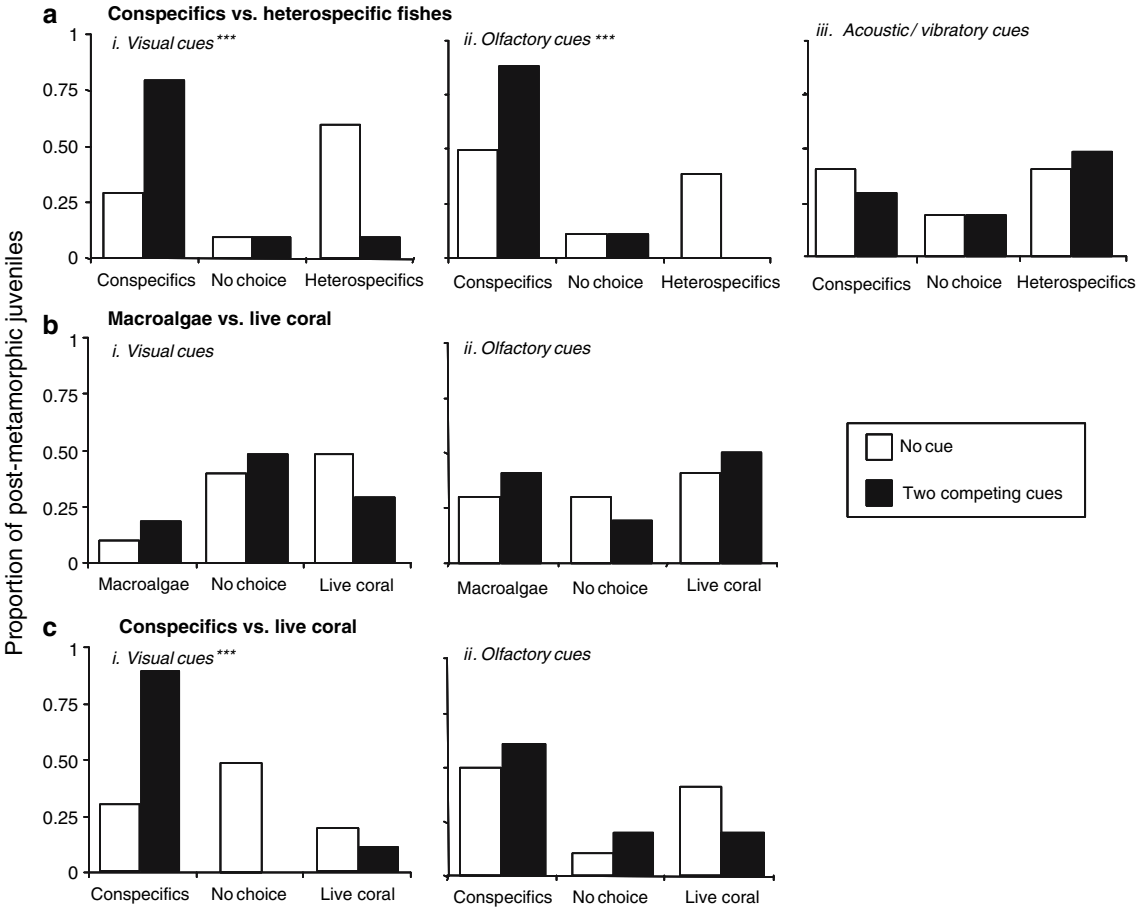

Fig. 6 Distributions of choices of juvenile *Thalassoma hardwicke* presented with no cues (*unshaded bars*), or two competing cues (*black bars*) in Experiment 3. Effects of **a** conspecifics (juveniles of *T. hardwicke*) vs. heterospecifics (juveniles of *Acanthurus triostegus*), **b** macroalgae vs. live coral, and **c** conspecifics vs. live coral, when cues were (*i*) visual, (*ii*) olfactory, and (*iii*) acoustic/vibratory. Ten juveniles were tested in each type of trial. Significant deviations (chi-square test; $P < 0.05$) between distribution with no cues (null distribution) vs. distribution with two competing cues (observed distribution) are indicated by "***". Choices offered in chambers **b** and **c** are indicated by "macroalgae", "live coral", "conspecifics" or "heterospecifics", whereas "No choice" refers to *T. harwicke* juveniles that remained in compartment *A* (see Fig. 2)

conspecifics over live coral ($X_1^2 = 3.75$, $P = 0.05$) using visual cues, but showing no discernible preference when only olfactory cues were present.

Discussion

Larval *T. hardwicke* were attracted to benthic macroalgae, while post-metamorphosed juveniles were attracted to live coral. For larvae, habitat preference was not mediated by the presence of older conspecifics, and choices were made based upon visual cues of macroalgae. In contrast, juveniles selected live coral colonies, were attracted to older conspecifics, and used both visual and olfactory cues to locate conspecifics. Previous field observations in Moorea support these results. *T. hardwicke* larvae remain relatively inactive within clumps of macroalgae and/or within interstices of branching corals while they complete their metamorphosis. Older juveniles range over larger areas and regularly shelter within branching corals (Shima 1999a, b; Lecchini 2003).

These laboratory results that larvae and 3-week-old juveniles of *T. hardwicke* preferred different microhabitats generate a number of additional questions that have received relatively little attention in the coral reef fish literature. For example, we know that habitat strongly influences the growth and/or survival of young fishes (for review, see Doherty 2002). Assuming that behavioral preferences of young *T. hardwicke* are adaptive, the disparity in choices between larvae and juveniles, suggests that, "good" habitat for settling larvae is not necessarily "good" habitat for juveniles. Post-settlement migration might, in theory, help to mitigate this potential fitness conflict, although field observations suggest that migrations among patch reefs within this system are relatively rare (Shima 1999a, b; Lecchini 2003). Shifts in microhabitat use, however, could be accomplished by heterogeneity on small spatial scales: e.g.,

patch reefs that contain both macroalgae and live coral. As a consequence, the demographic effects of resource requirements that vary through ontogeny will depend on the spatial configuration of the available microhabitats.

Some of the results appear inconsistent. This may reflect limited power to document effects (especially in Experiment 3) or possible artifacts of the experimental approach (e.g., aberrant behaviors resulting from the laboratory setting, sequential testing, and/or maintenance regime for fishes between trials). For example, results suggest that settling *T. hardwicke* are not attracted to conspecifics whereas older juveniles are. One possible explanation for this result is that it represents an artifact of housing post-larval fish together in groups (increasing their tendency to choose conspecifics more as juveniles compared to larvae). This possibility cannot be ruled out; however, larval *T. hardwicke* were together in the cod-ends of the crest nest prior to collection and were subsequently maintained in groups for at least 11 h prior to the initial set of experiments. Furthermore, the extent to which larval stages of reef fish may aggregate in pelagic environments is relatively unknown, although recent evidence suggests that larvae may be dispersed pelagically in groups (Selkoe et al. 2006). Despite some conditioning with conspecifics, larvae in our studies were not attracted to conspecifics. In contrast, juveniles preferred conspecifics. This result is consistent with behavioral observations of *T. hardwicke* on natural reefs (Shima 1999a, b; Lecchini 2003), where juveniles routinely aggregate and interact. Under similar field conditions, settlers typically shelter alone and rarely interact. Because survival of young *T. hardwicke* is strongly density dependent (Shima 2001; Shima and Osenberg 2003), and the effect of density is likely greater for younger fish (Schmitt and Holbrook 1999), the absence of a preference of larvae for conspecifics may help reduce the deleterious effects of density dependence. Indeed this neutral response may stem from a conflict between potentially beneficial cues provided by conspecifics (as indicators of "good" habitat) and density dependent effects (Shima and Osenberg 2003). In contrast, the attraction of older *T. hardwicke* to conspecifics could intensify effects of density dependence on natural reefs while facilitating the development of social interactions required for reproduction.

In at least one case, the laboratory results were inconsistent: juveniles exhibited a preference for live coral (over macroalgae) in Experiment 1 but not in Experiment 3 when presented with only visual or only olfactory cues. Three possible explanations for this apparent discrepancy include: (1) limited statistical power in Experiment 3 (which examined discrimination among three options using only ten trials); (2) fish had been tested so often since capture that they were overly stressed and could not make a choice; or (3) juveniles might require a combination of visual and olfactory cues (or other senses) to discriminate between coral and macroalgae and therefore the preference was not expressed when only vision or olfaction are available (as in Experiment 3) but was when all cues were present (as in Experiment 1). All factors may have contributed and only further study can distinguish these possibilities. In other cases, the laboratory results were consistent across experiments and clearly highlight the differential role of different sensory systems. For example, *T. hardwicke* larvae preferred macroalgae in Experiment 1 (when all sensory cues were available), and showed no response to conspecifics in Experiment 2 (when all sensory cues were available). Experiments 3B and 3C suggested that this preference for macroalgae was mediated by vision and not olfaction. Despite possible concerns about the approach taken here, this approach has been validated by in situ field experiments using a different species (Lecchini et al. 2005b).

Although other studies have highlighted changes in habitat choice early in the life history of marine organisms (for review, see Gillanders et al. 2003), we continue to lack understanding of the sensory capabilities and behavioral responses underlying these ontogenetic habitat shifts. For some taxa, metamorphosis and ontogenetic shifts in habitat use are associated with changes in sensory abilities (shrimp: Strasser and Felder 1999; crab: Diaz et al. 2001; insect: Strebler 1989). In fish, some physiological studies have demonstrated that larvae have good visual abilities, but these abilities could improve after metamorphosis for some species (Kotrschal et al. 1990; Lara 2001). Other studies have demonstrated that chemoreceptors of teleost larvae developed quickly during the oceanic phase, but that the density of these receptors at larval stage is lower than at juvenile and adult stages (summarized by the reviews of Doving and Knutsen 1993; Myrberg and Fuiman 2002).

These studies note a difference in sensory abilities between pre- and post-settlement fish, but this difference varies greatly according to the species and their ecological requirement (for review, see Myrberg and Fuiman 2002). For example, *Upeneus tragula* (goatfish) experienced a shift in sensory acuity and associated structures over 12 h during metamorphosis (McCormick 1993; McCormick and Shand 1993). Similarly, larval scarids (but not labrids) have fewer microvillous and ciliated receptor cells than adults (Lara 1999). In contrast, the sensory abilities of pre- and post-settlement damselfish (*Pomacentrus nagasakiensis*) have similar olfactory abilities (Wright et al. 2005). Our study has explored the use of different sensory cues of *T. hardwicke* on both sides of the settlement transition, and the apparent differences were small at best and might reflect the small disparity in age (3 weeks). The importance of sensory development in settlement-stage larvae of fish has not received enough attention to draw generalization about the differences between pre- and post-settlement fish. Future studies

should examine a wider range of ages and conduct more detailed physiological studies (e.g., Wright et al. 2005) to accurately determine the full trajectory on sensory ontogeny.

Overall, a successful "settlement-transition" (sensu McCormick and Makey 1997) may require the use of multiple cues that operate over different spatial scales (for reviews, see Pawlik 1992; Myrberg and Fuiman 2002). A growing body of evidence suggests that the larvae of some species of coral reef fish may use olfactory cues and/or sound to help them locate reefs at larger distances (Cato 1992; Kingsford et al. 2002; Leis et al. 2002). Other cues (and senses) may become more important at shorter scales. The laboratory results with *T. hardwicke* suggest that larvae are attracted to macroalgae via visual cues (or a combination of visual and other cues), and because macroalgae is a dominant component of the reef crest structure on Moorea, this behavior pattern could facilitate entry of larval *T. hardwicke* into the relatively sheltered lagoon system of the island (separate from the cues they use to get to the reef crest). Once inside the lagoon, larvae could then switch to yet other cues to locate appropriate benthic microhabitats. For example, *T. hardwicke* larvae tested in the first experiments (i.e., collected while in the process of colonizing the lagoon) might be relying on the "initial cues" that enabled them to gain entry to the lagoon, while older juveniles may have shifted to "subsequent cues" that serve as more reliable indicators of quality of benthic microhabitats. Further study of this apparent shift in behavior (and its underlying causes) could enhance the understanding of settlement of marine larvae in coral ecosystems (see also Kaufman et al. 1992; Finn and Kingsford 1996; McCormick and Makey 1997; Qian 1999; Lecchini 2005).

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