Coral Reefs (2011) 30:667–676
DOI 10.1007/s00338-011-0739-1

REPORT

# Coral larvae settle at a higher frequency on red surfaces

**B. Mason · M. Beard · M. W. Miller**

Received: 6 August 2010 / Accepted: 17 February 2011 / Published online: 4 March 2011


**Abstract** Although chemical cues serve as the primary determinants of larval settlement and metamorphosis, light is also known to influence the behavior and the settlement of coral planulae. For example, *Porites astreoides* planulae settle preferentially on unconditioned red substrata. In order to test whether this behavior was a response to color and whether other species also demonstrate color preference, settlement choice experiments were conducted with *P. astreoides* and *Acropora palmata.* In these experiments, larvae were offered various types of plastic substrata representing three to seven different color choices. Both species consistently settled on red (or red and orange) substrata at a higher frequency than other colors. In one experiment, *P. astreoides* settled on 100% of red, plastic cable ties but failed to settle on green or white substrata. In a second experiment, 24% of larvae settled on red buttons, more than settled on six other colors combined. *A. palmata* settled on 80% of red and of orange cables ties but failed to settle on blue in one experiment and settled on a greater proportion of red acrylic squares than on four other colors or limestone controls in a second experiment. The consistency of the response across a variety of plastic materials suggests the response is related to long-wavelength photosensitivity. Fluorescence and reflectance spectra of experimental substrata demonstrated that the preferred substrata had spectra dominated by wavelengths greater than 550 nm with little or no reflection or emission of shorter wavelengths. These results suggest that some species of coral larvae may use spectral cues for fine-scale habitat selection during settlement. This behavior may be an adaptation to promote settlement in crustose coralline algae (CCA)-dominated habitats facilitating juvenile survival.

**Keywords** Coral · Planulae · Metamorphosis · Color · *Porites astreoides* · *Acropora palmata*

Communicated by Biology Editor Dr. Andrew Baird

B. Mason (✉)
Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA
e-mail: bmason@rsmas.miami.edu

M. Beard
Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA

M. W. Miller
National Oceanic and Atmospheric Administration, Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, FL 33149, USA

## Introduction

Coral recruitment is the result of success during three sequential early life history phases: planktonic dispersal, settlement, and post-settlement growth and survival (Ritson-Williams et al. 2009). Habitat selection by larvae plays a critical role in determining post-settlement survival (e.g., Mundy and Babcock 2000; Harrington et al. 2004; Birrell et al. 2005). Evidence suggests that multiple sensory cues are involved in habitat selection (e.g., Raimondi and Morse 2000), yet many details regarding the link between sensory biology and settlement ecology of coral larvae remain elusive.

Chemical cues are known to influence larval behavior, settlement, and metamorphosis in many species. For example, *Porites astreoides* larvae swim downward in response to water-soluble cues originating from the reef (Gleason et al. 2009), and many species settle and metamorphose in response to crustose coralline algae (CCA) and/or microbial

films (e.g., Morse et al. 1988; Harrington et al. 2004; Webster et al. 2004). The larvae of many species also display substratum preferences that are congruent with the depth and habitat distribution of adults. For example, two species of *Goniastrea,* with an adult distribution restricted to reef flat environments, preferred to settle on terracotta tiles conditioned at 2 m depth, in contrast to two species of *Fungia* whose adult distributions are restricted to the base of reef slopes and preferred to settle on tiles conditioned at 12 m (Baird et al. 2003). Similarly, larvae of *Goniastrea retiformis,* and *Stylaraea punctata*, settle at higher densities on substrata common in habitats where the adults are abundant (Golbuu and Richmond 2007).

While chemical cues are clearly important in determining settlement and metamorphosis, light is also known to influence larval behavior and habitat selection. For example, Kawaguti (1941) described positive phototaxis in zooxanthellate planulae, noting a several-fold difference in sensitivity among species. Since *Symbiodinium* display phototaxis in culture, it had been hypothesized that zooxanthellae are responsible for phototaxis in coral planulae (e.g., Zahl and McLaughlin 1959). However, *Montastraea faveolata* larvae, which lack zooxanthellae, swim at the surface in the light but remain at the bottom of cylinders in the dark (Szmant and Meadows 2006). In addition, light quality and intensity have been shown to influence settlement in both spawned and brooded larvae. *Porites astreoides* larvae prefer environments sheltered from UV radiation (Gleason et al. 2006), while species-specific preferences for light intensity at settlement have been shown in *Goniastrea aspera, Acropora tenuis,* and *Oxypora lacera* (Babcock and Mundy 1996; Mundy and Babcock 2000), and preference for light quality is apparent in larvae from *Goniastrea favulus* and *Montipora peltiformis* (Mundy and Babcock 1998). Lastly, a previously undocumented photosensitive behavior was observed while conducting experiments with *P. astreoides* larvae (BM and MB, pers. obs.): Larvae settled on a red, fluorescent, plastic cable ties (Fig. 1) but failed to settle on other colors of identical cable ties, unconditioned coral rubble, and/or surfaces of plastic containers. This apparent preference for red settlement surfaces prompted the study described in this paper.

The color of an object is a function of the light emerging from the subject. The emerging light can result from addition (e.g., bioluminescence and fluorescence) and/or subtraction radiances (e.g., pigment colors) (Lythgoe 1979). Fluorescence contributes little to coloration in the terrestrial environment, but in aquatic environments, where water spectrally filters both the incident and the reflected light, fluorescence appears to play a more significant role. Fluorescent pigments contribute to the coloration of many corals and sea anemones (e.g., Dove et al. 2001; Mazel and Fuchs 2003; Oswald et al. 2007), and fluorescence has been shown to enhance the brightness and visibility of signaling in mantis shrimp (Mazel et al. 2004). Unlike red and orange color resulting from subtraction radiances (pigments absorbing blue and green wavelengths but reflecting red or orange), red and orange fluorescence can be brighter than the incident light of those wavelengths and, at deeper depths, can create vivid color displays that contrast dramatically with the predominantly blue surroundings (Lythgoe 1979). If substrate color is influencing the settlement ecology of coral larvae, the effective stimulus may result from reflectance and/or fluorescence of potential settlement habitat.

The aim of this study was to experimentally test the influence of substrata color on the settlement of coral larvae. Specific objectives were (1) to investigate whether the preferential settlement on red substrata, previously observed in larvae of *P. astreoides* (a brooder), is a response to color; (2) to determine whether larvae of *Acropora palmata* (a representative broadcast spawner) also distinguish among substrata using color as a cue; and (3) to determine the reflectance and fluorescence spectra of experimental substrata in order to characterize the spectral signature responsible for cuing larval settlement.

## Materials and methods

In this study, larval settlement preferences were tested by conducting choice experiments with two species—*Porites astreoides* and *Acropora palmata.* In experiments, larvae were offered plastic substrata representing a range of color choices. Separate experiments were conducted with each species in 2006 and again in 2009; each experiment had a slightly different design and employed different substrata types. Following the experiments, reflectance and fluorescence spectra were measured for a subset of the settlement materials to characterize and compare the spectral signatures of stimuli provided in the individual experiments.

### Larval collection

Eight adult *Porites astreoides* (a brooding species with zooxanthellate larvae) colonies were collected from Little Grecian reef in Key Largo, FL, 2 days prior to the new moon in June 2006 (Experiment I) and in June 2009 (Experiment III). Colonies were transported to the University of Miami and maintained in a flow-through seawater table. Upon release, larvae were collected by pipet and transferred to containers with filtered seawater until experiments were set up. Larvae were less than 24 h old when introduced to the experimental treatments. Gametes were collected from *A. palmata* (a spawning species with azooxanthellate larvae) in Key Largo, FL in August 2006

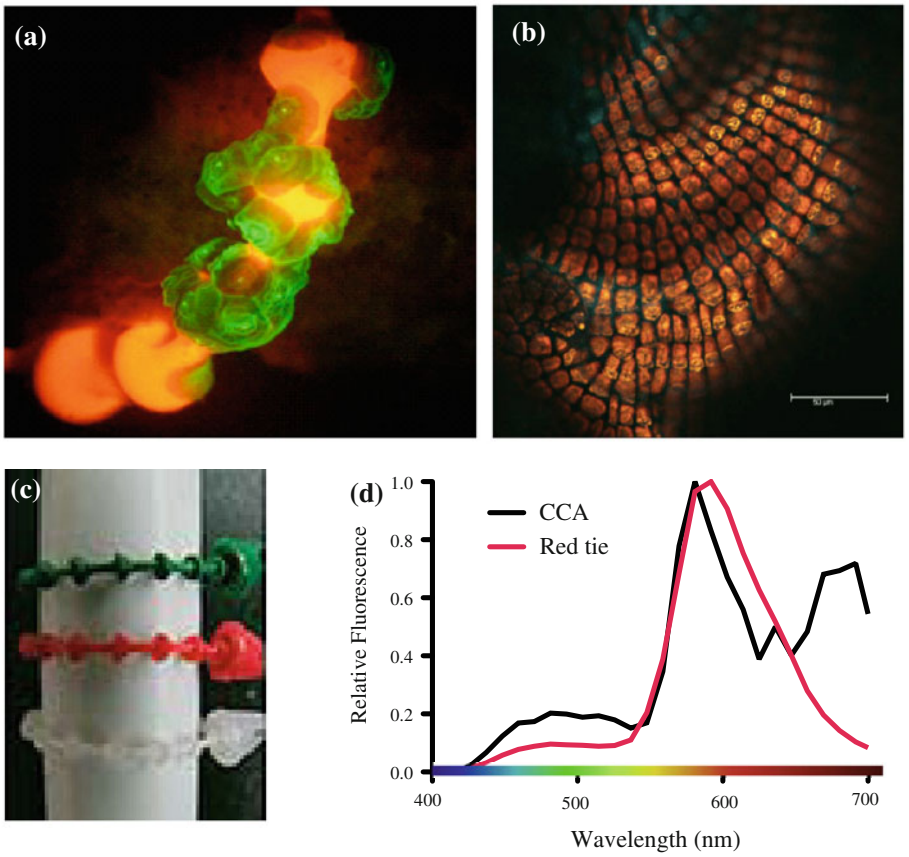


**Fig. 1** Images and fluorescent spectra of substrata. **a** *Porites astreoides* spat attached to *red/fluorescent orange* cable tie (middle tie shown in panel **c**) used in Experiment I. **b** Three-channel (405, 546, and 588 excitation) fluorescent confocal micrograph of CCA (*T. prototypum*). (see **d**) combine to produce a bright orange fluorescence. **c** Beaded cable ties used in Experiment I, as they appear under white light. **d** Fluorescence emission spectra of CCA, including chlorophyll fluorescence and phycoerythrin fluorescence, and the red-beaded cable tie (corresponding to images **a** & **b**)

(Experiment II) and August 2009 (Experiment IV) by placing weighted nets over adult colonies immediately prior to spawning. Upon release, positively buoyant gamete bundles were collected in plastic containers secured to the top of the nets. Intact bundles were returned to the research vessels, and gametes from colonies representing three different genotypes were combined to facilitate fertilization. Larvae were reared to competency following the methods described by Miller and Szmant (2006).

## Settlement experiments

### *Experiments I and II*

The design of the Experiment I was based on the apparent selection of red, fluorescent nylon cable ties in ancillary observations of *P. astreoides* larvae. In both Experiments I and II, larvae were offered arrays of clean (i.e., unconditioned) nylon cable ties representing three colors secured around white fiberglass rods. In Experiment I, 10 *P. astreoides* larvae were placed in each of 12 replicate 100 ml dishes containing one each of red, green, and white cable ties (Avery Dennison, Secure-A-Tie® fasteners, 127 mm, Nylon 6/6 and polypropylene), secured around one rod, and 1-μm-filtered seawater. The order of the colors was randomized, and fiberglass rods were positioned in the center of each dish. Six dishes were kept in the dark, and six were placed under 120 cm, fluorescent strip light (GE Ecolux®) and maintained on a 12 h:12 h light/dark cycle. Irradiance (PAR) at the surface of the laboratory bench on which experiments were conducted, across the area in which dishes/aquaria were placed, ranged from 20 to 30 μmol photons $m^{-2}$ $s^{-1}$. Irradiance measurements were made using a LiCor (LI-189) light meter with a terrestrial, quantum, cosine sensor (LI-190). Partial water changes were conducted daily to minimize effects of evaporation and respiration. After 48 h, substrates were examined under a dissecting microscope, and the number of settled (attached and metamorphosed) larvae was recorded. Metamorphosis here was defined as larval transition to an orally aborally flattened primary polyp with secretion of a basal plate.

In Experiment II (conducted in August 2006), the above-mentioned experiment was repeated with *A. palmata.* Ten larvae were placed in each of 10 replicate, 100 ml dishes, with filtered seawater and arrays of cable ties, assembled as above. The cable ties used in this experiment ("Standard Duty, 7 inch, 50 lb", nylon; BuyCableTies.com) were a different type and represented different colors (red, orange, and blue) than those used in Experiment I. Larvae were 5 days old when they were introduced to the experimental

treatments, dishes were placed in constant darkness or under the light regime described above, and larvae were allowed 5 days to settle.

*Experiment III*

The goal of this experiment was to provide *P. astreoides* larvae with a choice of plastic (nylon) substrata (16 mm diameter buttons; Jesse James Embellishments Basics Small Dyed-to-Match Hearts) representing a broader range of the visible light spectrum. Colors included red, pink, orange, green, blue, and purple. One button of each color was placed in each of 10 plastic petri dishes containing 25 ml of 1-μm-filtered seawater. The positions of the buttons were randomized within dishes, 10 larvae were added to each dish, and dishes were positioned on a laboratory bench beneath two 120 cm, fluorescent strip lights (GE Ecolux®). Irradiance levels were the same as above. Lights were maintained on a 12 h:12 h light/dark cycle, and the larvae were allowed 48 h to settle.

*Experiment IV*

This experiment investigated behavior of *A. palmata* larvae, and settlement substrata consisted of 5 cm × 5 cm square acrylic tiles cut from 6.35-mm-thick translucent cast acrylic sheet (www.delviesplastics.com). Four intersecting grooves (a tic-tac-toe pattern) were carved into the upward-facing surfaces of all tiles using a soldering gun to provide texture known to facilitate settlement of some species (e.g., Petersen et al. 2005). Limestone tiles of equivalent dimension were cut from travertine floor tile and textured as above. Colors of substrates included red, orange, yellow, green, blue, and stone (limestone). One tile of each color was haphazardly arranged in the bottom of each of ten 20-l aquaria, 12 l of filtered seawater (1 μm), and 30 larvae were added to each. Water was lightly aerated, and salinity was monitored daily during the course of the experiment. Salinity was adjusted by the addition of distilled water and maintained between 35 and 37 ppm. The ten aquaria were arranged beneath two ballasts (five aquaria beneath each) containing two 120 cm, fluorescent strip lights (one GE Ecolux; one All-Glass Aquarium 32 W/8,000 K). Irradiance levels were in the range reported above (20–30 μmol photons $m^{-2}$ $s^{-1}$). Lights were maintained on a 12 h:12 h light/dark cycle during the experiment, and larvae were allowed 1 week to settle.

Spectral measurements of settlement substrata

*Fluorescence spectra*

Fluorescent emission spectra were measured using a Leica TCS SP5 confocal microscope. Fluorescent emission spectra were generated for all plastic substrata (cable ties, heart-shaped buttons, and acrylic squares) and CCA (*Titanoderma prototypum*, a species shown to induce settlement of *A. palmata* larvae (Ritson-Williams et al. 2010)) by scanning materials from 415 to 700 nm at 10-nm interval using the xy$\lambda$ scan mode (Leica Application Suite) with 405 nm (UV) excitation. Fluorescence spectra were normalized as percent change in fluorescent emission (percent change relative to the baseline detection).

*Reflectance spectra*

Diffuse reflectance of plastic buttons and acrylic squares was measured using a diffuse reflectance measurement system (Optronic Laboratories (OL), LLC). Components of the system included an integrating sphere reflectance attachment (OL 740-70), a diffraction grating monochromator (OL 750-M-D), and an OL 200/IR source attachment. A 150-W quartz halogen lamp served as the radiation source during measurements. A digital motor control interface and drive motor were used to position diffraction gratings and control the wavelength of incident radiation with 0.1-nm precision. Reflected radiation was measured using a silicon photodiode detector, and data were collected using OL 750/754 DOS Application Software. The diffuse reflectance of materials was measured at 10-nm intervals from 400 to 700 nm. Data were transformed to relative diffuse reflectance (diffuse reflectance at each interval/maximum reflectance from 400 to 700 nm).

Statistical analysis

Since aggregative settlement has been observed in both *P. astreoides* and *A. palmata* (personal observation), the settlement of individual larvae within experimental dishes was not considered independent. The pooling of larvae from multiple colonies likely reduced any bias resulting from this aggregative settlement effect but dishes were considered to be the smallest independent units, and statistical analyses were performed only on the counts of dishes with or without settlement for each color. This count frequency data was analyzed using a categorical (contingency table) model to determine whether settlement was contingent upon substrate color. Pearson chi-squared tests were used to determine whether color had a significant effect on settlement within each experiment.

## Results

Larvae of *Porites astreoides* and *Acropora palmata* showed a higher settlement response to red (or red and

orange) substrata (Table 1). When kept in the light, *P. astreoides* settled on all red cable ties but failed to settle on green or white. In the same experiment, larvae failed to settle when dishes were kept in the dark (Table 1, Experiment I). In Experiment III, a total of 24 *P. astreoides* larvae settled on red buttons, greater than the total number that settled on all other colors combined. *A. palmata* larvae also preferred red (or red and orange) substrata. In Experiment II, *A. palmata* larvae settled in equal numbers on the red and orange cable ties but failed to settle on blue, and in Experiment IV, the total number of settlers observed on red acrylic squares was greater than twice the number observed on any other color (Table 1). In all experiments, larvae settled predominantly in cryptic locations (i.e., below rods where the cable ties contacted the dishes; on the undersides of buttons; in grooves carved into the surface of acrylic tiles).

Since larvae of these species display aggregative settlement, the settlement of individuals on different treatments within each dish was not considered independent. Treating dishes as independent replicates, the number of dishes in which settlement was observed on red (or red and orange) substrata was consistently greater than on other colors (Fig. 2). Chi-square tests of contingency, between substrate color, and the frequency of dishes in which settlement was observed indicated that settlement was contingent upon color in Experiment III (Fig. 2b) with *P. astreoides* ($\chi^2 = 19.41, P = 0.0035$) and Experiment IV (Fig. 2d) with *A. palmata* ($\chi^2 = 11.77, P = 0.0381$) (Fig. 2). Statistical analysis was not performed on the results from Experiments I and II (Fig. 2a, c) since differential settlement response was evident by inspection and low replication made the application of Chi-square tests inappropriate.

Spectral measurements revealed that a natural, preferred CCA species, and experimental plastic substrata had common spectral attributes (Fig. 1). The fluorescence emission spectra of CCA (*Titanoderma prototypum*) and the red, beaded cable tie used in Experiment I had similar fluorescent emission peaks at 580 and 590 nm, respectively (Fig. 1d). Due to the size and surface irregularities of the CCA and the cable ties, measurement of the diffuse reflectance was not possible using the instrumentation available. However, reflectance and fluorescent emission spectra were obtained for the plastic buttons and acrylic squares. The preferred substrata from each of these experiments, the red buttons and red squares, had reflectance spectra dominated by radiation greater than 600 nm and little or no reflectance or fluorescence of wavelengths in the blue or green spectral regions. Substrata experiencing little or no settlement in experiments, including the pink button, had reflectance spectra dominated by, or fluorescent peaks with,

**Table 1** Summary of larval settlement experiments[a]

| | Year | Spp.[b] | Substrate | # larvae | # dishes | l/d[c] | Substrate Color | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Red | Green | White | | | | | |
| I. | 2006 | Pa | Cable ties | 60 | 6 | l | 32(7) | 0(0) | 0(0) | | | | | |
| | | | | 60 | 6 | d | 0(0) | 0(0) | 0(0) | | | | | |
| | | | | | | | Red | Orange | Blue | | | | | |
| II. | 2006 | Ap | Cable ties | 50 | 5 | l | 8(3) | 8(4) | 0(0) | | | | | |
| | | | | 50 | 5 | d | 0(0) | 0(0) | 0(0) | | | | | |
| | | | | | | | Red | Orange | Pink | Green | Blue | Purple | White | |
| III. | 2009 | Pa | Buttons | 100 | 10 | l | 24(10) | 3(2) | 0(0) | 0(0) | 1(1) | 10(10) | 1(1) | |
| | | | | | | | Red | Orange | Yellow | Green | Blue | Stone | | |
| IV. | 2009 | Ap | Squares | 300 | 10[d] | l | 16(4) | 6(3) | 6(4) | 4(3) | 1(1) | 6(2) | | |

[a] Total # settled (max. settled/replicate)

[b] Species—*P. astreoides* (Pa)/*A. palmata* (*Ap*)

[c] Light environment—light(*l*)/dark(*d*)

[d] Experiments conducted in 20-l aquaria

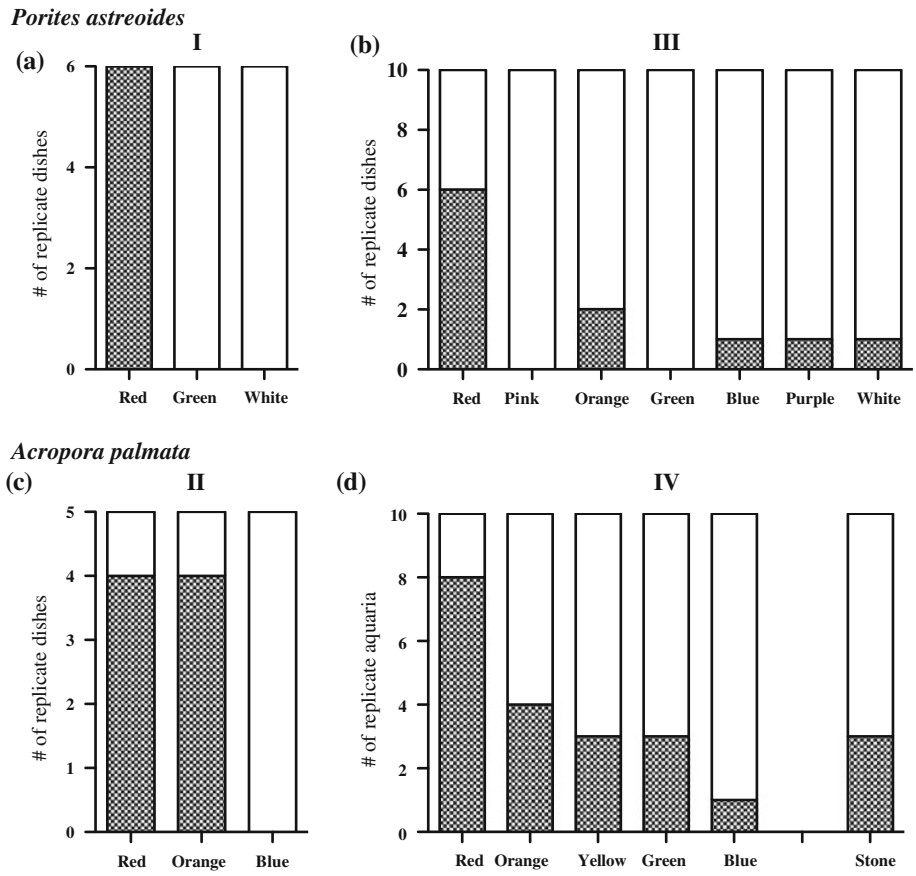


**Fig. 2** Results of settlement experiments. *Bars* represent the number of replicate dishes in which settlement was observed for each color of substrata. **a** Experiment I, cable tie experiment conducted in June 2006 (dishes kept in the light). **b** Experiment III conducted in June 2009 (plastic buttons). **c** Experiment II, cable tie experiment conducted in August 2006 (dishes kept in the light). **d** Experiment IV conducted in August 2009 (acrylic squares)

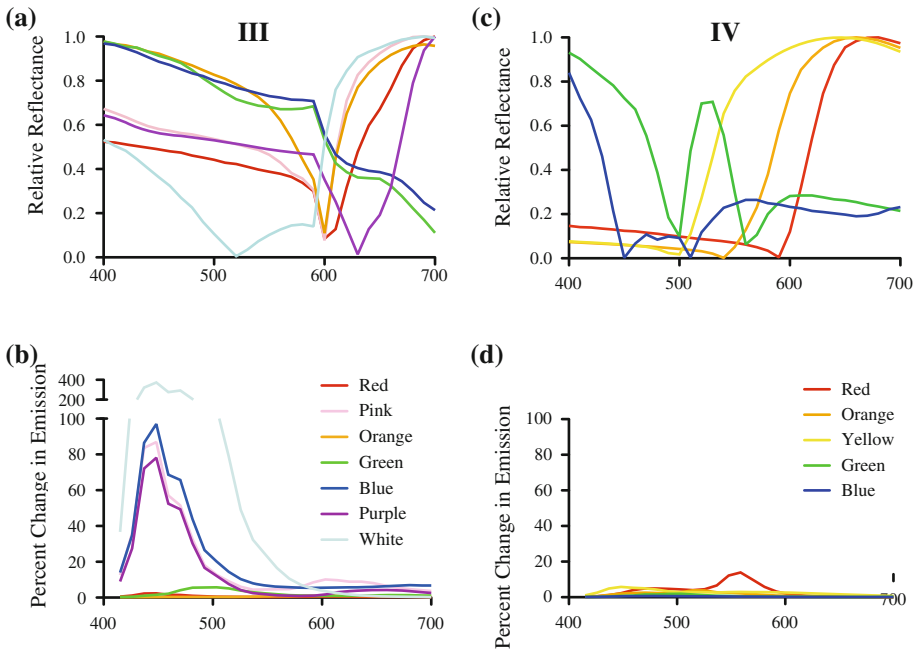


**Fig. 3** Reflectance and fluorescence emission spectra of settlement substrata. **a** Relative diffuse reflectance plastic buttons (Experiment III). **b** Percent change in fluorescence emission of plastic buttons, generated with UV (405 nm) excitation. **c** Relative diffuse reflectance of acrylic squares (Experiment IV). **d** Percent change in fluorescence emission of acrylic squares, generated with UV (405 nm) excitation

wavelengths between 400 and 550 nm (Fig. 3). While some colors of the buttons had strong fluorescent peaks (Fig. 3b), the red buttons did not exhibit detectable fluorescence and the acrylic squares were only weakly or non-fluorescent (Fig. 3d). The percent change in emission of these materials only ranged from <1 (blue) to ~14% (red) and likely contributed little to their overall spectral signatures (Fig. 3).

## Discussion

These results demonstrate that larvae of at least one brooding, *Porites astreoides*, and one spawning coral, *Acropora palmata*, settle and metamorphose at a greater frequency on red substrata than on similar substrata of other colors. Since this tendency was observed on a variety of plastic materials and only when experiments were conducted in light, it suggests the larvae are responding to color rather than a chemical unique to dyes in a particular material. Elevated rates of settlement and metamorphosis did not appear to be restricted to red fluorescent materials, as the red buttons used in Experiment III with *P. astreoides* were not fluorescent, and red acrylic squares used in Experiment IV were only weakly fluorescent with peaks in the yellow region of the spectrum. However, the response observed in Experiment I (Fig. 2a) may have been enhanced by fluorescence, since the strong fluorescent peak of the red cable tie likely contributed to the overall red–orange color of this substrate. It is possible that long-wavelength fluorescence also contributed to the overall spectral signatures of preferred red (or red and orange) substrata in other experiments, but was not detected in the characterizing measurements reported here. Since fluorescence was measured using a single (UV; 405 nm) excitation wavelength, long-wavelength fluorescent pigments, with excitation spectra well above 405 nm, may not have been excited during measurements.

### Potential function of color response

Red-photosensitivity (and/or avoidance of spectral environments dominated by blue) could serve as a mechanism for locating habitat suitable for the survival of juveniles and adults. Although substrate color previously has not been hypothesized to influence settlement or metamorphosis in corals, some species show preference for specific light qualities (Mundy and Babcock 1998) or intensities (Babcock and Mundy 1996; Mundy and Babcock 1998). These authors suggested that a possible ecological function of light-dependent settlement observed in some species is to concentrate larvae at a depth optimal for adult survival. While vertical positioning may be one function of larval photosensitivity, the settlement behavior demonstrated here suggests that these species use photosensitivity and color cues for fine-scale discrimination and selection of substrata, or microhabitats. Future work should investigate whether other species demonstrate color preference during settlement.

The composition of the plastic materials used in these experiments is unlike substrata that naturally occur on coral reefs, but red surfaces are common components of the coral reef benthos. CCA are conspicuous and important functional components of coral reefs and can be especially abundant on shallow, high-energy reef crests (reviewed by Steneck 1986). While CCA is not required for the induction of settlement and metamorphosis in *P. astreoides* (pers. obs.) and *A. palmata* (Ritson-Williams et al. 2010), both species have been shown to settle on the surface of some CCA species, and in a laboratory experiment, 7-day-old *A. palmata* settled at a higher frequency in response to the CCA *Hydrolithon boergesenii* than to fragments of *A. palmata* skeleton, or the CCAs, *Paragoniolithon solubile*, *Porolithon pachydermum*, or *Titanoderma prototypum* (Ritson-Williams et al. 2010). Although the colors of CCAs vary, all species possess phycoerythrin (an accessory pigment abundant in Rhodophytes), which is responsible for orange fluorescence, seen here in *T. prototypum*, and closely resembles the fluorescent emission of the red plastic cable tie used in Experiment I of this study (Figs. 1 and 3). The spectral signatures of the red/orange plastics and CCA may provide similar spectral cues that enable fine-scale habitat selection during settlement.

The selection of red surfaces may be driven by strong selective pressure resulting from high rates of post-settlement mortality. Settlement on CCA or in CCA-dominated habitat does not necessarily increase a larva's probability for survival since some species of CCA can overgrow spat or dislodge them by sloughing their outer tissue layer. However, other species of CCA appear to facilitate early post-settlement survival (e.g., Ritson-Williams et al. 2009; Arnold et al. 2010; Price 2010), and the distribution of CCA is inversely correlated with other known sources of juvenile coral mortality (for example, macroalgae and sediment; e.g., Birrell et al. 2005). It is not yet known whether spectral signatures and larval settlement preferences correspond among various species of CCA.

While the coral species investigated here are common in shallow reef habitats where red and orange wavelengths of light remain abundant, the orange fluorescence of CCA could provide a cue for habitat selection in deeper reef environments. The fluorescence of phycoerythrin is maximally excited by green light with a wavelength $\sim$546 nm but is also by blue (French and Young 1952). In oligotrophic water, blue and green wavelengths of light penetrate well below 50 m (Levine and MacNichol 1982). Downwelling light dominated by blue and green wavelengths could excite fluorescence of phycoerythrin pigments in CCA. If spectral cuing of settlement and metamorphosis in coral larvae involves a photopigment spectrally tuned to absorb in the orange/red region of the spectrum, the orange phycoerythrin fluorescence of CCA could serve as a beacon against an otherwise dark background.

## Potential mechanism of color response

The induction of metamorphosis by long-wavelength light is not unprecedented. Incubation in red light resulted in more rapid metamorphosis of frog tadpoles (Joshi and Mohinuddin 2003). The acceleration of metamorphosis occurred in both intact and experimentally blinded tadpoles indicating that the response was mediated by extraocular photoreceptors. Opsins are the only long wavelength–sensitive photopigments known to occur in animals (van der Horst and Hellingwerf 2004) and are likely responsible for the accelerated metamorphosis observed in tadpoles. While opsins are traditionally associated with visual structures (rod and cone cells of the retina), non-ocular opsins (e.g., melanopsins, vertebrate ancient opsins, peropsins, neuropsins, pinopsins) with biological functions other than vision (e.g., cell division, photoentrainment of circadian clocks, the regulation of reproduction) have been described in various cell types (other than rods and cones) in both vertebrates and invertebrates (e.g., Terakita 2005; Shichida and Matsuyama 2009).

To date, the only photosensitive molecules described in corals are cryptochromes (Levy et al. 2007), but these are strictly blue-light-sensitive molecules that function at the terminal end of circadian gene networks and are therefore unlikely to play a role in this long wavelength, settlement response. Since opsins are the only long wavelength–sensitive photopigments known to occur in animals, they are most likely responsible for the behavior observed here. Opsins recently were discovered in other cnidarians (for example, the starlet sea anemone, *Nematostella vectensis*, *Hydra magnapapillata* and two hydrozoans; Suga et al. 2008), and a melanopsin-like transcript is present in the *Acropora millepora* larval transcriptome (Vize 2009). Efforts to identify and describe opsins in *A. palmata* larvae are currently in progress.

## Integration of multiple cues by coral larvae

While the larvae in this study settled at a higher frequency on red substrates than on other colors, the proportions of larvae that settled were lower than those typical in other experiments. Settlement of *Porites astreoides* on unconditioned experimental substrata reported here (53% in Experiment I; 39% in Experiment III) was similar, but failed to match the maximum rates of settlement achieved in studies utilizing conditioned (for 5 weeks on the reef) terra cotta tiles or reef rubble (40–60%; e.g., Gleason et al. 2006; Kuffner et al. 2006; Ross et al. 2010). Settlement of *A. palmata* in this study (33% in Experiment II and 13% in Experiment IV, though the 13% in Experiment IV did not account for substantial numbers of larvae that settled on the bottom or side of the aquaria) also did not approach the upper range of experimental settlement rates observed for this species (60–80%, e.g., Ritson-Williams et al. 2010; Albright et al. 2010), when provided with conditioned tiles or natural substrata (e.g., CCA chips). This lower settlement suggests that color may be only one in a suite of cues responsible for habitat selection and metamorphosis in these species.

As research continues to reveal more about the sensory biology of coral larvae, a complex picture is emerging. Raimondi and Morse (2000) described a complex suite of behavior in larvae of the brooding coral, *Agaricia humilis*, including swimming behavior to maintain depth range, orientation that facilitated cryptic settlement, and metamorphosis cued by a surface morphogens of specific CCA. These authors attributed such complex behaviors to a hierarchy of cues operating across different spatial scales. Other studies have elucidated and provided evidence for both coarse and fine cues leading up to settlement. At the coarse scale, some species of coral larvae may be capable of using sound to locate coral reefs (Vermeij et al. 2010). Once larvae are near reef habitat, water-soluble cues may be responsible for pre-settlement behaviors (Gleason et al. 2009), and at least some species of larvae appear capable of using light cues to locate and position themselves within depth strata that are optimal for their post-settlement survival (e.g., Mundy and Babcock 1998).

Once larvae contact the reef, the fine-scale sensory environment, in which habitat selection, settlement, and metamorphosis occur, likely becomes even more complex. Light may continue to influence larval behavior at fine spatial scales, as light quality or intensity has been shown to influence selection of cryptic versus exposed surfaces (e.g., Babcock and Mundy 1996). Some coral species require chemical cues associated with CCA to induce metamorphosis (e.g., Morse et al. 1996; Raimondi and Morse 2000; Baird and Morse 2004), while others display less stringency in their requirement for CCA (e.g., Morse et al. 1988; Heyward and Negri 1999; Baird and Morse 2004) or prefer biofilm (Golbuu and Richmond 2007). Among those tested, some species of larvae are capable of distinguishing among different types of CCA (e.g., Morse et al. 1988; Harrington et al. 2004; Ritson-Williams et al. 2010). While less is known about the response of coral larvae to chemical cues produced by other benthic invertebrates, secondary metabolites produced by cyanobacteria (*Lyngbya* spp.) have been shown to inhibit larval settlement (e.g., Kuffner and Paul 2004; Kuffner et al. 2006) and compounds produced by different macroalgae have been shown to result in mixed (either inhibiting or promoting) effects on larval settlement and metamorphosis (e.g., Heyward and Negri 1999; Kuffner et al. 2006; Birrell et al. 2008).

At both coarse- and fine-scale larvae may use multimodal signals to improve the accuracy of habitat selection.

Multimodality (communication involving multiple signals engaged simultaneously; reviewed by Partan and Marler 1999; Hebets and Papaj 2005), may involve either redundant (resulting in equivalent effects on the receiver when presented separately) and/or non-redundant signals (resulting in different or no effect when presented separately). Future research should investigate larval behavior within a multimodal framework as color and chemical cues may be redundant (e.g., red color and chemicals both acting to promote settlement on CCA), or non-redundant, (e.g., red color promoting but chemicals inhibiting settlement on sponge or red algae) components of a multimodal signal which serves to enhance the accuracy of habitat selection in some species of coral larvae.

**Acknowledgments** This project was facilitated by permits (FKNMS-2006-009, FKNMS-2006-026, FKNMS-20070114, FKNMS-2009-022) and logistic support from the Florida Keys National Marine Sanctuary. Funding was provided by the NOAA Coral Reef Conservation Program, Sanctuary Friends Foundation of the Florida Keys, and Henry Foundation. We gratefully acknowledge assistance from D.Williams, L. Johnston, and a multitude of others involved in fieldwork and larval culture for the project. R. Ritson-Williams assisted with the collection and identification CCA. G. Gaidosh and K. Voss enabled measurements of fluorescence and reflectance spectra. E. Borneman provided helpful discussion on methods. We also thank M. Schmale, R. Albright, A. Baird, and four anonymous reviewers for comments on the manuscript. M. Beard's contribution (published here post-humously) was part of an internship conducted at the Southeast Fisheries Science Center as part of the Florida State University Certificate Program in Marine Biology.

## References

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci USA 107:20400–20404

Arnold SN, Steneck RS, Mumby PJ (2010) Running the gauntlet: inhibitory effects of algal turfs on the processes of coral recruitment. Mar Ecol Prog Ser 414:91–105

Babcock R, Mundy C (1996) Coral recruitment: Consequences of settlement choice for early growth and survivorship in two scleractinians. J Exp Mar Biol Ecol 206:179–201

Baird AH, Morse ANC (2004) Induction of metamorphosis in larvae of the brooding corals *Acropora palifera* and *Stylophora pistillata*. Mar Freshw Res 55:469–472

Baird AH, Babcock RC, Mundy CP (2003) Habitat selection by larvae influences the depth distribution of six common coral species. Mar Ecol Prog Ser 252:289–293

Birrell CL, McCook LJ, Willis BL (2005) Effects of algal turfs and sediment on coral settlement. Mar Pollut Bull 51:408–414

Birrell CL, McCook LJ, Willis BL, Harrington L (2008) Chemical effects of macroalgae on larval settlement of the broadcast spawning coral *Acropora millepora*. Mar Ecol Prog Ser 362:129–137

Dove SG, Hoegh-Guldberg O, Ranganathan S (2001) Major colour patterns of reef-building corals are due to a family of GFP-like proteins. Coral Reefs 19:197–204

French CS, Young VK (1952) The fluorescence spectra of red algae and the transfer of energy from phycoerythrin to phycocyanin and chlorophyll. J Gen Physiol 35:873–890

Gleason DF, Edmunds PJ, Gates RD (2006) Ultraviolet radiation effects on the behavior and recruitment of larvae from the reef coral *Porites astreoides*. Mar Biol 148:503–512

Gleason D, Danilowicz B, Nolan C (2009) Reef waters stimulate substratum exploration in planulae from brooding Caribbean corals. Coral Reefs 28:549–554

Golbuu Y, Richmond RH (2007) Substratum preferences in planula larvae of two species of scleractinian corals, *Goniastrea retiformis* and *Stylaraea punctata*. Mar Biol 152:639–644

Harrington L, Fabricius K, De'ath G, Negri A (2004) Recognition and selection of settlement substrata determine post-settlement survival in corals. Ecology 85:3428–3437

Hebets EA, Papaj DR (2005) Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol 57:197–214

Heyward AJ, Negri AP (1999) Natural inducers for coral larval metamorphosis. Coral Reefs 18:273–279

Joshi BN, Mohinuddin K (2003) Red light accelerates and melatonin retards metamorphosis of frog tadpoles. BMC Physiol 3:9

Kawaguti S (1941) On the physiology of reef corals. V. Tropisms of coral planulae, considered as a factor of distribution of the reefs. Palao Trop Biol Stat Stud 2:319–328

Kuffner IB, Paul VJ (2004) Effects of the benthic cyanobacterium *Lyngbya majuscula* on larval recruitment of the reef corals *Acropora surculosa* and *Pocillopora damicornis*. Coral Reefs 23:455–458

Kuffner IB, Walters LJ, Becerro MA, Paul VJ, Ritson-Williams R, Beach KS (2006) Inhibition of coral recruitment by macroalgae and cyanobacteria. Mar Ecol Prog Ser 323:107–117

Levine JS, MacNichol EF (1982) Color vision in fishes. Sci Am 246:140–149

Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, Miller DJ, Hoegh-Guldberg O (2007) Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. Science 18:467–470

Lythgoe JN (1979) The ecology of vision. Clarendon Press, Oxford

Mazel CH, Fuchs E (2003) Contribution of fluorescence to the spectral signature and perceived color of corals. Limnol Oceanogr 48:390–401

Mazel CH, Cronin TW, Caldwell RL, Marshall NJ (2004) Fluorescent enhancement of signaling in a mantis shrimp. Science 303:51

Miller MW, Szmant AM (2006) Lessons learned from experimental key-species restoration. In: Precht WF (ed) Coral reef restoration handbook: Rehabilitation of an ecosystem under siege. CRC Press, Boca Raton, FL, pp 219–234

Morse DE, Hooker N, Morse ANC, Jensen RA (1988) Control of larval metamorphosis and recruitment in sympatric Agariciid corals. J Exp Mar Biol Ecol 116:193–217

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M (1996) An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191:149–154

Mundy CN, Babcock RC (1998) Role of light intensity and spectral quality in coral settlement: Implications for depth-dependent settlement? J Exp Mar Biol Ecol 223:235–255

Mundy C, Babcock R (2000) Are vertical distribution patterns of scleractinian corals maintained by pre- or post-settlement processes? A case study of three contrasting species. Mar Ecol Prog Ser 198:109–119

Oswald F, Schmitt F, Leutenegger A, Ivanchenko S, D'Angelo C, Salih A, Maslakova S, Bulina M, Schirmbeck R, Nienhaus GU, Matz MV, Wiedenmann J (2007) Contributions of host and symbiont pigments to the coloration of reef corals. FEBS Lett 274:1102–1109

Partan S, Marler P (1999) Behavior—Communication goes multimodal. Science 283:1272–1273

Petersen D, Laterveer M, Schuhmacher H (2005) Spatial and temporal variation in larval settlement of reef building corals in mariculture. Aquaculture 249:317–327

Price N (2010) Habitat selection, facilitation, and biotic settlement cues affect distribution and performance of coral recruits in French Polynesia. Oecologia 163:747–758

Raimondi PT, Morse ANC (2000) The consequences of complex larval behavior in a coral. Ecology 81:3193–3211

Ritson-Williams R, Arnold S, Fogarty N, Steneck RS, Vermeij MJA, Paul VJ (2009) New perspectives on ecological mechanisms affecting coral recruitment on reefs. Smithson Contrib Mar Sci 38:437–457

Ritson-Williams R, Paul VJ, Arnold SN, Steneck RS (2010) Larval settlement preferences and post-settlement survival of the threatened Caribbean corals *Acropora palmata* and *A. cervicornis*. Coral Reefs 29:71–81

Ross C, Ritson-Williams R, Pierce R, Bullington JB, Henry M, Paul VJ (2010) Effects of the Florida red tide dinoflagellate, *Karenia brevis*, on oxidative stress and metamorphosis of larvae of the coral *Porites astreoides*. Harmful Algae 9:173–179

Shichida Y, Matsuyama T (2009) Evolution of opsins and phototransduction. Philos Trans R Soc B 364:2881–2895

Steneck RS (1986) The ecology of coralline algal crusts: convergent patterns and adaptive strategies. Annu Rev Ecol Syst 17:273–303

Suga H, Schmid V, Gehring WJ (2008) Evolution and functional diversity of jellyfish opsins. Curr Biol 18:51–55

Szmant AM, Meadows MG (2006) Developmental changes in coral larval buoyancy and vertical swimming behavior: Implications for dispersal and connectivity. Proc 10th Int Coral Reef Symp 1:431–437

Terakita A (2005) The opsins. Genome Biol 6:213

Van der Horst M, Hellingwerf K (2004) Photoreceptor proteins, "Star actors of modern times": a review of the functional dynamics in the structure of representative members of six different photoreceptor families. Acc Chem Res 37:13–20

Vermeij MJA, Marhaver KL, Huijbers CM, Nagelkerken I, Simpson SD (2010) Coral larvae move toward reef sounds. PLOS ONE 5:e10660

Vize PD (2009) Transcriptome analysis of the circadian regulatory network in the coral *Acropora millepora*. Biol Bull 216:131–137

Webster NS, Smith LD, Heyward AJ, Watts JEM, Webb RI, Blackall LL, Negri AP (2004) Metamorphosis of a scleractinian coral in response to microbial biofilms. Appl Environ Microbiol 70:1213–1221

Zahl PA, McLaughlin JJA (1959) Studies in marine biology. IV. On the role of algal cells in the tissues of marine invertebrates. J Protozool 6:344–352