



Small-scale habitat selection by larvae of a reef-building coral

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ABSTRACT: When animals select habitats, they integrate positive and negative cues in the environment that shape their choices about where to live. We conducted a settlement experiment with the larvae of *Orbicella faveolata*, a reef-building scleractinian coral on Caribbean reefs. We investigated the settlement decisions of *O. faveolata* larvae in relation to communities of benthic spaceholders and explicitly investigated how benthic communities influence how larvae may make settlement decisions at small spatial scales. Settlement tiles that attracted at least one *O. faveolata* settler had a significantly different community composition than tiles with no settlers. Red filamentous algae and crustose coralline algae were abundant on tiles with no settlers, while bare substrate was abundant on tiles with settlers. When we analyzed the spatial patterns of coral settlement within tiles, coral settlers avoided areas with sediment, sponges, and red filamentous algae and preferred areas with green filamentous algae. Selection among individual taxa was dominated by selecting against rather than for taxa. Our results show that coral larvae make complex decisions about where to settle even down to the millimeter scale. Importantly, these coral larvae select their habitat by balancing contrasting forces to avoid risks.

KEY WORDS: Coral reefs · *Orbicella faveolata* · Coral settlement · Turf algae · Bare space

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1. INTRODUCTION

The larvae of many sessile marine invertebrates exhibit complex responses to biotic and abiotic cues in the environment that provide information about the quality of potential habitats (Connell 1985, Ritson-Williams et al. 2009). Larvae may respond to biotic cues to avoid settling near other taxa that are strong competitors for space (Grosberg 1981) or near predators (Johnson & Strathmann 1989). For example, coral larvae may avoid settling near fleshy macroalgae (Rit-

son-Williams et al. 2020), reducing the growing coral's competition for space early in ontogeny. In rocky intertidal communities, the larvae of barnacles can detect cues left on the substrate by predatory whelks and avoid these areas to reduce their future risk of predation (Johnson & Strathmann 1989). Positive selection in response to biotic cues can also be beneficial to settling larvae, such as coral larvae that preferentially settle on specific species of crustose coralline algae (CCA) that confer higher rates of post-settlement survival (Harrington et al. 2004, Price 2010).

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In addition to biotic cues, settling larvae also use abiotic cues including light (Mundy & Babcock 1998), sound (Vermeij et al. 2010), and water flow (Larsson & Jonsson 2006) to locate favorable habitats. For example, the larvae of barnacles avoid settling in high-flow habitats that are suboptimal for suspension feeding (Larsson & Jonsson 2006). Some species of corals preferentially settle in low-light habitats, reducing their risk of competition and predation (Raimondi & Morse 2000). Additionally, the larvae of many marine invertebrates preferentially settle on structurally complex substrates (such as in pits and grooves) which can reduce their risk of predation (Carleton & Sammarco 1987, Bourget et al. 1994, Doropoulos et al. 2016, Gallagher & Doropoulos 2017).

Corals are sessile marine invertebrates whose larvae respond to both positive and negative settlement cues during settlement (Ritson-Williams et al. 2009). For example, some coral larvae prefer to settle on or near certain species of CCA (Harrington et al. 2004, Ritson-Williams et al. 2014) but strongly avoid many species of fleshy macroalgae (Kuffner et al. 2006, Diaz-Pulido et al. 2010, Paul et al. 2011). The decisions that larvae make are a crucial step in the life cycle of corals because they determine their future competitors and success as adults. For example, larvae that settle on preferred CCA have significantly higher survival rates than larvae that settle on species of CCA that are typically avoided, which can overgrow and outcompete coral recruits (Harrington et al. 2004, Price 2010). Much of the foundational work on habitat selection by the larvae of marine invertebrates, including corals, elucidated larval preference or avoidance of individual taxa in lab experiments (Harrington et al. 2004, Kuffner et al. 2006, Ritson-Williams et al. 2009, 2014, Paul et al. 2011). Recruitment studies in the field have expanded on this work by asking how larvae select habitats in communities of organisms that include taxa that are preferred and avoided by larvae (Price 2010, Arnold & Steneck 2011, Doropoulos et al. 2016, Evensen et al. 2021). These studies have shown that differences in the composition of communities where corals recruit can determine rates of growth and survival (Vermeij 2006, Doropoulos et al. 2016, Evensen et al. 2021). How communities impact small-scale settlement decisions by larvae and the spatial arrangement of settlers relative to the rest of the benthic community is less well understood.

On reefs across the wider Caribbean, coral cover has been declining since at least the 1970s (Gardner et al. 2003, Jackson et al. 2014), including the loss of

important habitat-forming genera *Orbicella* and *Acropora*. As the abundance of colonies declines, successful recruitment of new individuals is key to the persistence of coral populations. However, recruits of *Orbicella* are rarely observed on natural substrate or on coral settlement tiles in the field (Hughes & Tanner 2000, Edmunds et al. 2011, Edmunds 2015), leading to questions about how larvae of *Orbicella* spp. choose settlement habitats. Given the threatened status of *Orbicella* and hypothesized recruitment limitation (Hughes & Tanner 2000), understanding the settlement ecology of *Orbicella* corals is highly important.

Our study investigated the settlement decisions of coral larvae within benthic communities at small spatial scales. First, we preconditioned settlement tiles on a coral reef for 2 yr to allow natural benthic communities to develop. We then used these tiles in an experiment examining settlement choice in larvae of the reef-building coral *Orbicella faveolata* (Fig. 1). We gave coral larvae the opportunity to settle on tiles for 48 h and then used high-resolution digital photographs to make maps of locations where each larva settled relative to other benthic organisms (e.g. CCA, turf algae) and bare space, which we hereafter collectively refer to as 'benthic categories'. We then used these maps to evaluate the spatial arrangement of settlers relative to the benthic community on tiles. Specifically, we asked 4 questions: (1) Does benthic community composition influence the likelihood of coral settlement? (2) Which benthic categories positively or negatively influence the willingness of larvae to settle on a tile? (3) Within a tile, how does the community composition around coral settlers differ from the overall community composition of the tile? (4) Within a tile, which benthic categories do coral larvae select for or avoid? Our results show that coral larvae make decisions at the millimeter scale about the benthic spaceholders they settle among, balancing the tradeoffs among benthic organisms that are either attractive or repellent.

2. MATERIALS AND METHODS

2.1. Gamete collection and rearing of coral larvae

We conducted our work on shallow spur and groove reefs in the upper Florida Keys, USA, and worked with larvae of the reef-building coral *Orbicella faveolata*. *O. faveolata* are long-lived corals that are ecologically important for building habitat across

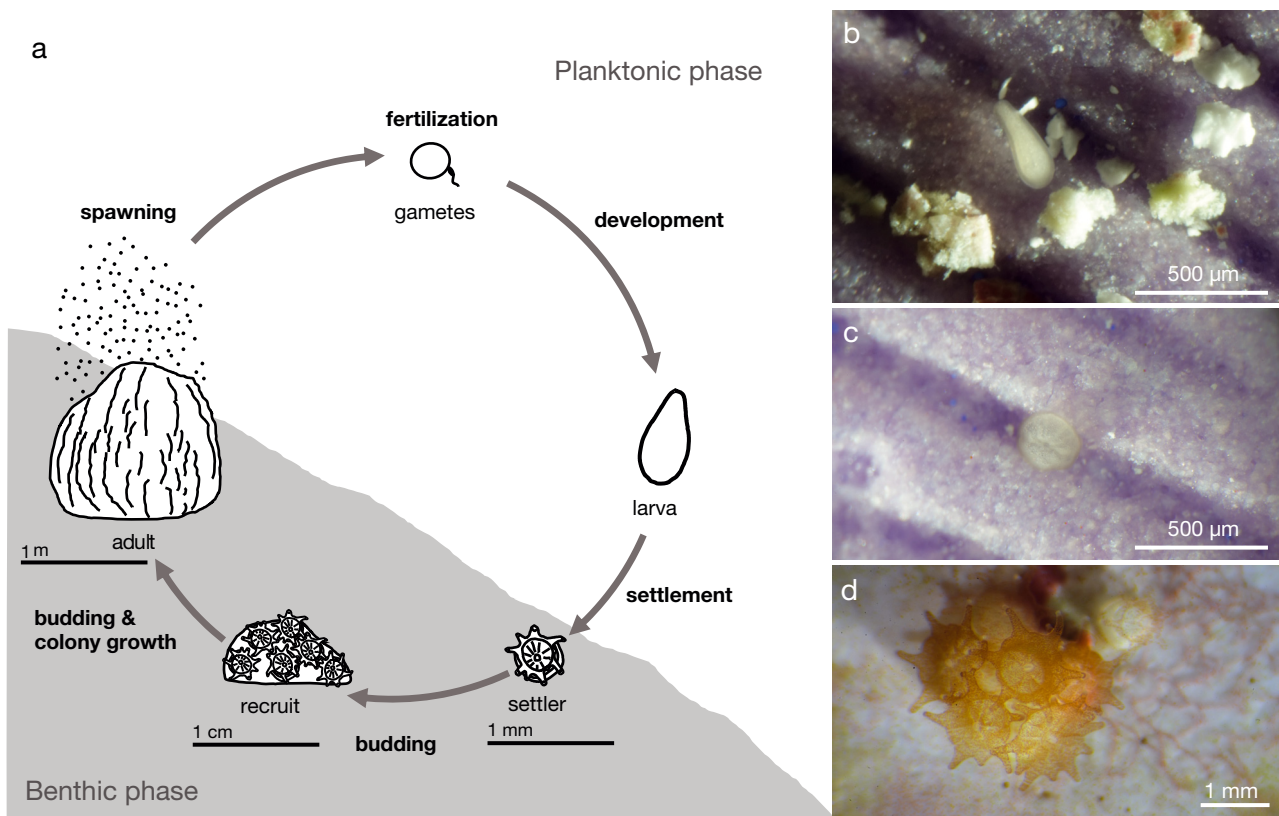


Fig. 1. (a) Life cycle of the broadcast spawning coral species *Orbicella faveolata* (adapted from Fig. 1 in Jones et al. 2015; published under a Creative Commons CC-BY 4.0 licence). Adult colonies of *O. faveolata* release spawned gametes that float to the ocean surface where they mix with gametes from other individuals and are fertilized. Fertilized eggs develop into swimming larvae after several days. Larvae then search the substrate before choosing a place to settle, where they undergo metamorphosis and begin building their calcium carbonate skeleton. Following settlement, newly settled corals reproduce asexually via budding, forming a coral colony. (b) *O. faveolata* larva searching for a place to settle; (c) newly settled *O. faveolata*, 1 d after settlement; (d) *O. faveolata* settlers that have grown into 5 polyps, 2 mo after settlement. Photos (b–d) courtesy of Kristen Marhaver

the reefs of the wider Caribbean. We collected gamete bundles of *O. faveolata* from parent colonies in the upper Florida Keys during the annual spawning period in August of 2017 and immediately transported gametes to our field laboratory in Key Largo, FL. Gametes from at least 10 parent colonies were crossed in a batch culture. Gametes were fertilized and reared in an outdoor laboratory with a recirculating water system with water collected from the reef and transported to the lab in carboys. Throughout the larval-rearing phase and experiment, the seawater system was maintained at $29.53 \pm 0.64^\circ\text{C}$ (mean \pm SD) and water was filtered with a $1 \mu\text{m}$ canister filter and UV sterilizer. See Miller (2014) for additional details on the seawater system methods. We conducted daily, independent competency assays to ensure that a high proportion of larvae were competent to settle and metamorphose in response to a positive settlement cue (chip of reef rubble) before starting the settlement experiments (e.g. Miller 2014).

Based on these observations, we began settlement experiments 6 d after fertilization of *O. faveolata* gametes.

2.2. Cultivation of benthic communities for settlement assays

We used $10 \times 10 \times 2$ cm coral limestone tiles (Keystone Productions) as settlement substrate. Coral limestone tiles closely mimic the natural limestone foundation of coral reefs, as they were quarried from Floridian Pleistocene reef formations. Limestone tiles were attached to plastic-coated mesh with cable ties, and the mesh was nailed to the benthos near Pickles Reef ($24^\circ 59.087' \text{N}$, $80^\circ 24.964' \text{W}$), where they were conditioned for 2 yr. Communities of turf algae, CCA, sponges, and macroalgae developed on the tiles, and these communities were very similar to the communities on the adjacent natural substrate.

Immediately prior to the settlement experiment (<24 h), we collected tiles from the reef and transported them to our field lab on Key Largo. Our objective was to understand how coral larvae select settlement habitats at small spatial scales. Therefore, we subdivided the large tiles into smaller 2.5 × 2.5 × 1 cm (L × W × H) settlement tiles using a tile saw. We cut the tiles such that one large side of each tile contained the community on the exterior of the tile, and the other 5 sides ('back' and 4 edges) were freshly cut limestone. Thus, each tile had only one preconditioned surface with benthic organisms. Past experiments have shown that many larvae settle on the undersides of settlement substrate (Raimondi & Morse 2000, Price 2010, Arnold & Steneck 2011), but our objective was to understand how larvae respond to the benthic communities on the tile surfaces. Therefore, we coated each of the 5 bare limestone sides with a thin layer of paraffin wax to make these sides unavailable to larvae, leaving only one preconditioned side available for larvae to settle, a method that we have previously used for coral settlement experiments (Speare et al. 2019). Our preliminary experiments showed that larvae will settle near but not on top of paraffin wax, making it a suitable, non-toxic barrier to prevent larvae from avoiding the benthic communities of interest by settling on the bare limestone surfaces (Speare et al. 2019).

Subdividing the preconditioned settlement tiles resulted in ~100 of the 2.5 × 2.5 × 1 cm tiles to choose from, of which we selected 30 for our settlement experiment with coral larvae. We selected tiles that represented a wide gradient in abundance of red filamentous algae, CCA, small sponges, green filamentous algae, and bare space to capture a range of benthic categories that likely both repel and facilitate settlement. The category 'bare space' referred to areas without macroscopic organisms, but these areas were likely colonized by microorganisms and biofilms that were difficult to characterize with our methods. Because coral larvae strongly avoid many species of fleshy macroalgae (Kuffner et al. 2006, Diaz-Pulido et al. 2010), we avoided tiles with large amounts of fleshy macroalgae. There were small amounts of several taxa of fleshy macroalgae (in the genera *Valonia*, *Ace-tabularia*, and *Dictyota*) on some of the tiles, but each taxon accounted for <2% cover on any tile. Before starting the experiment, we took high-resolution digital photographs (Olympus Stylus Tough TG-4) of each of the 30 settlement tiles while the tiles were submerged in seawater. Dig-

ital photographs of the tiles were used to quantify the community composition on each tile and map the location of coral settlers.

2.3. Settlement experiment with coral larvae

We carried out our settlement experiment in the temperature-controlled, recirculating seawater system used for rearing larvae (described above). Within the seawater system, we used clear acrylic cylindrical chambers (1 l volume; 10.2 cm diameter × 12.7 cm long) as experimental settlement chambers in which the bottom end was covered with 120 µm mesh and the top end extended above the water surface. Settlement chambers were submerged halfway in the recirculating water system with the mesh end down, resting on a submerged table made of egg crate panels. This setup elevated the settlement chambers off the bottom of the seawater system and allowed water to flow into and out of the chambers but did not allow larvae to escape.

We put one settlement tile and 30 larvae in each settlement chamber for 48 h. At the end of the settlement experiment, we counted the number of settled larvae on each tile using fluorescence microscopy in a dark room. Corals, including the aposymbiotic larvae and settlers in our experiments, appear green or orange when excited with ultraviolet light, whereas other chlorophyll-containing organisms, such as algae, appear red (Logan et al. 1990, Vermeij et al. 2002). Therefore, this method is robust for detecting settlers within communities because it distinguishes coral settlers from other organisms. One concern with this method is that on tiles with abundant filamentous algae, coral settlers may be obscured from view. However, in a series of related experiments with *O. faveolata* larvae using the same methods, we found no relationship between the number of settlers and the percent cover of filamentous algal turfs across a range of 2–92% cover (Speare et al. 2019). Therefore, our ability to detect settlers was not diminished in areas with high densities of other sessile organisms, especially filamentous algal turfs.

Individuals were classified as 'settlers' only if they displayed visible signs of settlement (i.e. attachment to the substrate) and metamorphosis (i.e. transition from pear shaped to flat/disc shape). For each settler, we digitally mapped its location onto the digital photograph of each tile (Preview for Mac OS). This

method allowed us to generate a location for each settler using an X,Y coordinate system that corresponds to pixels of the image of each tile. A ruler in each photo was used to calibrate pixels to physical distance.

2.4. Quantifying community composition of settlement tiles

We used the digital photographs of the tiles to quantify benthic community composition of each tile by manually identifying points using CoralNet software (Beijbom et al. 2015). Points were overlaid in a uniform grid on the tile photographs. Because the dimensions (the length of each side) of each settlement tile were slightly different (mean \pm SD of all side lengths: 2.4 ± 0.3 cm) for each tile, we adjusted the number of rows and columns of points so that the point density for each tile was $100 \text{ points cm}^{-2}$. We manually identified the community member under each point using benthic categories (e.g. sponge, calcareous invertebrates; see Table S1 in the Supplement at www.int-res.com/articles/suppl/m724p067_supp.pdf for descriptions of community members). We identified community members to the lowest taxonomic level possible given the limitations of identifying small organisms. Furthermore, because some community members were very low in abundance and only present on one or 2 tiles, it was necessary to lump some categories into functional groups (i.e. macroalgae) for statistical purposes. Each of the points was associated with an X,Y coordinate that corresponds to the location of that pixel of the digital photograph, making our benthic composition data spatially explicit.

2.5. Data analysis

2.5.1. Benthic community composition and categories that influence the likelihood of coral settlement

Statistical analyses were performed in R v.3.6.2 (R Core Team 2017). First, we sought to determine whether the community composition of tiles was associated with the likelihood that a tile had at least one settler. For each tile, the percent cover of each community member was calculated as the proportion of points identified as each community member relative to the total number of points on the tile. Using the R package 'vegan' (Oksanen et al. 2013), we first

calculated compositional dissimilarities using a Bray-Curtis dissimilarity matrix. We then used 2-dimensional nonmetric multidimensional scaling to visualize differences in tile community composition. We grouped tiles based on whether or not they had at least one coral settler and then determined whether tile community composition differed between tiles that had at least one *O. faveolata* settler and those that had no settlers using a permutational analysis of variance (PERMANOVA; 'adonis' function in 'vegan'; Oksanen et al. 2013). We then tested for differences in group dispersions using the 'betadisp' function in 'vegan' to evaluate whether significant differences in the PERMANOVA could have been driven by differences in group dispersions. Finally, we used a SIMPER analysis (Clarke 1993) to determine which community members drove dissimilarity between tiles with at least one coral settler and those with no coral settlers.

2.5.2. Community composition of neighborhoods around settlers vs. overall community composition of tiles

Next, we sought to determine whether coral larvae selected for or against certain benthic categories at scales smaller than the scale of the tile. To do this, we first determined community composition in concentric circular neighborhoods (hereafter 'neighborhoods') of 1–6 mm radii around each settler. For each neighborhood of a given radius, we calculated community composition by computing the distance between the location of the settler and the location of every point identified on that tile. Points whose distance from the settler was less than or equal to the circle radius were stored and used to determine the community composition of that neighborhood. This process was repeated for each size neighborhood for each settler (6 neighborhood sizes per settler). The high density of points identified on each tile ($100 \text{ points cm}^{-2}$) allowed us to evaluate community composition at these small spatial scales (see Table S2 for the number of points identified in each size neighborhood).

We were interested in understanding whether coral larvae were selecting for settlement neighborhoods that differed in community composition compared to the tile as a whole. Using the data on community composition of neighborhoods and community composition of whole tiles, we carried out a compositional analysis of habitat use, which compares the proportional use of habitat types (percent

cover of benthic categories) relative to their availability (Aebischer et al. 1993) using the 'compana' function within the 'adehabitatHS' package (Calenge 2006). We defined the use of a benthic category as the percent cover of a benthic category in a neighborhood around a coral settler, and its availability as the percent cover of that benthic category on the tile as a whole. We carried out separate compositional analyses of habitat use for each size neighborhood (see Fig. 3b, Table S2). Some of the coral settlers were located near the edge of the tile such that a large portion of the neighborhood circle was off of the tile. We restricted the compositional analyses of habitat use to only include settlers for which at least 90% of their neighborhood was on the tile (see Table S2 for the number of point IDs in each size neighborhood and the number of settlers included in data analysis for each size circle). Because neighborhood area increases as neighborhood radius increases, more of the large-radii neighborhoods were at least partially off the edge of the tile than the smaller-radii neighborhoods, and thus the number of settler neighborhoods included in the data analysis decreased as neighborhood size increased.

To evaluate the possibility that sampling small areas of the tiles (neighborhoods) returned significant results in the compositional analysis of habitat use due to randomness inherent in sampling small areas of the tile, we also carried out a separate control analysis using randomly selected areas on the tiles. For each size neighborhood, we randomly selected locations on the settlement tiles using a random number generator. We selected the same number of random locations for each size neighborhood as we used in the compositional analysis of habitat use for the settler neighborhoods (Table S2). We then conducted the compositional analysis of habitat use for the randomly selected neighborhoods described above to determine whether the communities in randomly selected neighborhoods were different from communities on the tile as a whole.

2.5.3. Benthic categories that larvae select for or avoid

Lastly, we evaluated selection for or against individual members of the tile community using Strauss' linear selection index (L): $L = r_i - p_i$; where r_i is the proportional cover of community member i within a neighborhood and p_i is the proportional cover of community member i on the tile (Strauss 1979). Positive values of L reflect selection for a given community member relative to its abundance, whereas neg-

ative values of L reflect avoidance of a given community member. Tiles that had 0% cover of a given community member were omitted from the data analysis for that community member because it is impossible to select for or against a community member that is not present. We calculated L for each community member within each neighborhood size and averaged selectivity indices by community member and neighborhood size, and calculated 95% confidence intervals.

3. RESULTS

3.1. Benthic community composition and categories that influence the likelihood of coral settlement

Of the 30 settlement tiles in this experiment, 23 tiles had at least one settler (Fig. 2a). We found that tiles with at least one coral settler had significantly different community composition compared to tiles that had no coral settlers (PERMANOVA, tile community composition ~ tiles with or without coral settlers, $F_{1,28} = 4.87$, $p = 0.01$, $R^2 = 0.15$). Multivariate group dispersions did not differ between tiles that had at least one settler and tiles that did not have settlers ($F_{1,28} = 3.35$, $p = 0.08$). On average, tiles with settlers had 22.3% more bare substrate, but 15.1% less thick red filamentous algae, 11.0% less CCA, and 4.8% less fine red filamentous algae compared to tiles with no settlers (Fig. 2b). Bare substrate, thick red filamentous algae, CCA, and fine red filamentous algae combined to account for 70.7% of the dissimilarity between tiles with at least one settler vs. tiles with no settlers (SIMPER; Table S3).

3.2. Community composition of neighborhoods around settlers vs. overall community composition of tiles

The compositional analysis of habitat use revealed that coral larvae selected neighborhoods that were significantly different from the community composition of the whole tile (i.e. the 'available' habitat) for neighborhoods of 1–6 mm radii (p -values given in Fig. 3b). When we conducted the same composition analysis of habitat use with neighborhoods that were selected by a random number generator (the control analysis), we did not detect significant differences from communities on the whole tile for any of the different sized neighborhoods (Fig. 3c). This result confirms that the significant habitat selection that we

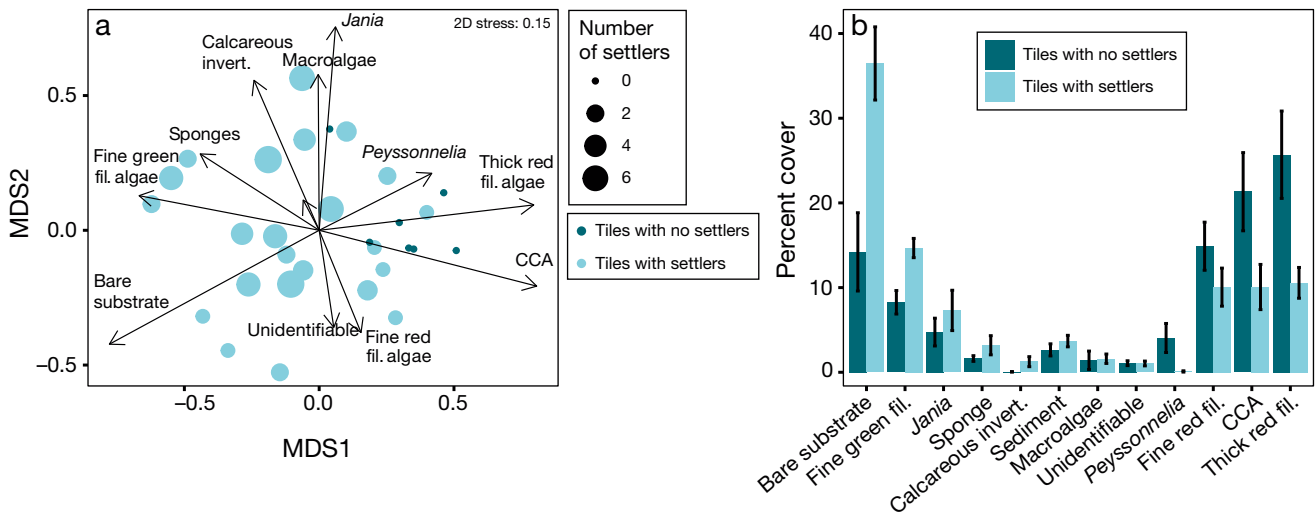


Fig. 2. (a) Two-dimensional nonmetric multidimensional scaling plot of settlement tile community composition. Points represent individual tile communities and are scaled to the number of coral larvae that settled on each tile. Points are colored to indicate whether there was at least one coral settler (light blue) or no coral settlers (dark teal) on each tile. Vectors correspond to the direction of increasing abundance of each benthic group. (b) Comparison of the mean (\pm SE) percent cover of each group on tiles with no settlers ($n = 7$) and tiles with settlers ($n = 23$). CCA: crustose coralline algae; fil: filamentous (algae)

observed was not driven by randomness associated with sampling small areas of the tiles.

3.3. Benthic categories that larvae select for or avoid

When we compared the abundance of individual benthic categories within each neighborhood to its abundance on the tile, we found that larvae exhibited strong preferences for or against individual com-

munity members and that these preferences often changed across neighborhood sizes (Fig. 4). Strauss' L showed that, generally, larvae exhibited stronger preferences against individual community members at smaller radii. For small neighborhoods, larvae strongly avoided thick red filamentous algae (1 mm radii), sediment (1–2 mm radii), and sponges (1–2, 4–5 mm radii). They also avoided fine red filamentous algae across most neighborhood sizes (1, 4–6 mm radii). Larvae also showed a trend towards a

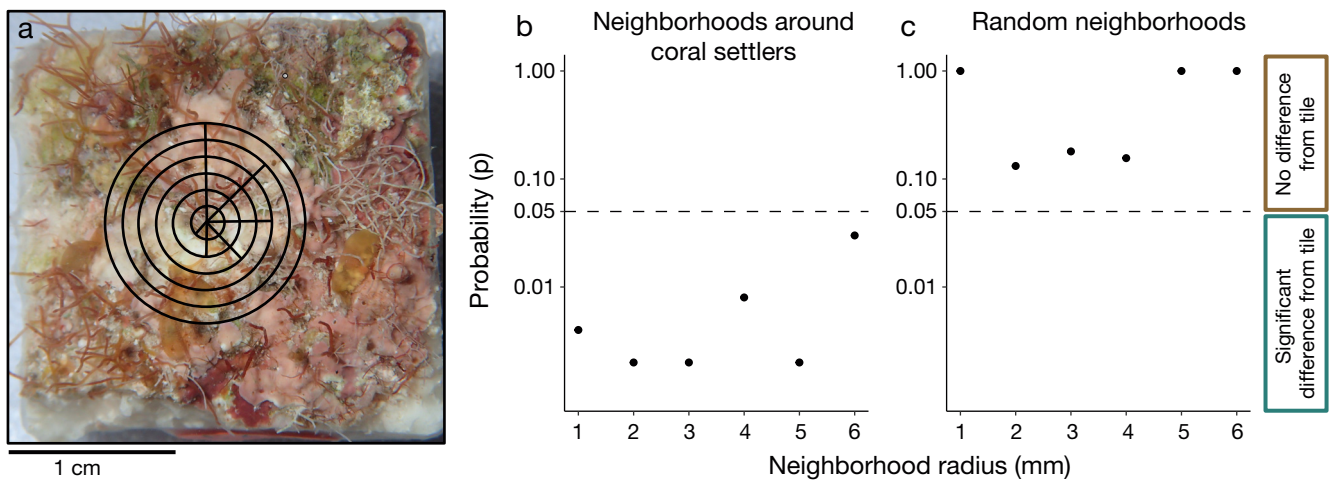


Fig. 3. (a) One of the settlement tiles, with overlaid concentric circles used to determine the community composition of neighborhoods around each coral settler. Compositional analysis of habitat use for neighborhoods (b) around coral settlers and (c) in randomly selected locations on the tiles. For (b) and (c), p-values represent the results of testing difference between the community within each neighborhood radius compared to the community on the tile as a whole; $p < 0.05$ suggests that neighborhoods within that radius differed from the community on the whole tile

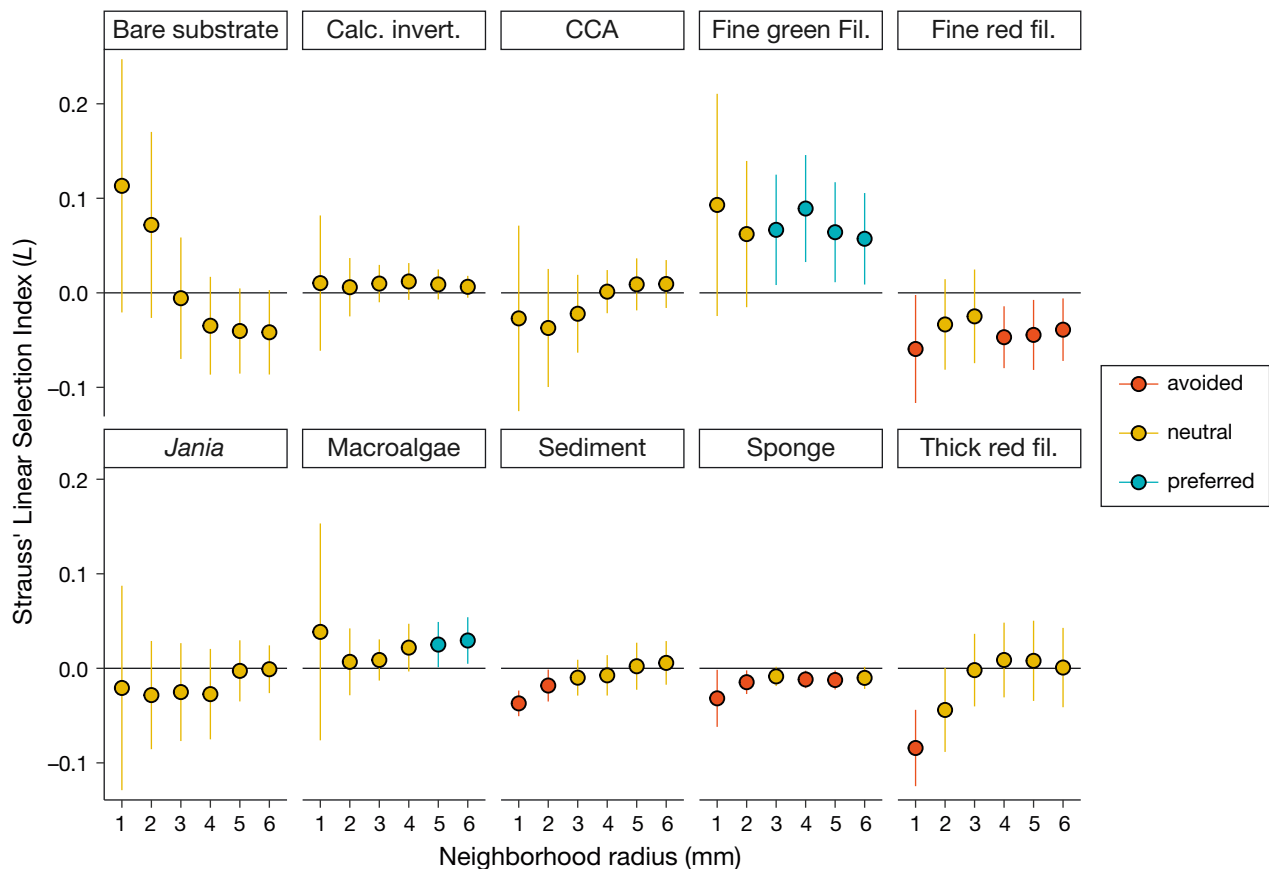


Fig. 4. Mean values of Strauss' linear selection index \pm 95% CI, showing preference or avoidance of taxa across neighborhoods of different radii. Teal dots: taxa that were present in coral settler neighborhoods in significantly higher abundance than on the tile as a whole, suggesting preference for these taxa; red dots: taxa that were present in coral settler neighborhoods in significantly lower abundance than on the tile as a whole, suggesting avoidance of these taxa; yellow dots: taxa that were present in coral settler neighborhoods in abundance that was proportional to the tile as a whole, suggesting no selection for or against these taxa. CCA: crustose coralline algae; fil: filamentous algae

preference for bare space within 1–2 mm radii neighborhoods, but this effect was not significant. Larvae preferred fine green filamentous algae across most larger neighborhoods (3–6 mm radii) with some preference also for macroalgae at larger neighborhoods (5–6 mm radii).

4. DISCUSSION

Here, we show that benthic communities strongly influence the complex decisions that coral larvae make when selecting a habitat on which to settle. At the scale of the whole tile, community composition significantly impacted the willingness of *Orbicella faveolata* larvae to settle. Red filamentous algae and CCA were abundant on tiles with no settlers, while bare substrate was abundant on tiles with settlers, suggesting that larvae were choosing for abundant

free space at the scale of the tile, which is consistent with the results of field-based recruitment studies (Arnold & Steneck 2011). Within tiles, coral settlers avoided regions with benthic categories that pose risks to newly settled corals (i.e. sediment, sponges, and red filamentous algae) while preferring areas with green filamentous algae, which is likely benign to newly-settled corals. Selection among individual benthic categories was strongest for the smallest neighborhoods (1–2 mm radii) and was dominated by avoidance rather than attraction to individual benthic categories. Ultimately, our results show that coral larvae make complex decisions at the millimeter scale about where to settle and how to navigate interactions with other benthic organisms.

Our study examined habitat selection in an experimental setting across relatively small spatial scales (mm to cm). However, environmental conditions and decisions that larvae make at broader spatial scales

before larvae reach the benthos also influence how coral larvae select habitats in nature. For example, currents dictate the large-scale transport of coral larvae, and other conditions such as sound, light, temperature, salinity, and chemical cues can dictate when larvae swim downward in the water column and toward the reef (Mundy & Babcock 1998, Vermeij 2006, Gleason et al. 2009, Vermeij et al. 2010). These environmental conditions can influence how far larvae travel, which reefs they encounter, and the habitats available to choose from when making settlement decisions. For example, water flow often influences coral settlement patterns, with higher flows often inhibiting coral settlement and lower flows facilitating settlement (Reidenbach et al. 2009, Levenstein et al. 2022). Like many experiments on coral settlement (e.g. Harrington et al. 2004, Ritson-Williams et al. 2014, 2016) we used experimental arenas with low flow that allowed us to study the complexities of larval settlement behavior in a laboratory setting. Furthermore, we tested the impact of benthic communities on larval settlement over small scales near the benthos where flow is often reduced (Reidenbach et al. 2009, Levenstein et al. 2022). It is also possible that lower flow resulted in higher concentrations of chemical cues in our experiment relative to what larvae experience on the reef. However, we acknowledge that the flow regimes that are necessary to conduct these small-scale experiments are artificial, though common across the field (e.g. Harrington et al. 2004, Ritson-Williams et al. 2014, 2016). Additionally, several studies have shown that coral larvae prefer to settle in pits and grooves of structurally complex substrate (Edmunds et al. 2014, Doropoulos et al. 2016, 2017, Evensen et al. 2021), but our study used flat limestone tiles so that we could more explicitly test the impact of benthic community composition and remove any confounding factors of structural complexity. We acknowledge that our laboratory experiment reduced much of the complexity that coral larvae encounter in nature, and therefore we cannot address what interactions may occur among these different factors that influence the settlement of coral larvae. However, by conducting our experiment in a seawater system, we controlled for water conditions and simplified the choices available to larvae, which likely improved our ability to detect patterns in the complex decisions that coral larvae make in navigating settling within benthic communities. Lastly, we used 900 coral larvae in our experiment, of which 74 chose to settle on our tiles. Like many settlement experiments, logistical constraints limited the number of larvae and the

scale of the experiment that we could conduct. We acknowledge that the results that we report reflect the settlement choices by a limited number of larvae and may or may not reflect the behavior of an entire species.

The abundance of benthic categories on our settlement tiles influenced coral settlement at both the scale of whole tiles and within tiles. At the scale of whole settlement tiles, larvae preferred tiles with a high abundance of bare substrate (i.e. devoid of macroscopic organisms), but a low abundance of CCA, thick red filamentous algae, and fine red filamentous algae. At smaller scales within tiles (neighborhoods of 1–6 mm radii around a settler), coral larvae preferentially settled near fine green filamentous algae but avoided thick red filamentous algae, fine red filamentous algae, sediment, and sponges. The amount of free space that is available for settlement can strongly shape settlement patterns for many sessile invertebrates (Gaines & Roughgarden 1985). Bare space is likely an ‘enemy-free’ resource to newly settled corals given the lack of macroscopic organisms, and bare space may host bacterial and microalgal biofilms that facilitate coral settlement and metamorphosis (Webster et al. 2004). In contrast, filamentous algae and sponges likely pose risks as competitors (Arnold & Steneck 2011), and sediment puts larvae at risk of being smothered and may make attachment to substrate difficult.

In the largest neighborhoods (5–6 mm) within tiles, there was also significant positive selection for fleshy macroalgae. This somewhat paradoxical positive selection likely reflects trade-offs rather than a real preference for macroalgae. Past studies have shown that larvae avoid many species of fleshy macroalgae and that macroalgae can increase post-settlement mortality of coral recruits (Kuffner et al. 2006, Diaz-Pulido et al. 2010, Paul et al. 2011). We intentionally selected tiles with minimal amounts of fleshy macroalgae because of the well-established negative effects of macroalgae on coral settlement. The most likely explanation for the positive selection that we observed is that larvae prioritized avoiding settling close to certain benthic categories (sponges, sediment, red filamentous algae), resulting in a trade-off of settling at an intermediate distance from fleshy macroalgae. As larvae balance avoiding the least preferred benthic categories and selecting for the most preferred benthic categories, their choices may result in selecting locations near some groups of intermediate preference. Our results add to the evidence that coral larvae make trade-offs when selecting habitats (Doropoulos et al. 2016). For example,

many species of coral larvae preferentially settle on *Titanoderma prototypum*, a species of CCA that is commonly found on the undersides of surfaces, including the undersides of settlement tiles. These cryptic habitats have less light availability compared to exposed habitats, but these locations also have less intense competition with neighboring organisms and less predation by fishes. By settling on *T. prototypum* in these cryptic habitats, coral larvae may maximize their chances of post-settlement survival by making trade-offs between light availability, competition, and predation. Similarly, others have shown that larvae preferentially settle in topographic refuges (pits and grooves) that provide protection from predators but also higher levels of competition with other organisms such as macroalgae (Doropoulos et al. 2016).

Our work advances our understanding of how coral larvae discriminate among communities of organisms that positively and negatively influence the settlement of larvae. A large body of work has shown that some species of CCA facilitate coral settlement and increase post-settlement survival (Harrington et al. 2004, Ritson-Williams et al. 2010, Price 2010), whereas other species repel coral larvae and compete with newly settled recruits (Babcock & Mundy 1996, Harrington et al. 2004, Ritson-Williams et al. 2014). Our data at the scale of whole tiles show that tiles with at least one settler had less CCA compared to tiles with no settlers. However, it is not possible to disentangle possible avoidance of CCA with possible selection for fine green filamentous algae or bare space, which were inversely correlated with CCA in our ordination. Yet given that coral larvae appeared to avoid CCA at the scale of tiles, the taxa of CCA present were likely those that inhibit coral settlement and pose risks to newly settled corals as neighboring competitors. Furthermore, the fact that coral larvae did not select for CCA at smaller scales suggests that the taxa of CCA present were not those that may facilitate settlement. One limitation of our study is that it was not possible to identify different taxa of CCA because the CCA on our tiles were small and lacked the reproductive structures necessary for their identification. Coral larvae exhibit species-specific preferences for and against different species of CCA (Harrington et al. 2005, Ritson-Williams et al. 2014), and had other species of CCAs been present, we may have seen different preferences by larvae.

In a previous study on coral settlement in this system, we showed that filamentous algal turf did not impede coral settlement at the scale of whole tiles (Speare et al. 2019). Here, however, we show that the

effects of filamentous turf algae on settling coral larvae depend on the spatial scale and characteristics of the algal turfs. Coral larvae avoided thick red filamentous algae at the tile scale and the neighborhood scale, but they only avoided fine red filamentous algae at smaller scales. Larvae may strongly avoid thick red filamentous algae (with thallus diameter >200 μm) because these algae pose a physical barrier to settling coral larvae (Birrell et al. 2005), or if these turfs included species that exude allelopathic chemical defenses (e.g. Kuffner et al. 2006) that deter settling larvae. However, the taxonomy of filamentous algal turf is extremely difficult because turfs are often multi-species assemblages of morphologically similar species (e.g. Connell et al. 2014). It is also noteworthy that the abundance of thick red filamentous turfs and CCA were positively correlated at the tile scale, so the apparent avoidance of tiles with abundant thick red filamentous turfs could be influenced by larvae avoiding CCA, thick red filamentous algae, or both. By contrast, larvae preferentially settled near green filamentous turfs, suggesting that these turfs may be favorable habitat for newly settled corals. Our results show that the effects of algal turfs on coral settlement vary with different types of turfs and are consistent with past literature showing that different types of algal turfs may have different effects on coral settlement (Birrell et al. 2005, Arnold et al. 2010, O'Brien & Scheibling 2018). It is also important to note that our results and the preferences of larvae for and against individual taxa may be influenced by bacterial biofilms associated with the tiles or individual taxa (Morse et al. 1988). Although it was not the aim of our study to investigate mechanisms by which individual taxa are preferred or avoided by larvae, it is possible that the bacterial biofilms associated with these taxa played a role in larval preference or avoidance.

Understanding how the settlement of benthic marine invertebrates is influenced by the environment and behavior of larvae has been a goal of marine ecologists for decades (Thorson 1964, Meadows & Campbell 1972, Connell 1985, Rodriguez et al. 1993, Hadfield & Paul 2001). Scientists have identified some of the major biotic and abiotic drivers of habitat selection for many species of marine invertebrates (Pawlik 1992, Hadfield & Paul 2001, Ritson-Williams et al. 2009, Gleason & Hofmann 2011), yet there is still a limited understanding of the relative importance of habitat selection versus post-settlement processes in driving the settlement patterns that are observed in the field, in part because studying small, mobile larvae that have high rates of post-settlement

mortality is logistically challenging. Settlement plates deployed in the field are commonly used to estimate rates of settlement of sessile marine invertebrates on timescales of weeks to months. However, it is difficult to use settlement plates to make inferences about habitat selection because the observed settler abundance reflects both the choices made by larvae and their early post-settlement mortality (Hunt & Scheibling 1997). For example, important work on well-studied taxa such as corals and barnacles suggests that habitat selection can drive patterns of abundance of sessile invertebrates (Thiyagarajan et al. 2006, Arnold & Steneck 2011). Yet it is difficult to disentangle habitat selection from the supply of larvae and post-settlement mortality in these studies without also quantifying settler abundance immediately after settlement or quantifying post-settlement mortality on short timescales, which is logistically difficult (but see Jenkins 2005). In the future, using mechanistic lab experiments to assess habitat selection immediately after settlement (e.g. Vermeij & Sandin 2008, Doropoulos et al. 2016, Evensen et al. 2021, and as we have done in this study) combined with field-based assessment of settlement patterns would be a useful tool for evaluating the role of habitat selection in driving patterns of settlement. Additionally, future studies that condition settlement substrate in multiple locations (i.e. different reefs) or microhabitats would be an interesting next step for experiments evaluating small-scale choices by coral larvae within ecologically meaningful benthic communities.

Our work shows how coral larvae select settlement locations among communities of benthic spaceholders and how larvae can discriminate among habitat characteristics at the scale of millimeters to centimeters. At the scale of whole settlement tiles, *O. faveolata* larvae were more likely to settle on tiles with more bare space. This is consistent with the hypothesis that as benthic organisms colonize free space, the recruitment window for corals closes and recruitment becomes less likely (Arnold & Steneck 2011). The tiles in our experiment were conditioned on the reef for 2 yr, which is late in the timeline of succession of benthic organisms according to results from Arnold & Steneck (2011). An interesting next avenue for research would be to condition substrates for different lengths of time to compare fine-scale settlement choices in different types of communities. In our experiment, conditioning tiles for multiple years resulted in benthic communities that are likely reflective of the communities that larvae encounter in Florida and the Caribbean as they are searching

for a place to settle. If the habitats available to settling larvae on modern reefs host similar late-successional communities that larvae avoid, avoidance of these habitats or competition by the community may contribute to the scarcity of *O. faveolata* recruits on reefs in Florida and the Caribbean.

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