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Soluble settlement cue in slowly moving water within coral reefs induces larval adhesion to surfaces

M.A.R. Koehl[a,*], M.G. Hadfield[b]

[a] *Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA*
[b] *Kewalo Marine Laboratory, University of Hawaii, 41 Ahui Street, Honolulu, HI 96813, USA*

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Abstract

Larvae of many benthic marine animals are induced to settle and metamorphose by dissolved chemical cues released by organisms on the substratum. Can dissolved cues released into the turbulent, wave-dominated flow typical of many shallow coastal areas affect the adhesion of settling larvae to benthic surfaces? We addressed this question using larvae of the nudibranch, *Phestilla sibogae*, which settle and metamorphose in response to a water-borne, species-specific metabolite of their prey, *Porites compressa*, abundant corals forming reefs in shallow habitats in Hawaii. Field measurements of water velocities showed oscillatory wave-driven flow above reefs with peak instantaneous velocities of 0.10–0.40 m/s, much slower back-and-forth water movement through the spaces within reefs with peak velocities of 0.02–0.04 m/s, and net shoreward transport of water through reefs of ~ 0.01 m/s. We used a water channel in the laboratory to measure the wall shear stresses required to dislodge larvae of *P. sibogae* from various surfaces. We found that cue from *P. compressa* is necessary for the larvae to attach to surfaces and, if cue is dissolved in water bathing the larvae, they can adhere to surfaces other than living *P. compressa*. After 2 h of exposure to cue and a surface, the adhesive strength of the larvae reached its peak value and did not change during the next 20 h. The mean nominal wall shear stress required to dislodge larvae of *P. sibogae* attached to *P. compressa* tissue (1.59±0.64 Pa, n=10 experiments) was not significantly different from that necessary to wash them off coralline algae (2.53±2.45 Pa, n=8) encrusting coral skeleton collected within reefs, but they stuck more tightly to glass (4.26±1.04 Pa, n=13). It is likely that most *P. sibogae* larvae initially settle onto surfaces within reefs because (1) settlement cue released by *P. compressa* and sinking larvae of *P. sibogae* accumulate in the slowly moving water within a reef; (2) larvae exposed to cue are able to stick to surfaces such as coralline algae that are common within the reef; and (3) *P. sibogae* develop their full attachment strength slowly, and the magnitude and frequency of peak shear stresses due to turbulent sweeps that might wash larvae off surfaces are much lower within than at the top of the reef.

Keywords: Larvae; Adhesion; Coral reef; Recruitment; Chemical cue; Flow

1. Introduction

Many benthic animals produce planktonic larvae whose dispersal and recruitment to benthic sites can affect population dynamics and community structure (reviewed by Ólafsson et al., 1994; Palmer et al., 1996). The first step in larval recruitment is settlement

* Corresponding author. Tel.: +1-510-642-8103; fax: +1-510-643-6264.
E-mail address: cnidaria@socrates.berkeley.edu (M.A.R. Koehl).

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("settlement" is attachment of a larva to the substratum; "recruitment" is survival of a juvenile resulting from the metamorphosis of a settled larva until counted by an observer; Keough and Downes, 1982). Although both settlement and post-settlement events affect recruitment (e.g. Ólafsson et al., 1994), there are many cases in which temporal and spatial patterns in recruitment were produced by the transport of larvae by moving water and their settlement onto the substratum (e.g. reviewed by Roughgarden et al., 1991; Rothlisberg and Church, 1994).

1.1. Larval settlement in flowing water: contact and adhesion

Settlement involves the contact of a larva with the substratum and its anchoring to the substratum (e.g. Abelson, 1997; Crimaldi et al., 2002). The movement of water in the benthic boundary layer (the layer of water in which the velocity gradient between the substratum and free-stream ambient flow occurs) affects both the delivery of larvae to the substratum and the forces tending to dislodge them from it (reviewed by Nowell and Jumars, 1984; Butman, 1987; Eckman et al., 1990; Abelson, 1997). The turbulent flow in a benthic boundary layer mixes mass (water and the dissolved substances, particles and larvae it carries) and momentum between the free-stream flow and the bottom. The faster the flow, the higher the average shear stress in the water moving across the substratum, thus the greater the chance that particles and larvae on the bottom are rolled along or swept away. Turbulent eddies sometimes "sweep" through the thin viscous sublayer of laminar flow along the bottom and fluid near the substratum "bursts" up into the overlying flow. The faster the free-stream velocity, the more frequent these random burst-sweeps and the higher the instantaneous wall shear stresses when they occur (Eckman et al., 1990; Abelson, 1997; Crimaldi et al., 2002).

Although most of the studies of larval settlement in moving water have been done in unidirectional currents, many shallow coastal habitats are subjected to wave action, where water motion along the substratum is oscillatory. Boundary layers are thinner in waves, shear stresses along the bottom are higher, and waves are more effective at washing particles and larvae off the substratum than is unidirectional flow at the same free-stream velocity (Charters et al., 1973; Nowell and Jumars, 1984). Even though instantaneous velocities and turbulent mixing in waves can be high, net horizontal transport across a habitat in back-and-forth, wave-dominated flow is slow (e.g. Koehl and Powell, 1994).

Many benthic habitats are characterized by groups of organisms (e.g. corals, bivalved mollusks, macrophytes) that protrude above the substratum and provide habitats in which other species live. Such aggregations affect ambient flow and the transport of larvae. Structures protruding above the substratum can enhance turbulent mixing, thereby increasing the flux of larvae to the substratum (e.g. Eckman, 1990), but also reducing their settlement by raising the frequency of burst-sweeps along the bottom (Crimaldi et al., 2002). However, if the density of organisms in a group is high, skimming flow occurs in which most of the ambient water moves above rather than through the group (reviewed in Nowell and Jumars, 1984). For example, coral reefs retard the movement of water through the spaces within the reef (e.g. Oberdorfer and Buddeneier, 1986; Parnell, 1986; Black et al., 1990; Koehl and Dobbins, 1998). Although the effects of coral reefs on large-scale patterns of water flow and larval recruitment have been described (reviewed in Sammarco, 1994; Wolanski, 1994), the consequences of small-scale flow through a reef on larval settlement have received less attention.

Although initial contact of larvae with benthic surfaces is often due to physical processes, behavior of larvae after contact can affect where they settle (reviewed by Butman, 1987; Abelson, 1997). After initial contact, larvae of some species can explore the substratum on spatial scales of millimeters by crawling. Furthermore, larvae can drift passively along the substratum until they encounter a suitable location to stick, or can abandon unfavorable touchdown spots and move back up into the water column.

In summary, the position on a surface where a larva settles depends not only on its site of first contact (determined by water flow near the substratum), but also on whether the larva chooses to stick to the surface, and whether its adhesion is stronger than the hydrodynamic forces tending to wash it away. Although the effects of flow on the delivery of larvae to the substratum has received considerable attention, less is known about larval adhesion, which is the focus of this study.

1.2. Larval adhesion

Both the strength and speed of attachment to a surface affect where a larva can settle. To settle, a larva must anchor itself during the brief stress lulls between burst-sweeps (Crimaldi et al., 2002). Larvae that stick to surfaces upon contact (e.g. larvae of barnacles, bryozoans, some cnidarians) can settle on surfaces exposed to high water velocities (Yule and Walker, 1987; Eckman et al., 1990; Mullineaux and Butman, 1991; Abelson, 1994, 1997), whereas larvae that develop their adhesion to surfaces more slowly are able to settle only in spots where peak local shear stresses during burst-sweeps are too low to dislodge them (Abelson, 1997).

Despite the importance of adhesion to patterns and rates of larval settlement (reviewed in Abelson, 1997), the process of larval adhesion has rarely been quantified. Two approaches have been used to measure adhesive strength: (1) direct measurement with a transducer of the force to push, pull or peel an organism off a surface (e.g. Yule and Walker, 1984; Edlund and Koehl, 1998); and (2) measurement of the water velocity or wall shear stress required to dislodge from a surface microscopic bodies such as algal spores (Charters et al., 1971; Callow et al., 2001), tissue culture cells (Benoliel et al., 1994) or larvae (Eckman et al., 1990; Ackerman et al., 1995; Orlov, 1996).

1.3. Role of dissolved chemical cues in larval settlement and metamorphosis

Larvae of many types of benthic marine animals are induced to settle and metamorphose by chemical cues of organisms such as conspecifics, prey or biofilms on the substratum (reviewed by Hadfield and Paul, 2001). Although older studies emphasized the importance of surface-adsorbed inducers, recent research has also shown that chemical cues dissolved in the water column can induce settlement and metamorphosis for many species. Most investigations of larval responses to dissolved chemical cues were conducted in still water and focused on metamorphosis induction, although a flume study showed that larvae in unidirectional flow moved downwards in response to dissolved inducer (Tamburri et al., 1996). Little is known about effects of dissolved cues on the development of adhesive strength by larvae.

We used larvae of the nudibranch *Phestilla sibogae* to investigate the effect of a water-borne settlement cue on the ability of larvae to stick to surfaces. *P. sibogae* can be reared in the laboratory, their metamorphic process is well-described, and their competent larvae (i.e., larvae that are developmentally capable of metamorphosis) settle and metamorphose in response to a water-borne species-specific metabolite of their prey, *Porites compressa*, an abundant coral that forms reefs in shallow, wave-dominated habitats in Hawaii (Hadfield, 1977).

2. Methods

2.1. Water velocity measurements above and within coral reefs

Water velocities were measured above and within *P. compressa* reefs at two sites in Kaneohe Bay on the island of Oahu, HI (N 21°27′ , W 157°47′), during July 2000. The physical oceanography of Kaneohe Bay was described by Bathen (1968). Large ocean swells break at the mouth of the bay, so small waves drive water movement across the many patch reefs (composed mainly of *P. compressa*) in the Bay. Our water flow measurements at each site were made in the middle of a reef, halfway between its seaward and shoreward edges. Measurements were made midday, 1–2 h before high tide, when water depths above the reefs were 0.7–1.0 m, and when mean wind velocities were 4.6–5.3 m/s (weather station in Kaneohe Bay of the Hawaiian Institute of Marine Biology, University of Hawaii). Water flow above the reef was measured by a Marsh-McBirney (Frederick, MD) Model 511 electromagnetic water velocity meter (spherical probe diameter=3.81 cm, diameter of area sampled ~11.4 cm). Simultaneous measurements of flow at the surface of the reef or in the spaces within the reef were made using a Marsh-McBirney Model 523 electromagnetic water velocity meter, which has a smaller probe (spherical probe diameter=1.27 cm, diameter of area sampled ~3.8 cm). Each probe was held in position by a stiff aluminum rod (diameter=1.3 cm) supported by a scaffolding positioned so that it did not interfere with the water flow measured by the probe. We oriented the probes to measure horizontal velocities in the seaward-shoreward direction, parallel to the axis of the fastest

flow in the wave-driven water movement characteristic of these field sites. The 511 probe was positioned 27 cm above the top surface of the reef, and the 523 probe was positioned 5 cm above the reef, at the tips of the coral branches at the surface of the reef, or in spaces within the reef that were 5–25 cm below the reef surface (reef geometry determined the probe positions possible at each site). Probe positions relative to the reef surface were measured to the nearest centimeter using a tape measure glued to an aluminum rod.

Cables from the probes were run to a boat anchored nearby, where the velocity data were recorded by a Texas Instruments (Dallas, TX) TM 5000 laptop computer. Analog to digital data acquisition was accomplished using a DAQ-Card 1200 controlled by Labview 5 software (National Instruments, Austin, TX). The time constant for the Marsh-McBirney meters was 0.2 s and the data acquisition rate was 60 Hz. The power spectral density of each velocity record was calculated by Welch's method (Welch, 1967; Rabiner and Gold, 1975) using Matlab Signal Processing Toolbox 4.3 software.

General patterns of water flow around and through each reef were visualized by releasing fluorescein-labelled seawater from syringes at various positions near and within the reef.

2.2. Preparation of test solutions

In the experiments on adhesion and metamorphosis described below, we examined the effects on larval adhesion of the following solutions: (1) "filtered seawater", seawater from the Kewalo Marine Laboratory's continuously flowing system passed through a 0.45 μm Millipore filter; (2) "*P. compressa* cue", a standardized strong settlement inducer produced by placing healthy branches of *P. compressa* densely in beakers of filtered seawater, aerating them overnight at room temperature (23–25 °C), removing the coral, and decanting the water through a Whatman #50 paper filter (details given in Hadfield and Scheuer, 1985; Hadfield and Pennington, 1990); and (3) "30% cue", a 30% solution of *P. compressa* cue in filtered seawater that yields metamorphosis rates (see Section 2.4 below) similar to those produced by water collected from the spaces within *P. compressa* reefs at our field sites (Hadfield and Scheuer, 1985; Koehl and Hadfield, unpublished data).

2.3. Culture of larvae for assays of adhesion and metamorphosis

Juvenile and adult *P. sibogae* were maintained at the Kewalo Marine Laboratory in shallow water tables supplied with flowing seawater. These slugs were provided with small heads of living *P. compressa*, their prey, which were collected bi-monthly from the field. Adult *P. sibogae* deposited egg masses on the coral. Egg masses were collected on the day they were laid and maintained in aerated beakers of filtered seawater kept at 25 °C in an incubator until they hatched 6–7 days after deposition. Newly hatched larvae were then transferred into small baskets kept partially submerged in glass beakers (600 ml) that were also maintained at 25 °C in the incubator. Air-lifts circulated the water through the baskets. Details about these methods for culturing the larvae of *P. sibogae* are provided in Miller and Hadfield (1986). In this study, each beaker contained the larvae of ten different egg masses, so a single "batch" of larvae was composed of a variety of genotypes.

Larvae used for measurements of adhesion and assays of metamorphic induction were 11–12 days post-fertilization (4–5 days post-hatching) and old enough to be competent (i.e., developmentally capable of metamorphosis). Actively swimming larvae were selected from these cultures for metamorphosis assays and adhesion experiments. A culture beaker was observed using a dissecting microscope. Larvae swimming in the water near the top of the basket were gently captured using a Pasteur pipette and transferred into to a small (20-ml) beaker of filtered seawater. These larvae were then gently pipetted into culture wells for metamorphosis assays or into petri dishes for adhesion experiments, as described below.

Larvae used in some of the experiments were labeled with a vital stain, methylene blue, so that they would be more visible on test surfaces in the water channel (see Section 2.6 below). Swimming larvae captured from a culture beaker were transferred into a dish (100-ml) containing a solution of 0.0003% methylene blue in filtered seawater. After incubation in the stain solution for 75 min, actively swimming larvae were selected from the dish, as described above, for metamorphosis or adhesion assays.

2.4. Assays of induction of metamorphosis

Larval metamorphosis was used as a bioassay of the strength of the *P. compressa* cue used in each adhesion experiment, and as an indicator of the responsiveness of animals from each batch of larvae. Metamorphosis assays (details given in Hadfield and Pennington, 1990; Hadfield and Scheuer, 1985; Pennington and Hadfield, 1989) were conducted in 30-well microtiter plates. Larvae were added to 2 ml of test solution (filtered seawater or *P. compressa* cue) in each well (mean number of larvae per well=23±14, n=80 wells). For each batch of larvae, four replicates were conducted for each solution using unstained larvae. In addition, on days when adhesion experiments were conducted using stained larvae, four additional replicates per solution were done using stained larvae. The percentage of the larvae that had undergone metamorphosis after 24 h was recorded for each well.

2.5. Preparation of surfaces used in adhesion experiments

The adhesive strength of larvae to three types of surfaces was measured: (1) smooth glass, (2) living *P. compressa* and (3) coralline algae growing on *P. compressa* skeleton. New glass microscope slides were washed in distilled water. Some slides were used as the glass surfaces in adhesion experiments, while others were used to fabricate supports for the *P. compressa* or coralline algal surfaces. Such a support was two slides thick with a rectangular well 3 cm long cut out of the middle of the top slide. The slides were held together by Devcon's (Danvers, MA) High Strength 5-Minute Epoxy.

A small hacksaw was used to cut thin (~1-mm-thick) wafers (~2 cm wide, ~3 cm long) of skeleton and living coral tissue from flat surfaces of *P. compressa* branches. Similar wafers of skeleton were cut from flat surfaces of dead *P. compressa* branches collected from within the coral reef. These branches were overgrown by a variety of organisms; the most common of which were crustose coralline algae. Wafers were cut from regions of the dead coral branches that were covered predominantly by coralline algae. Each wafer of skeleton, bearing either living *P. compressa* polyps or coralline algae, was glued with 5-min epoxy into the well of a support so that the upper surface of the wafer was flush with the upper surface of the glass support. Gaps in the wells were then filled with 5-min epoxy to yield a flat, continuous surface between the glass support and the coral or algal tissue. Although the surfaces of the support and the epoxy were fabricated to be smooth, the natural surface textures of the *P. compressa* tissue and coralline algae were not altered.

After the wafers of living *P. compressa* or coralline algae were mounted in the supports, they were placed in water tables supplied with flowing seawater and left for 3–4 days. Only those wafers with clean, pigmented living tissue after this period were used in adhesion experiments.

2.6. Adhesion experiments in a water channel

The wall shear stress necessary to dislodge larvae of *P. sibogae* attached to test surfaces was measured in an enclosed water channel in which fully developed turbulent flow was produced (the design and dimensions of this apparatus are described in detail by Schultz et al., 2000). Our microscope slides or supports were mounted flush with the floor of the channel and exposed to wall shear stresses that depended on the volume flow rate of the seawater moving through the channel. The wall shear stress in the test section of the water channel was calculated from measurements of the pressure drop along the length of the channel, as described by Schultz et al. (2000). Calibration of this apparatus showed that the repeatability of the wall shear stresses produced in the test section of the channel was within 4% across the range of flow rates used (Schultz et al., 2000). Since the biological test surfaces had fine texture, and since newly settled larvae of *P. sibogae* (~200 μm tall) were greater in height than the viscous length scale in the test section of the channel (6–35 μm for the range of flow rates available; Schultz et al., 2000), the actual wall shear stresses encountered by larvae during our experiments may have been slightly higher than those determined for the smooth test section during calibration. Thus, we use the term "nominal wall shear stress" when reporting the results of our larval adhesion experiments, since it represents the stress determined during calibration of a smooth test

section rather than the actual stresses encountered by individual larvae.

Microscope slides or supports bearing experimental surfaces were submerged in test solutions in disposable petri dishes (150×50 mm, polystyrene) and kept at room temperature (23–25 °C). About 100 actively swimming competent larvae were gently pipetted into each petri dish. (Since large numbers of larvae were swimming around in big dishes, and since larvae settled onto the dishes and supports as well as the test surfaces, an accurate determination of the proportion of the larvae that settled onto each type of surface as a function of time was not feasible.) After a timed interval of larval exposure to the test solution and test surface, a slide or support was gently removed from the test solution, with attention given to keeping the slide level and maintaining a meniscus of water over the test surface, and mounted flush with the floor of the water channel, which had been filled with seawater at 24–26 °C. The flow rate in the channel was then adjusted to expose the test surfaces to the following sequence of nominal wall shear stresses: 0.2, 0.4, 0.6, 1.0, 1.7, 2.7, 3.8, 5.1, 6.5, 8.1, 9.8, 11.7, 13.7 and 15.8 Pa. A test surface was exposed to each stress for 1 min, and then the flow rate was increased to produce the next higher stress in the series. A "run" refers to the exposure of a single specimen of a test surface and the larvae on it to this series of nominal wall shear stresses in the water channel. The number of larvae in each run was the number of larvae on the test surface in the field of view of the microscope at the start of the experiment, before the flow was turned on in the water channel. Each specimen of a test surface was used only once. During each run, the larvae on the test surface were videotaped through the ceiling of the water channel by a SPI Minicam Model OS-70D (Southern Precision Instruments, San Antonio, TX) mounted on the ocular of a Wild dissecting microscope. These videotapes were then analyzed frame by frame so that the number of larvae dislodged at each nominal wall shear stress could be tallied, and the data were used to calculate the mean nominal wall shear stress required to dislodge larvae from the experimental surface after a timed exposure to a test solution (Section 2.2, above).

Statistical analyses were conducted using Statview 5.0 software. Means are presented ±1 standard deviation.

3. Results

3.1. Water flow above and within P. compressa reefs

The *P. compressa* reefs we studied in Kaneohe Bay were exposed to wave-driven water flow. Typical records of horizontal water velocity measured at various positions above and within a reef are shown in Fig. 1, examples of spectra are presented in Fig. 2, and

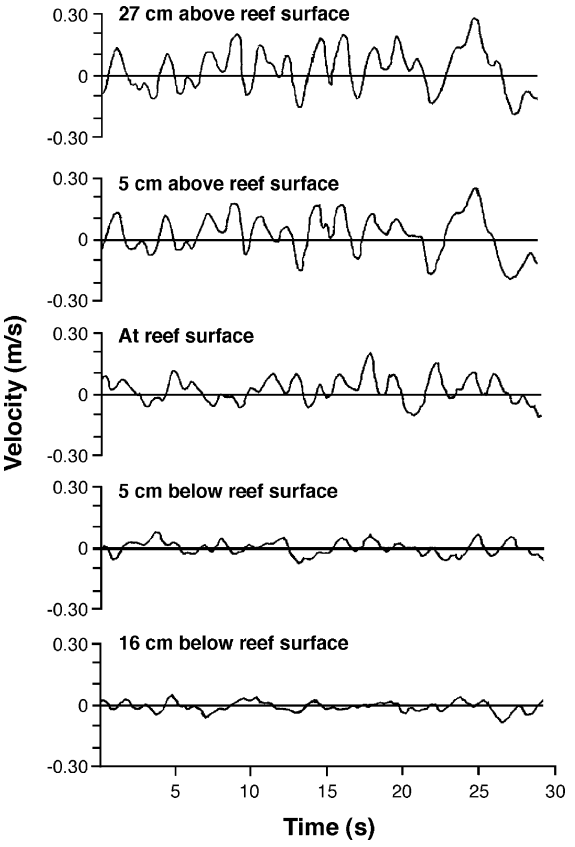

Fig. 1. Examples of water velocities typical of those we measured in the oscillatory wave-driven water flow above and within coral reefs (*P. compressa*) in Kaneohe Bay, HI (site 2; see Table 1). The horizontal component of water velocity in the shoreward (+) and seaward (−) directions is plotted as a function of time. The top graph shows velocities recorded at a position 27 cm above the top of the reef, while the second graph shows the slower velocities recorded simultaneously at a position 5 cm above the reef that was directly below the first position. The lower graphs show that flow at the reef surface and down in the spaces within in the reef (recorded at the same spot on the reef within 15 min of the time that the top two recordings were made) was also oscillatory, and that water movement within the reef was much slower than the flow above it.

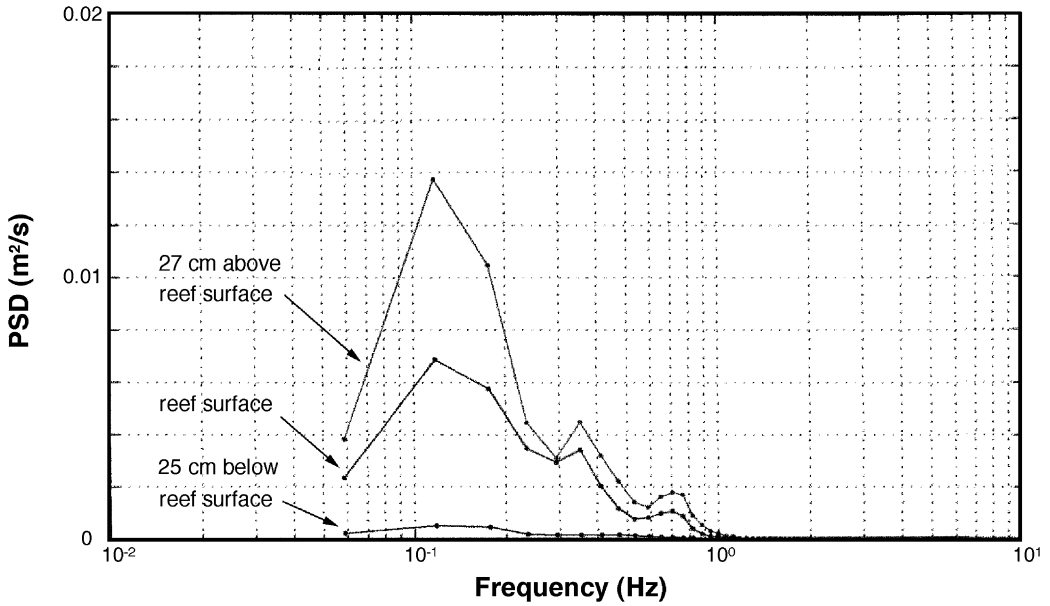

Fig. 2. Power spectral density (PSD) of some typical records of horizontal velocity measured as a function of time at various heights relative to the surface of a coral reef (*P. compressa*) in Kaneohe Bay, HI (site 1; see Table 1). The top line shows the spectrum for velocities recorded at a position 27 cm above the top of the reef, while the middle line shows the spectrum for velocities recorded simultaneously at a position at the tips of the coral branches at the surface of the reef that was directly below the first position. The bottom line shows the spectrum for velocities recorded within 5 min of the time that the other two recordings were made, but at a position within the reef directly beneath them that was 25 cm below the reef surface. Each spectrum was calculated using a velocity record of 10,000 points recorded at 60 Hz. Because the time constant for the low pass filter on the flow meter was 0.2 s, these spectra are attenuated at frequencies greater than 2.5 Hz and thus do not provide a measure of the small-scale turbulence. The dominant peak in each spectrum is due to the velocity fluctuations associated with the back-and-forth flow in the waves. The periods of the waves in this example were about 8–9 s.

the mean velocities measured within and above the reef at each site are listed in Table 1. The wave-driven flow was oscillatory, with periods (e.g. Fig. 2) of 5–10 s, depending on wind and surf conditions. Peak instantaneous shoreward velocities at 27 cm above the tops of the corals were generally 0.10–0.14 m/s (Table 1), with some waves peaking as high as 0.30 (e.g. Fig. 1) to 0.40 m/s. Peak seaward velocities of this back-and-forth water movement were slightly lower than the shoreward velocities, thus there was a net slow advection of water shoreward across the reefs at mean velocities of about 0.01–0.04 m/s (Table 1). The water moved back and forth more slowly close to the surface of the reef, with peak velocities typically only 0.08–0.11 m/s near the tips of the coral branches (Table 1). Water flow through the spaces within the reef was slower still (Fig. 1), with maximum instantaneous velocities of only 0.02–0.04 m/s and very sluggish net transport (Table 1).

Observations of the paths of dye released into the water at various positions around and within *P. compressa* reefs revealed that some water flowed into the seaward edge of a reef, while most was diverted over the top of the reef. The water that entered a reef moved slowly through the spaces within the complex reef structure; some of that water eventually flowed up and out of the middle of the reef. Our study of waves, turbulence, and water transport through and above reefs in Kaneohe Bay will be published elsewhere (Koehl, Cooper and Hadfield, unpublished data).

3.2. Metamorphosis of competent larvae of P. sibogae in response to P. compressa cue

Larvae in all the batches used in our adhesion experiments underwent metamorphosis in response to *P. compressa* cue, but not to filtered seawater

Table 1
Means of water velocities recorded above and within coral reefs in Kaneohe Bay, HI

Site, run[a]	Height above (+) or below (−) reef surface (cm)	Mean±S.D.[b] of velocity peaks shoreward (+) (m/s)	(*n*)[c]	Mean±S.D. of velocity peaks seaward (−) (m/s)	(*n*)[d]	Mean[e]±S.D. velocity (m/s)	(*n*)[f]
1, A	27	0.10±0.07	(74)	−0.11±0.07	(75)	0.00±0.09	(10,869)
1, B	27	0.10±0.06	(68)	−0.08±0.05	(69)	0.01±0.08	(10,913)
1, C	27	0.13±0.06	(48)	−0.07±0.04	(49)	0.03±0.08	(10,856)
1, D	27	0.11±0.07	(62)	−0.07±0.06	(62)	0.03±0.08	(10,755)
2, A	27	0.13±0.07	(61)	−0.10±0.05	(61)	0.02±0.09	(10,845)
2, B	27	0.13±0.06	(60)	−0.08±0.06	(60)	0.02±0.09	(10,813)
2, C	27	0.13±0.08	(65)	−0.09±0.06	(65)	0.03±0.09	(10,676)
2, E	27	0.11±0.08	(56)	−0.09±0.06	(55)	0.02±0.09	(10,884)
2, F	27	0.14±0.08	(50)	−0.09±0.05	(51)	0.04±0.09	(10,874)
1, C	5	0.08±0.05	(57)	−0.07±0.05	(57)	0.00±0.07	(10,856)
2, A	5	0.11±0.06	(55)	−0.10±0.06	(55)	0.01±0.09	(10,845)
1, D	0	0.07±0.04	(63)	−0.06±0.04	(63)	0.01±0.06	(10,755)
2, C	0	0.09±0.05	(56)	−0.09±0.05	(56)	0.00±0.07	(10,676)
1, A	−5	0.02±0.01	(70)	−0.02±0.01	(70)	0.00±0.02	(10,869)
2, D	−5	0.04±0.02	(74)	−0.03±0.02	(74)	0.01±0.03	(10,884)
2, E	−16	0.03±0.01	(57)	−0.04±0.02	(57)	−0.01±0.02	(10,874)
1, B	−25	0.02±0.01	(52)	−0.02±0.01	(52)	0.01±0.02	(10,913)

[a] A run is a record of flow velocity made simultaneously at two heights relative to the reef surface.
[b] S.D. is one standard deviation.
[c] Number of velocity peaks recorded in the shoreward direction (each velocity peak shoreward was the highest positive velocity recorded between two successive times when velocity=0).
[d] Number of velocity peaks recorded in the seaward direction (each velocity peak seaward was the largest negative velocity recorded between two successive times when velocity=0).
[e] Mean velocity is the net speed of horizontal transport of water above or through the reef.
[f] Total number of velocity data points, recorded at 60 Hz.

(Table 2). For each batch of larvae, a significantly greater percentage of the individuals bathed in cue underwent metamorphosis than did the larvae in filtered seawater (ANOVA, $p<0.05$). The mean of the mean metamorphosis rates of larvae in cue was 95.6±3.6%, $n=7$ batches of larvae, whereas that of larvae in filtered seawater was only 0.3±0.8%, $n=7$ batches of larvae.

Table 2
Percent of competent larvae of *P. sibogae* undergoing metamorphosis in filtered seawater (FSW) or *P. compressa* cue (Cue)

Batch of larvae	FSW, mean[a]±S.D.[b] (%)	FSW+ stain[c], mean[a]±S.D.[b] (%)	Cue, mean[a]±S.D.[b] (%)	Cue+ stain[c], mean[a]±S.D.[b] (%)
1	0±0		97±2	
2	2±3		91±3	
3	0±0		95±5	
4	0±0		92±4	
5	0±0	0±0	100±0	100±0
6	0±0	0±0	94±6	95±4
7	0±0	0±0	100±0	100±0

[a] Mean of four replicates; mean number of larvae per replicate=23±14, $n=80$ replicates.
[b] S.D. is one standard deviation.
[c] 0.0003% methylene blue.

Larvae stained with methylene blue responded to *P. compressa* cue and to filtered seawater in the same way as did unstained larvae (Table 2). On each of the three dates on which stained larvae were used, there was no significant difference between the percentages of stained and unstained larvae that metamorphosed in cue, and no significant difference between those of stained and unstained larvae in filtered seawater, while there were significant differences between percentages of larvae (both stained and unstained) that metamorphosed in cue and that metamorphosed in filtered seawater (ANOVA, Fisher's PLSD, $p<0.05$ for significance). The mean of the mean metamorphosis rates for unstained larvae in *P. compressa* cue was 98.1±2.8%, $n=3$ batches of larvae, and for stained

larvae in cue was 98.4±%2.9, n=3 batches. In contrast, no individuals, stained or unstained, from these three batches of larvae underwent metamorphosis in filtered seawater.

3.3. Adhesive strength of competent larvae of *P. sibogae* to various substrata

The dissolved settlement cue from *P. compressa* induced competent larvae of *P. sibogae* to adhere to glass and coralline algae, as well as to *P. compressa*. The larvae did not stick to glass or coralline algae when in filtered seawater. Thus, contact with *P. compressa* tissue was not required for larvae of *P. sibogae* to attach themselves to surfaces if dissolved cue from this species of coral was present in the water, as has been previously reported (e.g. Hadfield and Paul, 2001; Hadfield and Pennington, 1990; Hadfield and Scheuer, 1985).

Larvae had to be exposed to the cue from *P. compressa* and a test surface for at least 2 h to develop an attachment to the surface strong enough to be measured. Larvae incubated with a test surface and dissolved cue for shorter periods fell off the test surface during transfer into the water channel or washed off at flow rates lower than the one producing the lowest nominal wall shear stress that we tested (0.2 Pa).

Fig. 3 shows the mean nominal wall shear stress required to wash larvae of *P. sibogae* off glass, coralline algae or *P. compressa* tissue, plotted as a function of the duration of larval exposure to one of these surfaces plus dissolved cue from *P. compressa*. Once larvae attached to a surface, their adhesive strength did not change with time during the ensuing 20h (Kendall correlation, τ_K, of nominal wall shear stress to dislodge a larva and duration of attachment to a test surface in cue: glass, τ_K=0.242, p=0.27, n=12 runs; *P. compressa* tissue, τ_K=−0.333, p=0.18, n=10 runs; coralline algae, τ_K=0.429, p=0.14, n=8 runs).

The mean nominal wall shear stress required to dislodge larvae of *P. sibogae* attached to *P. compressa* tissue was 1.59±0.64 Pa, n=10 runs, to coralline algae was 2.53±2.45 Pa, n=8 runs, and to glass was 4.26±1.04 Pa, n=13 runs (Fig. 3). There was no significant difference between the nominal wall shear stresses required to remove larvae from the coral or

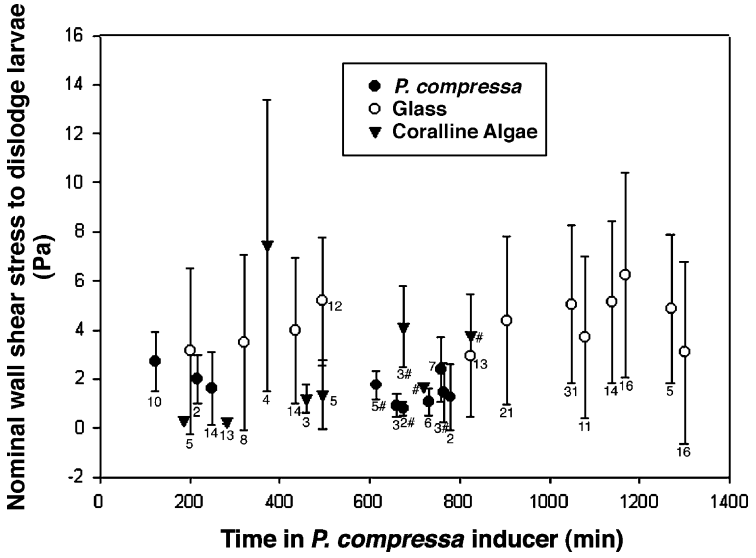

Fig. 3. Adhesive strength of the attachment of competent larvae of *P. sibogae* to various test surfaces: clean, smooth glass (open circles), living *P. compressa* (black circles) and coralline algae growing on *P. compressa* skeleton (black triangles). The nominal wall shear stress due to water flow along the surface to which the larvae were attached was used as the measure of adhesive strength. Then mean nominal wall shear stress required to dislodge larvae is plotted as a function of time since the larvae were first exposed to the surfaces and dissolved settlement cue from *P. compressa*. Error bars indicate one standard deviation, and the number next to each is the number of larvae monitored in that run. Larvae were exposed to 30% solutions of *P. compressa* cue, except in those runs indicated by #'s, during which larvae were exposed to 100% cue.

from the algal surfaces (p=0.18), whereas the strength of larval attachment to glass was significantly higher than to *P. compressa* (p=0.002) and to coralline algae (p=0.015) (ANOVA, Fisher's PLSD).

Larvae bathed in 30% cue (representative of cue strengths measured in field-collected water from within *P. compressa* reefs; see Section 2) stuck to natural surfaces just as tightly as those exposed to 100% *P. compressa* cue. The mean nominal wall shear stress necessary to remove larvae from *P. compressa* surfaces was 1.70±0.69 Pa, n=7 runs, if the larvae had been bathed in 30% cue, and was 1.32±0.50 Pa, n=3 runs, if the larvae had been bathed in 100% cue (Fig. 3). These stresses were not significantly different (ANOVA, p=0.42). Similarly, the mean nominal wall shear stress to dislodge larvae from coralline algae was 2.12±3.02 Pa, n=5 runs, for larvae that had been incubated in 30% cue and was 3.21±1.32 Pa, n=3, for larvae had been incubated in 100% cue. These stresses were not significantly different from each other (ANOVA, p=0.58).

Some of the specimens of coralline algal surfaces that we tested had fine cracks across or between the algal crusts. For a given nominal wall shear stress in the working section of the water channel, larvae sitting in such cracks might have experienced small-scale local water flow slower than that encountered by larvae sitting out on flat algal surfaces (e.g. Snelgrove et al., 1993; Abelson, 1997). Larvae wedged into cracks might also have been more difficult to dislodge than larvae sitting on flat surfaces (e.g. LeTourneux and Bourget, 1988). Since there were no significant differences between the mean attachment strengths of larvae in the runs done with coralline algal surfaces (ANOVA, Fisher's PLSD, p>0.05 in all cases), we pooled data for the runs in which some of the larvae were sitting in cracks so that we could assess the effect of cracks on how difficult it was to wash away larvae. Surprisingly, we found that there was no significant difference between the mean nominal wall shear stress in the water channel at which larvae in cracks were dislodged (4.9±5.15 Pa, n=7 larvae) from that at which larvae out on flat surfaces were washed away (2.3±2.2 Pa, n=10 larvae) (ANOVA, p=0.17). However, the variance in the nominal wall shear stresses at which larvae in cracks washed away was significantly greater than that for larvae on flat algal surfaces (F-test for homogeneity of variance, p=0.03).

4. Discussion

4.1. Dissolved cue induces larval adhesion to a variety of surfaces

We found that the larvae of *P. sibogae* adhere to surfaces other than living tissue of *P. compressa* if settlement cue from *P. compressa* is dissolved in the water bathing the larvae and the surface. Many of the surfaces within *P. compressa* reefs are overgrown by a variety of other organisms such as crustose coralline algae, to which *P. sibogae* adhere as tightly as they do to *P. compressa* tissue. Therefore, the larvae of *P. sibogae* in the field should not only be able to settle onto living *P. compressa*, but also onto non-*Porites* surfaces if they are surrounded by water containing settlement cue from *P. compressa.*

4.2. Retention of cue and larvae in the slowly moving water within coral reefs

Competent larvae of *P. sibogae* must be bathed in cue from *P. compressa* for at least 4–6 h for metamorphosis to be induced (Hadfield, 1977; del Carmen and Hadfield, unpublished data). The larvae of *P. sibogae* shed their swimming organ (the velum) about 10 h after exposure to cue begins, but the entire process of metamorphosis takes up to 24 h to complete (Bonar, 1974; Hadfield, 1977; del Carmen and Hadfield, unpublished data). Thus successful recruitment to a particular reef requires that larvae remain in cue for at least 4–6 h and within the reef for at least 24 h.

The settlement cue for *P. sibogae* accumulates in the slowly moving water within *P. compressa* reefs. Because the wave-driven water flow within the reefs in Kaneohe Bay is oscillatory, with low peak velocities, net transport of water through *P. compressa* reefs can be very slow (Table 1; Koehl, Cooper and Hadfield, unpublished data). The concentrations of substances released by reef organisms can build up in such slowly moving water (e.g. Atkinson and Atkinson, 1992). Cue concentrations in water collected within *P. compressa* reefs in the field are sufficient to induce competent larvae of *P. sibogae* to cease swimming and sink (Koehl et al., 2000; Koehl and Hadfield, unpublished data), and to

undergo metamorphosis (Hadfield and Scheuer, 1985).

Several lines of evidence suggest that competent larvae of *P. sibogae* are retained in the slowly moving water within a coral reef. We have shown that competent *P. sibogae* larvae immediately retract their ciliated velar lobes and sink when they encounter water containing the settlement cue from *P. compressa* (Hadfield and Koehl, 2001). As dissolved substances leaching from a *P. compressa* reef in wavy flow are mixed into the overlying water, fine filaments (mm in thickness) of the released substances swirl in the turbulent water above the reef (Reidenbach and Koseff, unpublished data). A computer simulation of the swimming and the cue-induced sinking of *P. sibogae* larvae as they encounter such distributions of filaments of cue while being transported by the turbulent, wave-driven flow above a reef, showed that sinking while in cue filaments enhanced the rate that the larvae entered into the structure of the reef (Strother et al., 2001). Once within the reef, bathed in cue-laden water, the larvae should continue to sink and be transported like passive particles. We found that larval mimics (particles that sink at the same velocity as *P. sibogae* larvae) released seaward of reefs in Kaneohe Bay remained in the water within the reefs long after the dyed water in which they were released had passed across the reefs (Koehl et al., 2000). The centimeter-scale topography within the reef is so complex that there are, no doubt, many local regions where larvae are likely to accumulate. For example, larvae may be entrained in recirculating eddies (e.g. Gallagher et al., 1983; Eckman, 1985; Mullineaux and Butman, 1990; Snelgrove et al., 1993; Abelson, 1997) that develop behind coral branches and in depressions, and larvae may be deposited in local areas of low shear stress on surfaces next to protruding bodies (e.g. Eckman, 1983; Eckman, 1990; Eckman et al., 1989; Wethy, 1986). Furthermore, asymmetric particles (like the larvae of *P. sibogae*) are less likely to roll away as bedload from the spot at which they are deposited than are symmetrical particles (Abelson, 1997). Thus, because coral reefs slow the flow rate of water through them, many of the larvae of *P. sibogae* that fall into the spaces within a reef are likely to be exposed to cue and surfaces long enough to adhere and undergo metamorphosis.

4.3. Adhesive strength of settling larvae of P. Sibogae

The nominal wall shear stresses required to wash the larvae of *P. sibogae* off surfaces after 2 h of exposure to settlement cue are comparable to those required to dislodge newly settled barnacle cyprids (0.2–8.7 Pa, calculated from boundary shear velocities reported by Eckman et al., 1990), and algal carpospores after 7–9 h of contact with the substratum ($\sim$2 Pa; Charters et al., 1973).

The larva of a *P. sibogae* must adhere to a surface in order to shed the larval shell during the process of metamorphosis (Hadfield, 1978). About 12 h after first exposure to cue from *P. compressa*, a larva positions the anterior-dorsal beak of its shell against the substratum and then contracts its retractor muscle so strongly that it pulls the muscle free from its attachment to the shell. The foot of the larva must be firmly attached to the substratum for this contraction to break the muscle's posterior connection to the shell, which is necessary for the emerging nudibranch to crawl from the shell (Hadfield, 1978). The maximum adhesive strength of a larva of *P. sibogae* takes about 2 h to develop after exposure to settlement cue begins, and then remains constant for at least the next 20 h. Thus, the larva is firmly attached to a surface long before it begins to shed its shell.

Not only does the time course of the development of adhesive strength by *P. sibogae* larvae affect whether they can successfully complete metamorphosis, but also where they can settle. A number of models have revealed the importance of the turbulent burst-sweep cycle to larval settlement in various types of habitats (Denny and Shibata, 1989; Eckman, 1990; Gross et al., 1992; Crimaldi et al., 2002). In turbulent water flow along the substratum, peak wall shear stresses during burst-sweeps can be more than an order of magnitude greater than the mean wall shear stress and can occur every few seconds (Eckman et al., 1990; Crimaldi et al., 2002). Therefore, to settle on a particular spot, a larva must be able to stick rapidly to the spot during the brief stress lulls between burst-sweeps, and its adhesive strength must be great enough to prevent dislodgement when the burst-sweeps occur over that spot (Crimaldi et al., 2002). Although it takes the larvae of *P. sibogae* several hours to develop their full adhesive strength, the larvae can probably stick to a surface upon contact (but the strength of that early attachment

is lower than we can measure in the water channel). The foot of a competent larva of a *P. sibogae* protrudes from its shell when the larva is induced by dissolved cue to sink, and the foot probably bears mucus (one of the attributes of metamorphic competence in the larvae of *P. sibogae* is development of the propodial mucus gland; Bonar (1974). Abelson (1997) review examples of other larvae that secrete mucus that enables them to adhere immediately to surfaces they contact. Nonetheless, the initial attachment strength of the veliger larvae of *P. sibogae* is clearly lower than that of the cyprid larvae of barnacles (Eckman et al., 1990) that are able to settle on surfaces exposed to rapid, turbulent water flow. Therefore, it is likely that settling larvae of *P. sibogae* can only remain in touchdown spots exposed to rare or weak burst-sweeps. However, because the larvae can crawl, they may move to surfaces exposed to more rapid flow once they have developed their full adhesive strength. Since metamorphosis takes ~24 h, juveniles may recruit to surfaces exposed to more rapid flow than those on which the larvae initially settled.

Our video records of the larvae of *P. sibogae* in the water channel revealed their behavior on the test surfaces when exposed to moving water. Some of the larvae actively crawled across the surfaces exposed to moving water, but there was no pattern in the orientations of their paths of locomotion relative to the flow direction. Typically, the larvae stopped crawling as the water flow was increased to produce nominal wall shear stresses approaching the magnitudes necessary to dislodge the larvae. The process of larval detachment was not instantaneous. Before each larva washed away, it separated from the substratum but was still anchored to the surface by a thread of material that looked like mucus. A larva dangling on a thread fluttered back and forth in the turbulent flow, and the amplitude of the fluttering increased as the thread stretched in length. When the thread broke, the larva washed away. This process of detachment and the appearance of the threads was similar for larvae on glass and on both types of natural surfaces we tested, suggesting that the thread material was secreted by the larvae rather than produced by other organisms on the surfaces.

We used a standard technique to assess the adhesive strength of larvae of *P. sibogae*: exposing them to steady-state unidirectional water flow that we could control in a laboratory water channel (e.g. Schultz et al., 2000). However, the wave-driven oscillatory flow across *P. compressa* reefs is unsteady. Therefore, the durations of high wall shear stresses that larvae on reef surfaces experience in nature are probably much shorter (lasting seconds or less) than in our water channel (each stress was applied for 1 min, while the whole sequence of stresses lasted ~15 min). Since larval dislodgement involves the stretching and rupture of an attachment thread (probably mucus), short pulses of a given wall shear stress may not detach a larva, whereas longer exposure to the same stress might wash the larva away. The time- and strain-dependent mechanical properties of the attachment thread need to be measured before we can assess whether larger stresses than those we measured are needed to dislodge larvae if the stresses are brief and intermittent.

Although we have not yet measured the timing or the magnitude of the maximum instantaneous wall shear stresses that occur during the turbulent burst-sweeps along surfaces at different locations in a complex coral reef in wave-driven flow, it is clear that the frequency and magnitude of pulses of high wall shear stresses are much greater at the tips of coral branches at the top of the reef than in the protected interstices of the reef. Therefore, it is more likely that *P. sibogae* larvae will be able to attach to surfaces within the reef than at the top of the reef. Since settlement cue released by *P. compressa* and sinking larvae of *P. sibogae* accumulate in the slowly moving water within a reef, and since larvae exposed to cue are able to stick to surfaces such as coralline algae that are common within the reef, the likelihood is great that most *P. sibogae* larvae initially settle onto surfaces within reefs. Unlike sessile animals such as barnacles and bryozoans that are glued to the substratum after metamorphosis, juvenile and adult *P. sibogae* can crawl across the substratum to find living tissue of *P. compressa* on which to feed.

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