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Rapid Behavioral Responses of an Invertebrate Larva to Dissolved Settlement Cue

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Abstract. Larvae of the nudibranch *Phestilla sibogae* were used to study whether a natural dissolved settlement cue (from their prey, *Porites compressa,* an abundant coral on Hawaiian reefs) induces behavioral responses that can affect larval transport to suitable settlement sites. As cue and larvae are mixed in the turbulent flow over a reef, cue is distributed in fine-scale filaments that the larva experiences as rapid (seconds) on/off encounters. To examine larval responses in this setting, individual larvae were tethered in a small flume with flow simulating water velocity relative to a freely swimming larva, and their responses to realistic temporal patterns of cue encounter were videotaped. Competent larvae quickly ceased swimming in cue filaments and resumed swimming after exiting filaments. The threshold cue concentration eliciting a response was 3%–17% of concentrations within heads of *P. compressa* in nature. When moving freely in filtered seawater, competent larvae swam along straight paths in all directions at ~0.2 cm s^{-1}, whereas in water conditioned by *P. compressa,* most ceased swimming and sank at ~0.1 cm s^{-1}. The ability of larvae to rapidly respond (by sinking) to brief encounters with dissolved settlement cues can enhance their rapid transport to the substratum, even in wave-driven turbulent flow.

Introduction

Successful larval settlement is critical to the long-term stability of benthic populations and communities. Planktonic larvae of benthic marine invertebrates are dispersed from their birth sites and depend on the vicissitudes of tides and currents to deliver them to suitable locations for recruitment. Once in or near such sites, successful settlement requires appropriate behavioral responses by larvae to reach or get very near the substratum and remain there long enough to complete metamorphosis. More than a half century of laboratory research has amply demonstrated that larvae from many different invertebrate phyla respond to chemical cues generated in the environment by settling in sites that are appropriate for successful metamorphosis, growth, and reproduction (reviewed by Hadfield, 1998; Hadfield and Paul, 2001).

Induction of settlement and metamorphosis by natural external chemical cues has been demonstrated for species as diverse as cnidarians (*e.g.,* Müller, 1973; Hofmann and Brand, 1987; Morse *et al.,* 1988; Morse and Morse, 1991; Leitz *et al.,* 1994; Fleck and Hofmann, 1995), polychaetes (Wilson, 1952; Jensen and Morse, 1990; Pawlik *et al.,* 1991; Hadfield *et al.,* 1994), sipunculans (Rice, 1988), bivalves (Grassle *et al.,* 1992), gastropods (Scheltema, 1961; Morse and Morse, 1984; Hadfield, 1984), barnacles (*e.g.,* Crisp and Meadows, 1962), crabs (Jensen, 1989; Brumbaugh and McConaugha, 1995), echinoids (Pearce and Scheibling, 1990a, 1991), phoronids (Herrmann, 1995), and ascidians (Young and Braithwaite, 1980). The search to understand the chemical nature of these cues has been a major component of research on invertebrate larval settlement (Hadfield and Paul, 2001), although much recent emphasis has been on signal-transduction mechanisms in the larvae (*e.g.,* papers in Clare and Jones, 1998).

Many earlier studies on invertebrate settlement focused on adsorbed, or surface-bound, cues. Crisp, a leader in such studies for more than 35 years, believed that larvae were too small to use dissolved cues to locate appropriate settlement sites (Crisp, 1974). However, studies on sea slugs (Hadfield and Scheuer, 1985; Hadfield and Pennington, 1990; Krug and Manzi, 1999), barnacles (Rittschof, 1985), blue crabs

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(Welch *et al.,* 1997), oysters (Zimmer-Faust and Tamburri, 1994), ascidians (Young, 1978), echinoids (Burke, 1984; Williamson *et al.,* 2000), and other taxa (reviewed in Hadfield and Paul, 2001) have demonstrated quite clearly that many larvae respond to dissolved chemical cues with decisive settlement from the water column, attachment to the substratum, and metamorphosis. Although less extensive, experimental evidence has also been presented to show that larvae of other opisthobranchs (Bahamondes-Rojas and Dherbonez, 1990; Lambert and Todd, 1994; Lambert *et al.,* 1997), prosobranchs (McGee and Targett, 1989; Boettcher and Targett, 1998), sipunculans (Rice, 1986), and other echinoids (Pearce and Scheibling, 1990b) are induced to settle and metamorphose by dissolved compounds arising from appropriate substrata, prey species, or conspecific individuals. With the exception of efforts by Turner *et al.* (1994), Tamburri *et al.* (1996), and Finelli and Wethey (2003), who studied responses of oyster larvae in unidirectional water flow in flumes, investigations of larval responses to dissolved inducers have been restricted to observations of the frequency of metamorphosis in stationary water.

Dispersion of dissolved settlement cues in marine habitats

A detailed understanding of the dispersion of dissolved substances from the substratum in ambient water flow is needed to determine how dissolved chemical cues affect larval settlement in nature. Many shallow coastal habitats are subjected to wave action, which renders water motion along the substratum oscillatory with high instantaneous velocities and accelerations (*e.g.,* Koehl, 1977; Denny, 1988). Even though instantaneous velocities are high, net advection of dissolved substances in back-and-forth, wave-dominated flow is slow, while turbulent mixing and shear dispersion can be high (Koehl and Powell, 1994). Canopies of attached organisms (*e.g.,* coral, kelp, sea grass) that provide a complex three-dimensional habitat in which many other species live, affect ambient flow and the dispersal of dissolved materials. For example, ambient water movement is retarded by coral reefs (*e.g.,* Black *et al.,* 1990), turbulent mixing in the water right above a reef is increased by the rough surfaces that coral and reef algae present to the flow (*e.g.,* Koehl *et al.,* 1997; Koehl and Dobbins, 1998), and some of the water hitting a reef flows through the porous reef framework rather than over the top of the reef (*e.g.,*Oberdorfer and Buddemeier, 1986; Parnell, 1986; Koehl and Hadfield, 2004). Chemical cues for larval settlement can be retained in water within reef canopies, as demonstrated by Hadfield and Scheuer (1985).

Advection-diffusion models are often used to describe the spatial patterns—on scales of centimeters to kilometers—of the concentration of dissolved substances released from the substratum and dispersed in coastal sites by currents, waves, and turbulent mixing (*e.g.,* Koehl and Powell, 1994; Okubo and Levin, 2001). However, instantaneous (~0.02 s), fine-scale (~200 μm) concentration measurements are required to understand the patterns of cue concentration encountered by larvae a few hundred micrometers in length swimming at a few millimeters per second in the water flowing above a cue-releasing substratum. Such fine-scale patterns of chemical dispersion can be measured by planar laser-induced fluorescence (PLIF) in a laboratory flume (*e.g.,* described by Crimaldi and Koseff, 2001). With this approach, a thin (~1-mm-thick) slice of the flow field is illuminated with a sheet of laser light, and video records are made of the brightness of fluorescent dye released from a substratum into the turbulent flow in the flume. PLIF studies have shown that chemical plumes near substrata in currents and waves are made up of fine filaments of high concentration swirling in clean water (*e.g.,* Crimaldi and Koseff, 2001; Koehl *et al.,* 2001; Mead *et al.,* 2003). The spatial distribution of such fine filaments above a coral reef has been revealed by PLIF measurements of fluorescent dye leaching from the surface of a reef composed of cleaned skeletons of *Porites compressa* in a flume in wave-driven flow (Reidenbach, 2004). Flow in the flume was designed to mimic field measurements of water movement and turbulence over reefs of *P. compressa* in Kaneohe Bay, Hawaii (Koehl and Hadfield, 2004). The resulting PLIF images revealed that microscopic larvae transported in the water above a cue-releasing substratum such as a coral reef may encounter fine filaments (hundreds of micrometers to millimeters in width) of high cue concentration interspersed in cue-less water (Fig. 1), rather than the diffuse gradient of cue concentration (scale of centimeters to meters) assumed in the past (*e.g.,* Crisp, 1974; Eckman *et al.,* 1994).

Research system

To better understand how larvae respond to dissolved settlement cues in habitats characterized by wave-driven, turbulent flow, where chemical cues arising from the substratum are rapidly and unevenly dispersed and diluted, we are investigating the settlement biology of a coral-eating nudibranch, *Phestilla sibogae* Bergh, 1905. This species serves as an excellent model organism for such research because its larvae are specifically induced to settle by a small, polar metabolite from its prey coral *Porites compressa* Dana, 1846 (Hadfield and Pennington, 1990), and because it can be rapidly and repeatedly reared in the laboratory throughout the year. Metamorphosis in the species is well understood at both light- and electron-microscopical levels (Bonar and Hadfield, 1974). The signal-transduction pathway has been extensively studied, including the identification of the site of the metamorphic inducer receptors on the larvae (Hadfield *et al.,* 2000).

The larvae of *P. sibogae* provide a model for larval

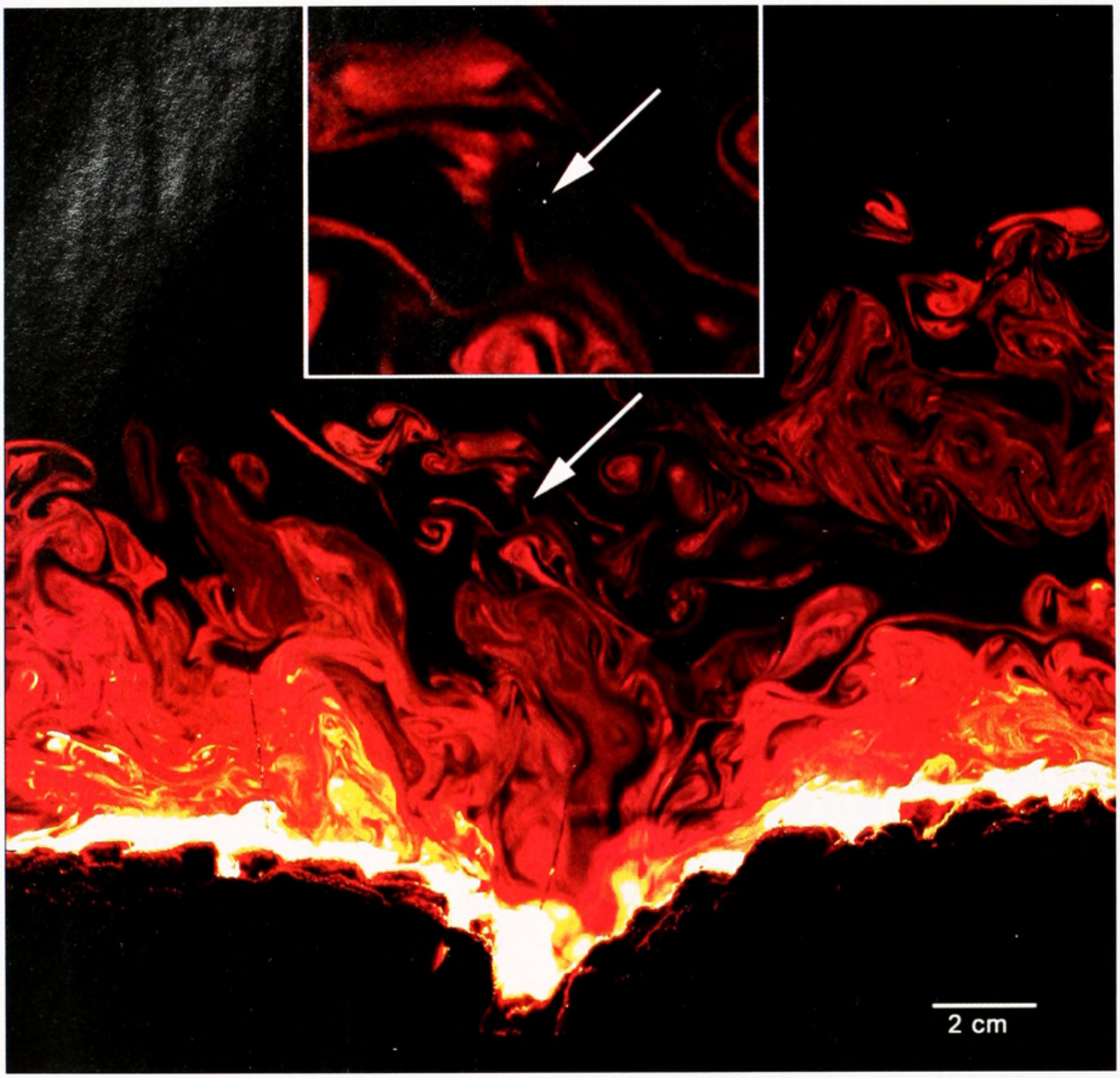

Figure 1. Planar laser-induced fluorescence (PLIF) image of rhodamine dye leaching from the surface of a reef of cleaned skeletons of *Porites compressa* exposed to waves in a flume (single frame of a video made by M. A. Reidenbach and J. R. Koseff, Environmental Fluid Mechanics Laboratory, Dept. of Civil Engineering, Stanford University). The brightness of the dye in the water is proportional to concentration (Crimaldi and Koseff, 2001). The arrow indicates the position of the modeled larva shown on the inset. Inset, segment of the PLIF image magnified 2.85× with a dot representing the size of a larva among the filaments of dye.

settlement into the complex three-dimensional communities of coral reefs. Coral-reef communities include not only the corals themselves, but also many algal species, reef fishes, and a host of invertebrates from many phyla living on and among the corals (Paulay, 1997). Most of the invertebrates in these communities have complex life cycles that include free-living larval stages. For many of the latter, successful recruitment depends on factors similar to those that confront larvae of *P. sibogae*. On Hawaiian reefs, these include the corals (Richmond, 1997), parasitic helminthes whose metacercariae encyst in the coral *Porites compressa* (Aeby, 1998), crustaceans that live on or within specific corals, coralliophilid snails that are obligate symbionts of corals (Kay, 1979), sessile vermetid gastropods (Hadfield *et al.*, 1972), sponges that live among or within the skeletons of the corals (Glynn, 1997), serpulid polychaetes whose tubes become embedded in living coral colonies, polychaetes and asteroids that feed on corals, sipunculans that burrow into coral skeletons, and many others (Paulay, 1997). Gaining information on larval transport into the complex interstices of coral reefs and their retention there will enhance understanding of metapopulation dynamics in all of these forms.

This first step in determining whether larvae of *P. sibogae* can utilize dissolved settlement cues in the turbulent, wave-

driven flow that characterizes coral reefs and many other coastal habitats focuses on the short-term behavioral responses of the larvae to dissolved cues from corals. Specifically, how rapidly do larvae respond behaviorally when they encounter the settlement cue? Larvae of *P. sibogae* require an exposure to cue of 1.5–2.0 h to develop a strong adhesion to a substratum (Koehl and Hadfield, 2004) and of nearly 6 h for metamorphosis to follow (Hadfield, 1977). Since larvae swimming or drifting above a coral reef are never likely to be in concentrations of coral cue long enough for morphogenetic induction to occur, we wanted to learn whether behavioral changes that bring about settlement from the water column occur more rapidly. If sinking or downward swimming behavior occurs rapidly and enhances transport of larvae into a reef, then larvae could remain in the cue-laden water within coral heads sufficiently long for metamorphic induction to occur (Hadfield and Scheuer, 1985).

Objectives of this study

The purpose of this study was to characterize the rapid behavioral responses of competent and precompetent larvae of *Phestilla sibogae* to brief encounters with dissolved chemical cues from *Porites compressa.* By videotaping swimming larvae through a microscope, we were able to monitor the behavior of the cilia, velar lobes, and foot of an individual larva as it entered and exited water containing different concentrations of cue from *P. compressa.* High-magnification video recordings of swimming larvae were made possible by tethering larvae in the field of view of a microscope while exposing them to the same rate of water movement relative to their bodies that they would experience when swimming freely. To translate cue-induced changes in the behavior of a tethered larva into differences in the velocities of untethered larvae, we also made and analyzed video recordings of larvae moving freely in aquaria filled with filtered seawater or seawater containing dissolved cue from *P. compressa.*

Materials and Methods

Culture of larvae for assays of behavior and metamorphosis

Phestilla sibogae is continuously maintained in Hadfield's laboratory at the University of Hawaii's Kewalo Marine Laboratory. Juvenile and adult animals are kept in shallow water tables supplied with flowing seawater, where they are provided with their living prey, *Porites compressa,* collected from the field 1–2 times per month. For this research, egg masses laid on the coral by adult *P. sibogae* were collected on the day they were deposited and maintained in bubbled beakers of filtered seawater in a 25 °C incubator until they hatched 6–7 days after deposition. Larvae were maintained at 25 °C as described in detail by Miller and Hadfield (1986). Competent larvae were typically used for studies on metamorphic induction when they were between 10 and 14 d post-fertilization (3–7 d post-hatching). In the experiments described below, precompetent larvae were tested on the day that they hatched from the egg mass. Each cohort of larvae used was a mixture of larvae from 5–10 different egg masses.

Test solutions

In the experiments on behavior and metamorphosis described below, the following solutions were tested: (i) seawater from the laboratory's continuously flowing system passed through a 0.45-μm Millipore filter (FSW); (ii) standardized strong cue from *P. compressa* ("*Porites* water" [100% PW]), 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 (iii) solutions of PW diluted with FSW (30% PW, 10% PW, 5% PW, and 1% PW). The metamorphosis assays described below were conducted in these solutions as well as in "reef water" collected within heads of living *P. compressa* on reefs in Kaneohe Bay, Hawaii, using procedures described in Hadfield and Scheuer (1985).

Tests of induction of metamorphosis

Assays to determine the biological activity of all batches of the solutions listed above were carried out in 30-well microtiter plates, with larvae placed approximately 20 per 2 ml of test solution in a well (Pennington and Hadfield, 1989). The percentage of larvae that metamorphosed after 24 h was tallied for each well and used as a measure of the relative inductive strength of the solution being tested (typically, 90%–100% of larvae metamorphose when exposed for 24 h to 100% PW [Hadfield and Scheuer, 1985; Hadfield and Pennington, 1990]). These tests, which were conducted on the days that behavioral experiments were done, also assessed the metamorphic responsiveness of each cohort of larvae used in the behavioral experiments.

Rapid behavioral responses of tethered larvae to brief encounters with cue filaments

Individual larvae of *Phestilla sibogae* were tethered in a small flume ("mini-flume"), and the water-flow velocity past the animal was adjusted to be about the same as the water velocity relative to an untethered swimming larva (0.2 cm s^{-1}). The acrylic plastic mini-flume had a working section (3 cm wide × 3 cm deep × 14.5 cm long) small enough to permit the larva to be viewed laterally through a

microscope. A steady flow rate of FSW through the flume was maintained by a constant-head tank; velocity was adjusted by raising or lowering the constant-head tank with a lab jack. The mini-flume was a flow-through system; because water was not recirculated through the test section, background levels of test solutions did not build up over the course of an experiment. Velocity past a larva in the mini-flume was measured by videotaping (60 frames s^{-1}) the movement of small, neutrally buoyant particles carried in the moving water. The microscope was focused on the midline of the flume (where the larva was positioned), and, to avoid errors due to parallax, only particles in sharp focus were digitized. Such particle movements in the focal plane of the tethered larva were videotaped using an SPI Minicam mounted on a 20× ocular on a Wild dissecting microscope positioned to give a lateral view of the larva at an objective magnification of 6×. These videos were analyzed using SCION Image software, ver. 1.62, and instantaneous velocities of the particles were calculated (as described below for velocity measurements of freely swimming larvae).

Individual larvae were tethered to the tip of a fine (0.24-mm diameter) stainless steel insect pin so that they could be held in a fixed position in the mini-flume in the field of view of the microscope. The tip of a pin, coated with a thin layer of petroleum jelly, was gently pushed against the dorsal surface of the shell of a submerged larva so that the hydrophobic shell stuck to the pin. The pin and larva were gently lifted from the larval culture dish and positioned with a micromanipulator in the mini-flume so that the larva could "swim" into the flow (*i.e.*, the water flow relative to the tethered larva was the same as the water flow relative to a freely swimming larva) (Fig. 2A). If the larva was positioned on the pin so that it did not contact the pin or the petroleum jelly when it extended its foot and velum from the shell, it quickly expanded its velar lobes and beat its velar cilia in the manner of a freely swimming larva. Only properly tethered larvae showing this "swimming" behavior in clean FSW in the mini-flume were used in our experiments.

Tethered larvae were exposed to filaments of test solutions (FSW, 100% PW, 30% PW, 10% PW, 5% PW, or 1% PW) labeled with 0.05% or 0.1% fluorescein that were carried past them in the flowing water (Fig. 2B, C). These filament encounters were designed to mimic the exposure to filaments of cue that a freely swimming larva would encounter in the turbulent flow above a coral reef (Fig. 1) (Koehl *et al.*, 2000). A computer simulation of larval motions (due to swimming, ambient waves, and turbulence) through the changing concentration fields recorded in PLIF videos (Reidenbach, 2004) was used to calculate the cue concentrations encountered by larvae as a function of time (Strother *et al.*, 2001). As a larva moves through the water, it passes into and out of cue filaments, and thus experiences an on-off temporal pattern of encounters with cue. Larvae close to the reef encounter filaments more often than do those higher above the reef (see Fig. 1). In the experiments reported here, a repeatable pattern of alternating exposure to test solution for 3–5 s (to simulate moving through a filament) and to clean FSW for 4–6 s was used so that larval responses to different test solutions could be compared

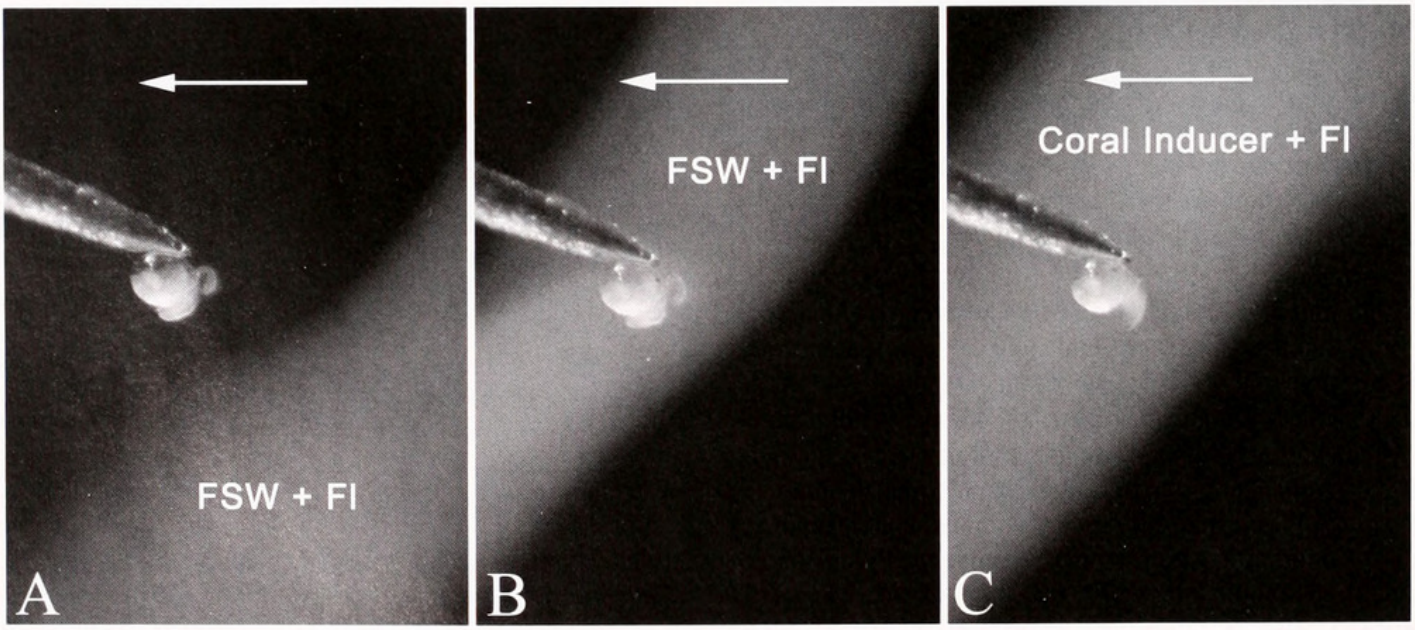

Figure 2. Photomicrographs of tethered larvae in the mini-flume. (A) Larva swimming in flowing seawater, with a filament of filtered seawater with fluorescein below larva. (B) Larva swimming in control filament (fluorescein in FSW). (C) Larva retracted in response to filament of seawater saturated with coral metabolites. FSW, filtered seawater; Fl, fluorescein. Arrow shows direction of seawater flow. The larval shell is about 210 μm long.

under standardized conditions. Filament exposures of such durations are typical at heights of about 5–8 cm above a reef dominated by *Porites compressa* (Koehl *et al.,* 2000; Strother *et al.,* 2001). A narrow stream, or filament, of test solution was gently released into the water through a syringe needle (bore diameter of 0.5 mm) positioned perpendicular to the flow upstream from the larva by a micromanipulator. The filament of test solution was carried downstream toward the larva by the flowing water in the mini-flume (*i.e.,* not by injection pressure), as though the larva were swimming toward the filament. An infusion pump (syringe pump model No. 351, Sage Instruments, Inc., Cambridge, MA) was used to produce a slow, steady rate of solution injection. By moving the syringe needle up and down slightly with the micromanipulator, the filament could be directed onto the larva (to simulate passage into a cue filament; Fig. 2C) or below the larva (to simulate passage out of a filament and into a band of clean water; Fig. 2A). Each larva was exposed to 18–21 filaments of a given test solution.

Both competent and precompetent tethered larvae were tested for responses to fluorescein-labeled FSW and PW. The behavior of the tethered larvae was recorded at 60 frames s^{-1} by the videocamera mounted on the ocular of the dissecting microscope positioned to observe the larvae in lateral view at 100×. Instantaneous responses could be observed, including cessation and resumption of beating of velar cilia, and partial or complete retraction or re-extension of the velum or foot. Frame-by-frame analyses of these video records permitted us to measure (to the nearest 0.017 s) the time when the edge of a filament encountered or left the chemoreceptive organ of a larva (Hadfield *et al.,* 2000) and the onset or cessation of any of these larval behaviors. These measurements were used (1) to calculate the lag time for larval responses to entering or leaving a cue filament and (2) to determine the percentage of filaments of test solution that caused a larva to react.

Effects of settlement cue (PW) on behavior of freely swimming larvae

The behavior of freely swimming larvae of *P. sibogae* was observed in acrylic plastic aquaria, 17.6 cm tall, 14 cm wide, and 4.5 cm thick, filled with either 100% PW or FSW at ~24 °C. An aquarium was set up in a darkroom, and a vertical plane midway between the front and back walls of the aquarium was illuminated by fluorescent lights shining through a 1-mm-wide slit on each side of the aquarium. The spectral range of the light in the water, determined with an LI-1800 underwater spectroradiometer (LI-COR, Inc., Lincoln, Nebraska), had peaks of irradiance at wavelengths of 400–725 nm, with the highest values at 550–650 nm. Similar measurements in shallow water in Kaneohe Bay, Hawaii (site of our field studies for this project), yielded high spectral irradiance at 325–700 nm, with greatest values at 350–600 nm (Gulko and Jokiel, 1995). Although these overlapping spectra are not identical, preliminary tests with newly hatched larvae of *P. sibogae* revealed that they swam toward the fluorescent lights in our aquaria, just as they do toward natural light (Miller and Hadfield, 1986). Miller and Hadfield (1986) determined that precompetent larvae of *P. sibogae* are attracted to light over a wide range of intensities (47–890 $\mu E\ m^{-2}\ s^{-1}$) in both the vertical and horizontal planes. As larvae of *P. sibogae* become metamorphically competent, their positive phototaxis weakens, until they become indifferent to light over the same range of intensities (Miller and Hadfield, 1986); trial tests with competent larvae in filtered seawater in the aquaria revealed the same response trend to the fluorescent lights.

For each "run," one of the test solutions was poured into an aquarium and allowed to come to rest, then larvae that had been in FSW were added to the aquarium, and the trajectories of larvae in the solution were videotaped at 60 frames s^{-1}. A Sony Hi8 Handycam TR101 video camera was mounted on a tripod and positioned sufficiently close to the aquarium that the field of view was an area 8.5 cm wide and 6.5 cm high in the lower half of the aquarium. The area filmed was equidistant from the side walls and the floor of the aquarium so that behavioral reactions to surfaces were not included in our analysis. The larvae in the vertical plane of illuminated water were clearly visible as bright spots in a dark field. For each run, about 300 larvae in 25 ml of water were gently poured into the aquarium. Videotaping began when larvae were introduced into the test water in the aquarium, but we waited 1 min before digitizing larval paths from the tapes. This provided the larvae with 1 min of exposure to the chemical cues, and allowed time for convection caused by adding the larvae to be sufficiently damped that larval swimming and sinking velocities were faster than convective velocities. Trajectories of all larvae that moved through the field of view during the subsequent 2 min were digitized using SCION Image software (sampling frequency 0.2 s) (Fig. 3). Because the movement of only those larvae in the illuminated plane in the aquarium could be digitized, errors due to parallax and to underestimation of velocity by digitizing larvae swimming toward or away from the camera were minimized.

Larval motion was quantified by analysis of trajectories such as those illustrated in Figure 3. "Instantaneous speed" was calculated by dividing the distance that a larva traveled between video frames by the time interval between those frames. The mean instantaneous speed for each larva was calculated using all of the instantaneous velocities measured for the trajectory of that larva. The length of the path followed by the larva was calculated as the sum of all the instantaneous speeds divided by the total duration of that individual's trajectory in the illuminated field of view. A vector was drawn between the first and last positions of a

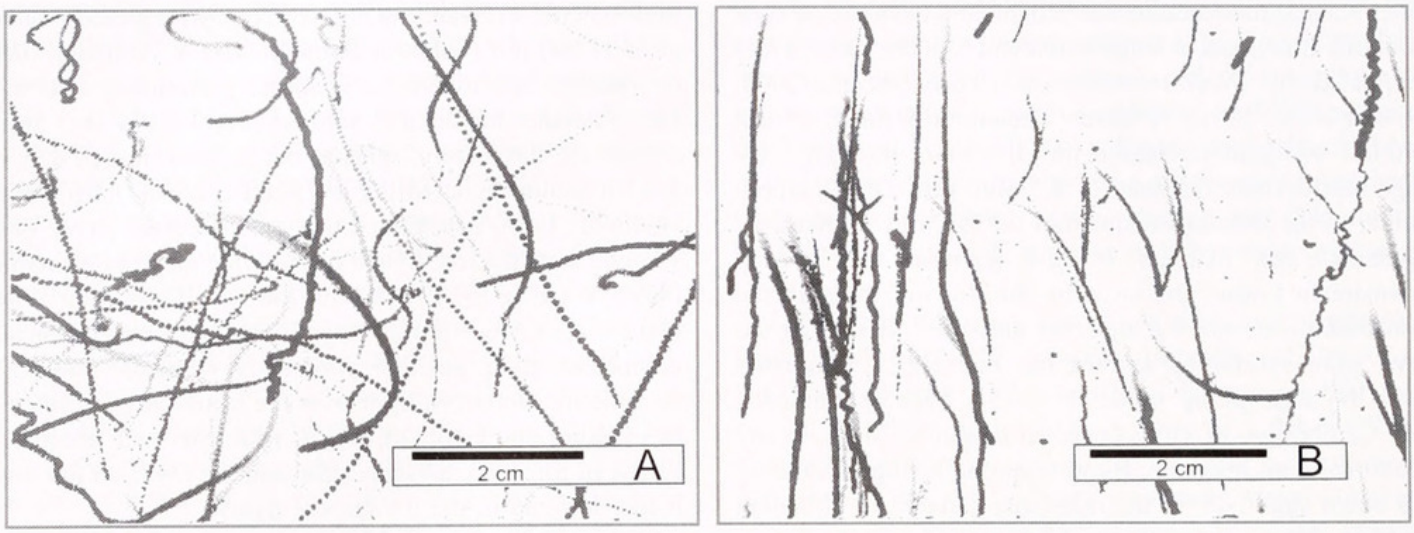

Figure 3. Trajectories of swimming or sinking larvae digitized from videotapes (duration = 50 s, sampled every 0.2 s). (A) Larvae moving in filtered seawater. (B) Larvae moving in settlement cue (seawater saturated with metabolites from the coral *Porites compressa*).

larva along its trajectory. The angle of the vector (where 0° was up, 90° was lateral to the left or right, and 180° was down) represented the net direction of movement by that larva. The length of the vector was the net displacement by the larva. We calculated "straightness index," a measure of straightness of the path followed by a larva, by dividing the net displacement of the larva by the length of the path followed by that individual. The "resultant speed" of the larva was calculated by dividing the net displacement of the larva by the duration of that individual's trajectory. As illustrated by the intersecting trajectories of the larvae in Figure 3A, the paths of individual larvae were not altered by the motions of the other larvae in the aquarium.

Sinking rate of dead larvae

The gravitational fall velocities of dead larvae of *P. sibogae* were measured to compare the passive sinking speeds of larvae with the velocities of larvae that moved downward in the experiments described above. Individually, actively swimming competent (11-day-old) larvae were gently captured from a culture beaker with a Pasteur pipette and transferred to a dilute solution of formalin (10 drops of 10% formalin solution in 35 ml of filtered seawater) to kill them. Prior to testing, each larva was inspected under a dissecting microscope to determine that it was entire and that its ciliary locomotion had ceased. These observations also revealed that every larva tested had retracted completely into its shell. Gravitational fall velocities of individual larvae that had reached terminal velocity while sinking through filtered seawater at 27 °C were measured using the technique described by Butman *et al.* (1988a).

Statistical analyses

ANOVA and Bonferroni/Dunn tests were conducted using Statview 5.0 statistical software, and means and standard deviations were calculated using Microsoft Excel 98 software.

Results

Metamorphosis in various concentrations of cue from Porites compressa

The biological activity of the *P. compressa* water (PW) used in our experiments was determined by metamorphosis assays using competent larvae of *Phestilla sibogae*. The results of these assays for 100% PW, 30% PW, and filtered seawater (FSW) are shown in Figure 4, where they are compared with results of metamorphosis assays for water collected within heads of *P. compressa* in Kaneohe Bay ("reef water"). No larvae underwent metamorphosis in FSW. Rates of metamorphosis in reef water were not significantly different from those we measured in 30% PW and were in the same range as those reported for reef water by Hadfield and Scheuer (1985), whereas metamorphosis rates in 100% PW were significantly higher, and those in FSW were significantly lower (ANOVA, Bonferroni/Dunn, significance level 5%). Therefore, 30% PW provides a reasonable laboratory mimic of the biological activity of inducer in water in reefs in the field.

Rapid behavioral responses of larvae to brief encounters with cue from Porites compressa

Tethered larvae "swimming" in the mini-flume (in which they were exposed to the same water motion relative to their

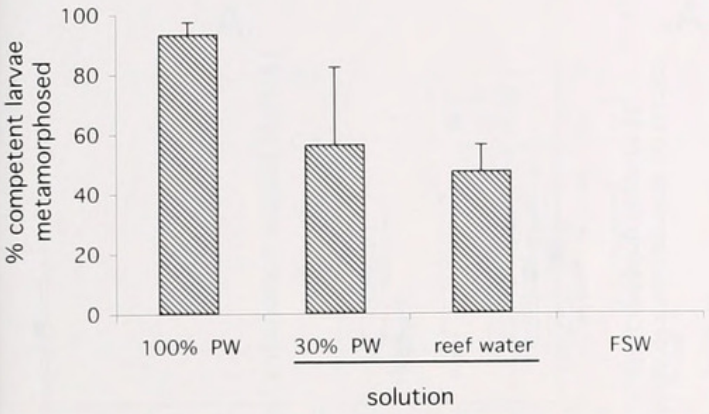

Figure 4. Percent of competent larvae of *Phestilla sibogae* that underwent metamorphosis in various solutions. Four replicate assays were conducted for each sample of a solution, and the mean was calculated for that sample. The mean of those sample means for each type of solution is plotted in this graph; error bars represent one standard deviation. For laboratory-prepared *Porites compressa* water (PW) and filtered seawater (FSW), a sample was taken on each day that experiments were conducted using tethered larvae. "Reef water" samples were collected within heads of *P. compressa* in Kaneohe Bay (n = 4 reefs). The bar below the horizontal axis indicates samples that were not significantly different from each other (ANOVA, Bonferroni/Dunn, significance level 5%).

bodies as they would have experienced if swimming freely through the water) extended their feet and velar lobes and beat their cilia for many hours in FSW (Fig. 2A). Frame-by-frame analysis of videotapes made through a microscope revealed the responses of such larvae "swimming through" filaments of fluorescein-labeled FSW or PW of various concentrations. The larvae continued to "swim" normally through control filaments of fluorescein-labeled FSW (Fig. 2B). In contrast, they often stopped beating their velar cilia and partially retracted their velar lobes upon entering a filament of PW (Fig. 2C). Typically, the larval foot remained at least partially extended, a posture important for immediate attachment when a settling larva encounters a surface.

The responsiveness of competent and precompetent larvae to encounters with filaments of different concentrations of PW is shown in Figure 5A. Typically, larvae did not retract when exposed to filaments of FSW or 1% PW. In contrast, larvae retracted their velar lobes in response to a high percentage of the filaments they encountered if the PW concentration in the filaments was 100% PW or 30% PW (30% is the concentration wherein metamorphosis-inducing activity is similar to that of water collected from within heads of *P. compressa* in the field). There was no significant difference between the percentage of filaments causing larval retraction in 100% PW and in 30% PW for both competent and precompetent larvae. A significantly lower percentage of filaments of 10% and 5% PW stimulated larvae to retract. There was no significant difference between the responsiveness of competent and precompetent larvae at any of the concentrations of PW.

Although both competent and precompetent larvae stopped "swimming" when they first entered a filament of cue from *P. compressa,* their behavior after that initial contact differed. Whereas competent larvae typically remained retracted while in a filament of PW, precompetent larvae more often re-expanded their velar lobes and re-

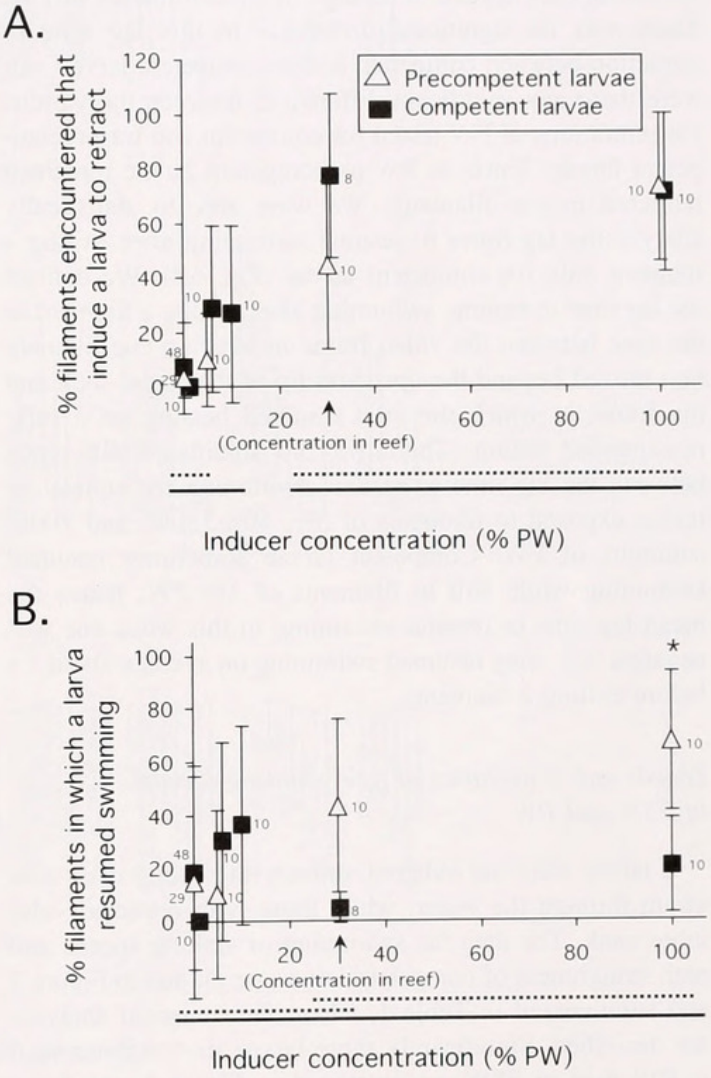

Figure 5. Responses of competent (black squares) and precompetent (open triangles) tethered larvae "swimming" through filaments of fluorescein-labeled filtered seawater or *Porites compressa* water (PW) in a small flume simulating untethered swimming. The small numbers next to each symbol represent the number of larvae in that treatment; error bars indicate one standard deviation. All of the data were compared using an ANOVA followed by Bonferroni/Dunn tests with a significance level of 5%. Solid lines below the horizontal axis indicate concentrations that were not significantly different for the competent larvae, and dotted lines indicate concentrations that were not significantly different for the precompetent larvae. An asterisk above the symbols for a particular concentration indicates that the competent and precompetent larvae were significantly different at that concentration. (A) "Responsiveness" of larvae to brief encounters with PW, measured as the percent of the filaments encountered that cause larvae to retract their velar lobes. (B) Percent of the filaments that stimulated retraction in which larvae re-expanded their velar lobes and resumed ciliary "swimming" while still in the filament.

sumed ciliary "swimming" while still in a PW filament (Fig. 5B).

There were lag times between when larvae first encountered or exited cue filaments and when their behavioral responses occurred. The lag time between the video frame when a filament of PW or FSW first reached a larva's apical sensory organ and the video frame when the cilia stopped beating and the velum started to retract is plotted in Figure 6A for larvae exposed to a range of concentrations of PW. There was no significant difference in this lag time to retraction between competent and precompetent larvae, nor were there any significant differences between the various concentrations of PW tested for competent and for precompetent larvae. Since so few precompetent larvae remained retracted in cue filaments, we were able to statistically analyze the lag times to resume swimming after exiting a filament only for competent larvae (Fig. 6B). We defined the lag time to resume swimming after exiting a filament as the time between the video frame in which a cue filament first moved beyond the upstream lip of the larval shell and the frame in which the cilia resumed beating on a fully re-expanded velum. There was no significant difference between the lag time to resume swimming for competent larvae exposed to filaments of 5%, 10%, 30%, and 100% solutions of PW. Competent larvae sometimes resumed swimming while still in filaments of 1% PW; hence the mean lag time to resume swimming in this weak cue was negative (*i.e.*, they resumed swimming on average about 1 s before exiting a filament).

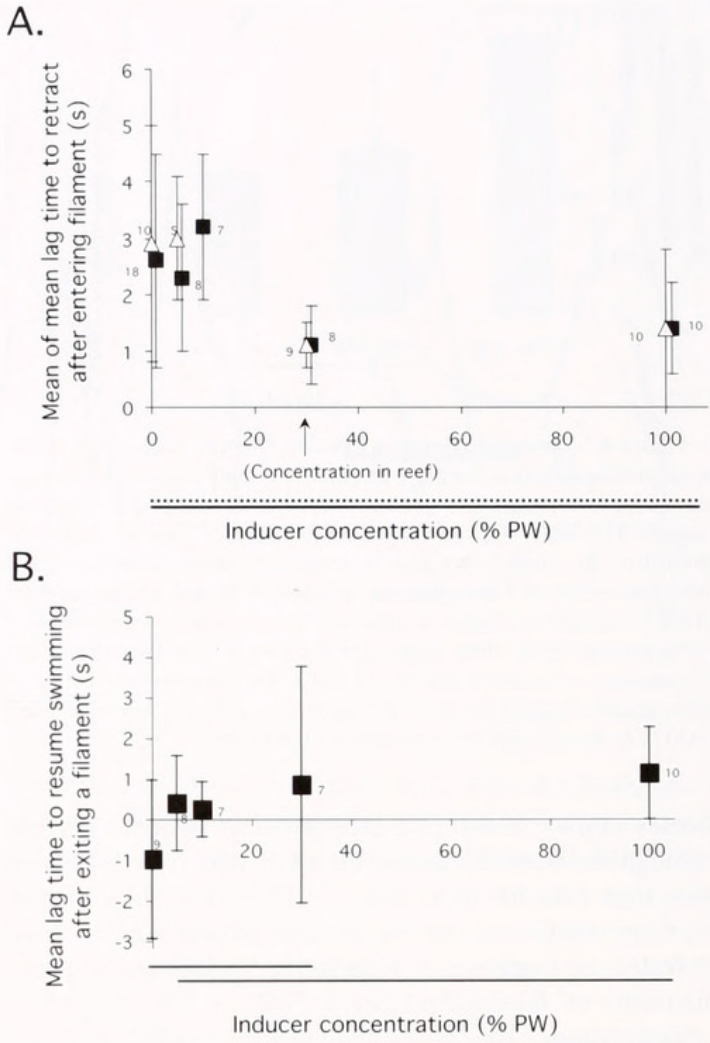

Figure 6. Mean lag times for larvae to respond to entering (A) or exiting (B) a filament, plotted as a function of *Porites compressa* water (PW) concentration for competent (black squares) and precompetent (open triangles) larvae. The small numbers next to each symbol indicate the number of larvae; error bars indicate one standard deviation. For precompetent larvae and competent larvae at PW concentrations of 10% PW, the *n* values are smaller than in Fig. 5 because not all of the larvae tested retracted when they encountered filaments. For each larva that retracted in each treatment, the mean lag time for all of its retractions was calculated, and the mean lag time for all its resumptions of "swimming" was calculated. The values plotted here are the means of those individual mean lag times. Statistical analyses (ANOVA, Bonferroni/Dunn, significance level 5%) were done as described in Fig. 5. Lines below different concentrations indicate that there was no significant difference between those concentrations for competent larvae (solid lines) and precompetent larvae (dotted lines). (A) Mean lag time to stop swimming. There were no significant differences between precompetent and competent larvae at any of the concentrations tested. (B) Mean lag time of competent larvae to resume swimming. Negative values indicate that larvae resumed swimming before exiting the filaments.

Speeds and trajectories of freely moving larvae in FSW and PW

If larvae were not tethered, those with beating velar cilia swam through the water, while those with retracted velar lobes sank. The data for swimming or sinking speeds and path straightness of competent larvae are plotted in Figure 7 and summarized in Table 1, where the statistical analyses are described. Significantly more larvae moved downward in PW than in FSW, as illustrated in Figure 3 and documented in Table 1. Our data underestimate the proportion of larvae moving downward, because many of the sinking larvae had already reached the bottom of the aquarium when the digitized section of each video began. Not only were the instantaneous speeds (Fig. 7A) of larvae in PW significantly slower than those of larvae in FSW, but so were the net speeds (Fig. 7B), since there was no significant difference between the straightness of the paths along which larvae in PW or FSW moved (Fig. 7C; Table 1).

Precompetent larvae swam faster and were less likely to move downward than competent larvae. Measurements of the motions of freely swimming precompetent larvae in aquaria containing FSW or 100% PW are plotted in Figure 8. We observed that precompetent larvae swam toward the light slits at the sides of our aquaria; hence only a small percentage of them moved downward in FSW (mean = 2%, SD = 1, n = 2 runs, number of larvae per run: 152 and 97). In contrast, competent larvae swam in many different directions in FSW in our aquaria, as illustrated in Figure 3a,

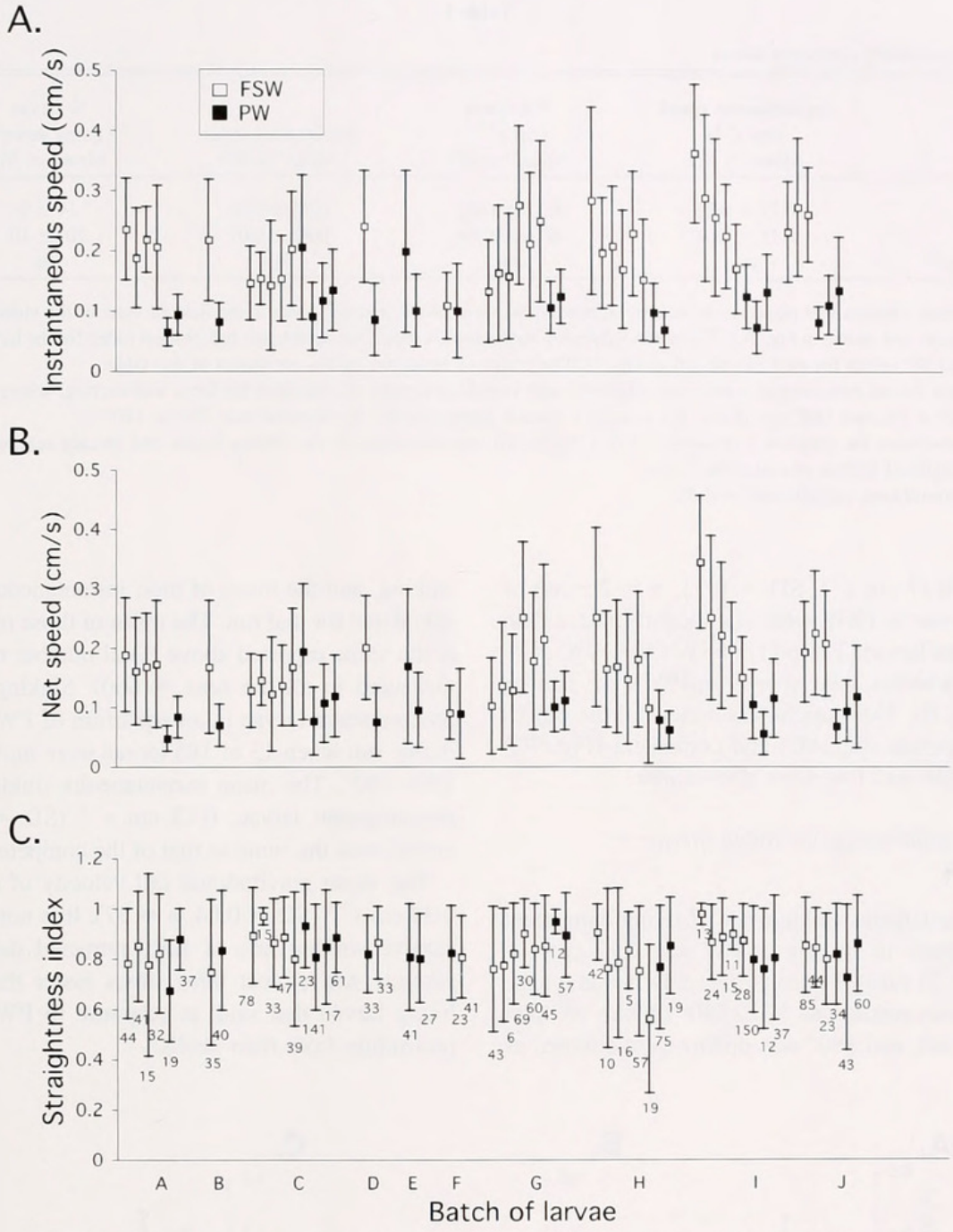

Figure 7. Measurements of competent larvae moving freely in aquaria containing filtered seawater (FSW; open squares) or 100% *Porites compressa* water (PW; black squares). Each symbol represents the mean value calculated for all of the larvae digitized in a run; error bars indicate one standard deviation. The small numbers below each symbol in Fig. 7C indicate the number of larvae digitized in that run in Figs. 7 A, B, and C. The letters along the horizontal axis indicate the cohorts of larvae used for the various runs. Statistical comparisons of these data are reported in Table 1. Since there were no significant differences between the cohorts of larvae (ANOVA, Bonferroni/Dunn, significance 5%), all of the runs were pooled for the analyses reported in Table 1. (A) The mean instantaneous speed for a run plotted here is the mean of the mean instantaneous speeds of all the larvae digitized in that run. (B) Mean net speed for all the larvae in each run. (C) Mean straightness index for all the larvae in each run. A straightness index of 1.0 represents a perfectly straight trajectory, whereas a lower straightness index indicates that a larva changed directions as it moved through the water, traveling along a curved or circuitous trajectory.

and thus significantly more competent larvae moved downward in FSW than did precompetent larvae (ANOVA, df = 32 where each sample was a run, $P < 0.05$). In the run we conducted with precompetent larvae in PW, 8% of the larvae moved downward (n = 165 larvae). The instantaneous speeds (mean = 0.32 cm s^{-1}, SD = 0.01, n = 2 runs)

Table 1

Movements of freely swimming competent larvae

Treatment	Instantaneous speed (cm s^{-1}) Mean[1] ± SD	Net speed (cm s^{-1}) Mean[1] ± SD	Straightness index Mean[1] ± SD	% larvae going down[2] Mean[1] ± SD	Number of runs[1]
FSW	0.21 ± 0.06	0.18 ± 0.05	0.83 ± 0.08	14 ± 9	32
PW	0.11 ± 0.04	0.10 ± 0.04	0.82 ± 0.07	20[3] ± 10	20
Significantly different?[4]	yes	yes	no	yes	

[1] For each run, a fresh solution was placed in an aquarium, new larvae were added, and all larvae in the field of view of the video were digitized (the numbers of larvae per run are shown in Fig. 7C). The mean values for instantaneous speed, net speed, and straightness index for the larvae in each run were calculated (means and SD values for each run shown in Fig. 7). The means of those run means are shown in this table.

[2] The angle between the net trajectory of a larva (see Methods) and vertical described the direction the larva was moving, where 0° was up, 90° was horizontal to the right or left, and 180° was down. We counted a larva as going down if its direction was 170° to 180°.

[3] These data underestimate the proportion of larvae moving downward, because many of the sinking larvae had already reached the bottom of the aquarium when the digitized section of each video began.

[4] ANOVA, Bonferroni/Dunn, significance level 5%.

and net speeds (0.27 cm s^{-1}, SD = 0.02, n = 2 runs) of precompetent larvae in FSW were significantly faster than those of competent larvae (Table 1) in FSW (ANOVA, df = 32, $P < 0.05$), whereas their speeds in PW were similar (Figs. 7A, B; 8A, B). The straightness indices of the trajectories of precompetent (Fig. 8C) and competent (Fig. 7C) larvae in both FSW and PW were also similar.

Sinking rates of dead larvae vs. *living larvae responding to PW*

The mean instantaneous sinking rate of living competent larvae moving freely in aquaria of PW was 0.12 cm s^{-1} (SD = 0.03, n = 21 runs). For each run, only larvae whose net direction of movement was 170°–180° (where 90° was lateral right and left, and 180° was down) were counted as sinking, and the mean of their instantaneous velocities was calculated for that run. The mean of those means for 21 runs is the value reported above (total number of sinking larvae measured in all the runs = 660). Sinking rates of living precompetent larvae in an aquarium of PW were measured in one run when 13 of 185 larvae were moving at angles of 170°–180°. The mean instantaneous sinking rate of these precompetent larvae, 0.12 cm s^{-1} (SD = 0.05, n = 13 larvae) was the same as that of the competent larvae in PW.

The mean gravitational fall velocity of dead larvae was 0.33 cm s^{-1} (SD = 0.04, n = 27). It is noteworthy that the passive sinking rate of fully retracted dead larvae of *P. sibogae* was almost three times faster than the speed of living larvae that sank in response to PW with their feet protruding from their shells.

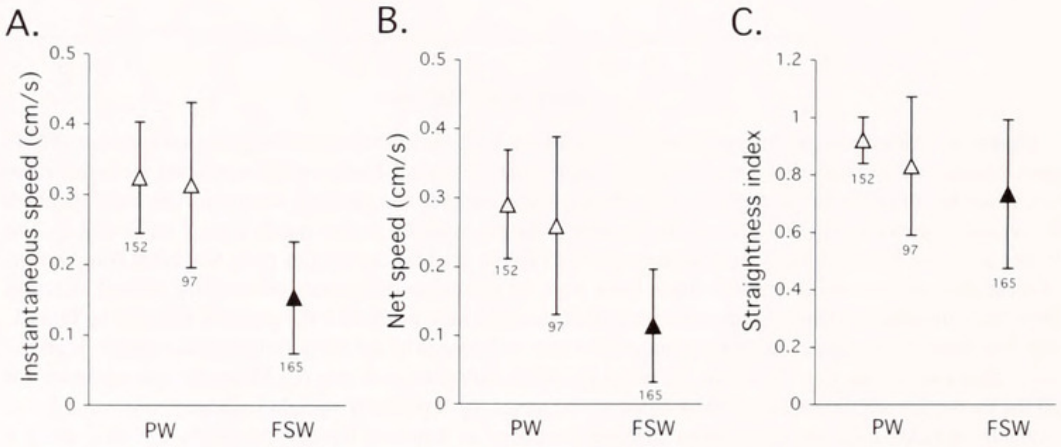

Figure 8. Measurements of precompetent larvae freely moving in aquaria containing filtered seawater (FSW; open triangles) or 100% *Porites compressa* water (PW; black triangles). All larvae were taken from the same cohort. Each symbol represents the mean value calculated for all of the larvae digitized in a run; the small numbers below each symbol indicate the number of larvae digitized in that run; and the error bars represent one standard deviation. (A) Mean instantaneous speeds, (B) net speeds, and (C) straightness indices for each run were calculated as described in Fig. 7; statistical analyses are reported in the text.

Discussion

Although many laboratory studies had revealed that competent larvae of *Phestilla sibogae* metamorphose in response to a dissolved substance from *Porites compressa* (*e.g.,* Hadfield and Pennington, 1990), a process that takes longer than 12 h, the dynamic behavioral responses of larvae of *P. sibogae* to encounters with coral cue had not previously been examined. Clearly, for coral cue to affect larval transport from the water column to the substratum in the turbulent flow across coral reefs, the larval behavioral responses to cue must be more rapid than the processes of metamorphosis. Thus, the present study assessed the behavioral responses of swimming larvae to encounters with water containing coral-produced settlement cue.

Rapid responses of larvae to settlement cue

This study focused on larval behavior over the time scale of tens of milliseconds to minutes. If chemicals are released from a surface in turbulent flowing water or air, fine filaments of fluid containing the chemical are formed in the high shear along the surface and are stirred into the surrounding water or air by swirling eddies (*e.g.,* Crimaldi and Koseff, 2001). Therefore, the distribution of concentrations of the released chemical in the turbulent, flowing fluid is very patchy. The intermittent nature of encounters with odors by insects flying in turbulent air flow has long been recognized (*e.g.,* Murlis, 1986; Murlis *et al.,* 1992; Vickers and Baker, 1992; Mafra-Neto and Cardé, 1994, 1998). Some studies of large benthic crustaceans tracking odor plumes have also documented the fluctuating, intermittent nature of chemical signals transported from a source by turbulent flowing water (reviewed in Weissburg, 2000), and recent PLIF studies have shown the fine-scale filamentous distribution of odors sampled by the olfactory antennules of these animals (Koehl *et al.,* 2000; Mead *et al.,* 2003). As microscopic larvae swim and are carried in the turbulent water flow above benthic habitats, they encounter dissolved substances released from the substratum (*e.g.,* Fig. 1) as a series of on-off pulses on a temporal scale of seconds (Strother *et al.,* 2001, unpubl. data). For this reason, we examined the rapid behavioral responses of swimming larvae exposed to a series of brief encounters, lasting a few seconds each, with filaments of settlement cue. We found that competent larvae of *P. sibogae* rapidly respond by sinking when they encounter a filament of dissolved cue from *Porites compressa,* but resume swimming when they exit the cue filament.

Settlement cue produced in the laboratory

Many studies of metamorphosis in *P. sibogae* have used a standardized "*Porites* water" (100% PW) produced in the laboratory (*e.g.,* Pennington and Hadfield, 1989). While this approach is suitable for studying the process of metamorphosis or isolating the inductive molecules in a chemical signal, investigations of behavioral responses should—if their results are to be useful in future analyses of larval transport and settlement—present larvae with a range of cue concentrations approximating those likely to be experienced in nature. The importance of using ecologically relevant concentrations of odors in studies of chemically mediated behavior is reviewed by Weissburg (2000).

The inductive chemicals in *Porites*-conditioned water (PW) are being isolated and characterized (V. Paul and Hadfield, unpubl.), but there is no chemical assay currently available to measure the molarity of inductive molecules in water samples. We have therefore used a bioassay—induction of metamorphosis—to measure the "strength" of inducer in our water samples. This bioassay has the advantage over a simple concentration measurement of incorporating any enhancement or reduction of cue activity by other substances that may in be in a water sample.

We found that water collected from coral heads in the field had metamorphosis-inducing strength similar to 30% solutions of laboratory-prepared PW. However, we found no significant differences between the instantaneous behavioral responses of larvae exposed to filaments of 100% PW or 30% PW. Furthermore, we found no difference between the adhesive strength of larvae induced to stick to surfaces by exposure to 30% PW or to 100% PW (Koehl and Hadfield, 2004). Thus, although we recommend that studies of larval behavioral responses to settlement cue be conducted at cue strengths comparable to those in nature, in the case of *P. sibogae* we found no difference between the behaviors of larvae in reef concentrations or in higher laboratory concentrations of cue from *P. compressa.*

Since the postures of larvae of *P. sibogae* induced to sink (velum retracted and foot expanded) by 100% PW were the same as those of larvae induced to sink in lower concentrations (5% PW–30% PW), our measurements of the fall velocities of larvae induced to sink in aquaria of 100% PW should be applicable to field conditions (fall velocity depends on the drag slowing the descent of a body, and drag depends on the size and shape of the body; *e.g.,* Vogel, 1994). Similarly, since the swimming velocities of larvae of *P. sibogae* in filtered laboratory seawater were the same as those of larvae in unfiltered water collected in the field upstream of coral reefs (Koehl and Hadfield, unpubl. data), our measurements of larval swimming velocities in filtered seawater should also be comparable to those of larvae in nature.

Downward velocities should be measured using unanesthetized living larvae

The downward velocities of living larvae responding to settlement cue are not necessarily the same as sinking

velocities measured for killed or anesthetized larvae. For example, when a larva of *P. sibogae* responds to coral cue by retracting its velum and sinking, its foot remains extended from the shell. The drag on the protruding foot slows the descent of the living larva relative to that of a fully retracted larva. Conversely, when near the substratum, oyster larvae may actively swim downward at speeds much greater than the sinking velocities of dead larvae (Finelli and Wethey, 2003). Therefore, measuring the sinking rates of anesthetized or dead larvae to determine the downward velocities of living larvae, as has often been done in the past (*e.g.,* Butman *et al.,* 1988a), may produce erroneous conclusions for some species.

Effects of larval behavior on their transport to and retention within a coral reef

On the scale of a larva, dissolved settlement cue released from a coral reef is very patchily distributed, but the frequency, thickness, and concentration of cue filaments are greater near the reef surface than higher above it (Fig. 1) (Reidenbach, 2004). A competent larva of *P. sibogae* responds within 1–2 s on most encounters with filaments of dissolved coral cue above threshold concentration by arresting the beat of its velar cilia, retracting its velum, and sinking. While microscopic larvae swim and sink through the water, the water in which they are moving is also transported toward and away from the reef surface due to the vertical orbital motion of water in waves and to swirling turbulent eddies (Koehl and Hadfield, 2004; Reidenbach, 2004). If the larva sinks, swims, or is transported closer to the reef, its encounters with cue filaments above threshold concentration increase in frequency and duration from one encounter every 10–20 s at a height of 12 cm above the reef to almost continuous exposure 1 cm above the corals (Strother *et al.,* 2001; Strother *et al.,* unpubl. data).

To examine whether the simple behavioral algorithm (sink in cue and swim in cue-free water) used by competent larvae of *P. sibogae* could enhance their settlement rates onto coral reefs exposed to turbulent, wave-driven flow, we employed a computer simulation (Strother *et al.,* 2001; Strother *et al.,* unpubl. data). In this individual-based model, we used measured values of the mean lag times, swimming and sinking velocities, and responsiveness to cue filaments of different concentrations. "Larvae" using this algorithm were randomly placed in the water column in PLIF videos (*e.g.,* Fig. 1) of changing concentrations of dye (an analog for cue) above a reef of *P. compressa* in a flume (Reidenbach, 2004) in wavy, turbulent flow like that measured over *P. compressa* reefs in the field (Koehl and Hadfield, 2004). Each "larva" swam in a particular direction randomly assigned at the beginning of the simulation. At each time step (*i.e.,* video frame), each "larva" swam or sank, depending on the cue concentration of the pixel in which it was located and on the history of cue exposure it had just experienced. The instantaneous net velocity of the "larva" was the vector sum of (i) its sinking or swimming velocity, (ii) the instantaneous velocity of the water at that height above the reef and phase of the wave cycle, and (iii) the random velocity fluctuation due to turbulence at that height and phase (values for ii and iii were measured in the flume; Reidenbach, 2004). The instantaneous net velocity of the "larva" determined its position in the next frame of the video, and so on. These calculations, done for thousands of "larvae," revealed that the simple behavioral algorithm used by the larvae of *P. sibogae* can enhance the rate of larval transport to the reef surface in turbulent, wavy flow by about 30% over that of larvae that do not alter their behavior in response to cue (Koehl *et al.,* 2000; Strother *et al.,* 2001; Strother *et al.,* unpubl. data).

The behavioral responses of competent larvae of *P. sibogae* to cue from *P. compressa* not only improve their transport down to the reef, but also enhance their chances of retention in a suitable habitat. Because larvae sink in cue concentrations like those found in the water between coral branches in *P. compressa* reefs, larvae that have been transported into a reef are likely to remain there, continuing to sink through the slowly moving (Koehl and Hadfield, 2004), cue-laden water within the reef. Furthermore, a larva that sinks in response to cue does so with its foot extended from the shell and thus is able to attach quickly on contact with a surface. Over the course of about 2 h of exposure to cue concentrations like those in coral heads in the field, larvae develop stronger adhesion both to living *P. compressa* tissue and to non-*Porites* surfaces (*e.g.,* coralline algae, glass) (Koehl and Hadfield, 2004), thereby further improving their chances of remaining in a suitable habitat bathed in inducer long enough for metamorphosis to occur.

The observation that precompetent larvae, not yet capable of undergoing metamorphosis, respond with about the same frequency as competent larvae to encounters with filaments of coral cue indicates that the chemosensitivity to the cue arises early in larval development. However, as indicated by the high percentage of times precompetent larvae re-extended their velar lobes and resumed swimming while within a cue filament, the sensitivity to coral cue is probably not sufficiently great to result in settlement and attachment. Furthermore, during daylight hours, the strongly photopositive precompetent larvae swim upward (Miller and Hadfield, 1986). Computer simulation of precompetent larvae in turbulent wave-driven flow above a reef of *Porites compressa* shows that such upward swimming when not responding to cue decreases larval encounters with cue filaments and enhances larval transport off the reef (Strother *et al.,* unpubl. data). By contrast, competent larvae are indifferent to light (Miller and Hadfield, 1986) and remain partially contracted in most of the cue filaments that cause them to stop swimming. Thus, in nature, competent larvae should

sink rapidly toward the coral reef, while precompetent larvae should be carried away.

Comparison of our results with those of other studies of effects of chemical cues on larval settlement in flowing water

Although many shallow coastal habitats are subjected to the oscillatory flow associated with waves, until now studies of the effects of chemical cues on larval transport to or settlement on the substratum have been done in unidirectional flow. The studies have typically determined the settlement positions of larvae onto different types of substrata after transport across the substrata in unidirectional laminar flow (*e.g.,* Butman *et al.,* 1988b; Pawlik *et al.,* 1991; Pawlik and Butman, 1993; Turner *et al.,* 1994). These studies provided solid evidence that larvae can discriminate among available substrata while being transported across them in flowing seawater. Additionally, Tamburri *et al.* (1996) videotaped paths of oyster pediveligers transported in a flume containing seawater only or seawater plus settlement-inducing substances, and reported downward movement of the larvae when the inducers were present. Our study contrasts with these earlier ones by focusing on wave-driven flow, by considering how dissolved cues are distributed on the spatial scale of microscopic larvae, and by examining the rapid behavioral responses of individual larvae to intermittent, brief encounters with cue. We found that larval responses are sufficiently rapid that repeated short encounters with cue filaments in turbulent, wave-driven flow can enhance larval transport into a suitable cue-releasing habitat. The behavior of larvae in cue can also enhance their retention in a flow-slowing, cue-releasing canopy such as a coral reef. Thus, this research on rapid behavioral responses of larvae in wave-driven flow is consistent with earlier studies of larvae in unidirectional flow in suggesting that larvae in flowing water can use chemical cues to enhance their settlement into appropriate habitats.

As in our computer simulations, another model of larval transport to the substratum also predicted enhanced settlement by larvae responding to chemical cues. Eckman *et al.* (1994) developed a one-dimensional model of the flux of larvae settling through a turbulent boundary layer when the vertical speed of the larvae is a function of their depth in the water column. In cases where steep gradients in the downward velocity of larvae occur near the substratum, which Eckman *et al.* (1994) suggested might be produced by larval responses to chemical cues associated with the bottom, settlement rate is enhanced.

Finally, the new data presented here demonstrate that a dissolved chemical cue from the prey coral *P. compressa* causes not only morphological metamorphosis in larvae of *P. sibogae,* a process requiring many hours of exposure (Hadfield, 1977), but also rapid behavioral responses that enhance chances of transport to and retention in a suitable habitat. Hence, settlement is not simply a result of the onset of metamorphosis, but rather is a behavioral phenomenon that results in placement of larvae within coral heads where dissolved cue is strong and consistent enough to induce adhesion to the substratum and metamorphosis (Koehl and Hadfield, 2004).

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