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DEVELOPMENTAL DIMORPHISM AND EXPRESSION OF CHEMOSENSORY-MEDIATED BEHAVIOR: HABITAT SELECTION BY A SPECIALIST MARINE HERBIVORE

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Summary

Developmental dimorphisms provide an opportunity to compare sensory systems and behavior patterns between different forms of a single species. Alternative morphs differing in dispersal ability often show behavioral differences that mediate life-history trade-offs. We measured the behavioral responses of both long-lived, feeding larvae and short-lived, non-feeding larvae of the specialist marine herbivore *Alderia modesta* during habitat selection. Larvae immediately responded to waterborne cues from the adult host algae by increasing their turning rate, by changing their swimming speed in the water and by moving in rapid hops or spiraling along the bottom. These behavior patterns retained larvae in areas where the dissolved cue was initially perceived, and prolonged exposure to the cue increased the percentage of larvae that initiated metamorphosis. Despite their differences in life span and trophic mode, both larval morphs displayed similar behavior patterns when stimulated by the waterborne cue. Long-lived larvae had a stronger response, however, suggesting that settlement behavior may offset the costs of a prolonged larval life. This is the first study to examine the effects of dimorphic development on chemosensory-mediated behavior.

Key words: dimorphism, larval settlement, metamorphosis, swimming behavior, *Alderia modesta*, opisthobranch.

Introduction

Developmental dimorphisms can result in discrete morphological differences between individuals of the same species (Roff, 1996). Examples of intra-sexual dimorphism are known from diverse animal taxa, including winged and flightless morphs in insects (Harrison, 1980; Crnokrak and Roff, 1995, 1998), the presence or absence of a male copulatory organ in snails (Schrag and Read, 1992; Schrag et al., 1994), male size variants in fish (Dominey, 1980; Gross, 1984) and horned *versus* hornless beetles (Eberhardt, 1982; Eberhardt and Gutierrez, 1991). Alternative morphologies are often associated with distinct behavior patterns that affect life-history trade-offs in mating strategy, reproduction, dispersal and resource competition (Gross, 1985; Brown and Bartalon, 1986; Mole and Zera, 1993; Crnokrak and Roff, 1998). Behavior underlies the fitness trade-off in crickets: wingless males compensate for limited foraging and dispersal abilities by producing more mating calls than winged morphs (Crnokrak and Roff, 1995). Male salmon maturing after 2 years are small and cryptic on breeding grounds, gaining access to females by sneaking into nesting sites, while males maturing after 3 years are larger and win access to mates through fighting (Gross, 1985). In the case of dispersal dimorphism, the migratory form of some species can preferentially locate and colonize high-quality food patches (Denno et al., 1980, 1989). However, previous studies have not addressed how developmental dimorphism affects chemoreception and behavioral responses to environmental signals during habitat selection.

Many marine invertebrates have a free-swimming larval stage that can disperse in the water column before eventually settling to the bottom and colonizing a new habitat (Thorson, 1950; Strathmann, 1978, 1985; Grahame and Branch, 1985; Pechenik, 1999). There are two contrasting modes of larval development, termed planktotrophy and lecithotrophy, which largely determine the amount of time larvae spend in the water column prior to settlement and metamorphosis (Wray and Raff, 1991; Levin and Bridges, 1995; Pechenik, 1999). Planktotrophic larvae must feed to become competent to metamorphose and often spend weeks or months maturing in the plankton, sometimes crossing entire ocean basins (Scheltema, 1962, 1971; Pechenik, 1999). Lecithotrophic larvae are non-feeding and are generally competent to metamorphose soon after hatching, usually settling close to the parental habitat (Levin and Bridges, 1995; Palumbi, 1995; Todd, 1998). Whether dispersing for minutes or years, larvae of both development modes ultimately face the same challenge: to locate and settle into an appropriate habitat on the ocean floor. Larval settlement and metamorphosis are often

induced by a species-specific chemical cue indicating a suitable juvenile environment (Pawlik, 1992). It is not known whether differences in the duration of the larval period affect behavioral responses or sensitivities to chemical settlement cues (Hines, 1986; Havenhand, 1991).

Single species expressing both development modes are extremely rare among marine invertebrates, limiting the opportunity for intraspecific comparisons between the two larval morphs (Hoagland and Robertson, 1988; Bouchet, 1989). The sea slug *Alderia modesta* (Lovén) is the only mollusc known to produce both planktotrophic and lecithotrophic larvae from the same population (Krug, 1998). Planktotrophic larvae of *A. modesta* feed on phytoplankton for 4 weeks before becoming competent to metamorphose, whereas lecithotrophic larvae can metamorphose immediately upon release from the egg capsule (Krug, 2000). This species therefore provides the opportunity to compare the behavior of long-lived and short-lived larvae within the same species. *A. modesta* is a specialist herbivore, found in temperate estuaries exclusively upon yellow-green algae of the genus *Vaucheria* (Xanthophyta: Xanthophyceae) (Hartog and Swennen, 1952; Bleakney and Bailey, 1967; Millen, 1980; Trowbridge, 1993). Larvae of *A. modesta* metamorphose specifically in response to bioactive carbohydrates from the adult host alga, *V. longicaulis* (Krug, 2000; Krug and Manzi, 1999). Living algae, sea water previously conditioned by the presence of *V. longicaulis* and aqueous extracts of the algae all induced metamorphosis of lecithotrophic larvae in laboratory assays (Krug and Manzi, 1999), but effects on swimming behavior and response thresholds were not determined.

Because of their small size and limited swimming abilities, most invertebrate larvae are considered to be passively transported and delivered to the bottom by ocean currents and local flow conditions (Eckman, 1983; Butman, 1987; Eckman et al., 1994). However, recent evidence has shown that dissolved chemical cues can trigger larval behaviors that influence settlement rates in moving water. Larvae of the oyster *Crassostrea virginica* showed dramatic behavioral responses to a waterborne chemical cue secreted by adult conspecifics, increasing settlement in both still and flowing water (Tamburri et al., 1992, 1996; Turner et al., 1994). Larval swimming behavior and chemoreception may therefore act cooperatively to facilitate settlement under natural conditions. The ecological implications of larval behavior during the settlement process are profound; larval recruitment is a critical factor in marine ecosystems, regulating population dynamics and community structure (Grosberg, 1982; Raimondi, 1988; Roughgarden et al., 1988; Underwood and Fairweather, 1989; Strathmann, 1990; Hurlbut, 1991).

We have used *Alderia modesta* to compare the behavior of developmentally distinct larvae in response to a key environmental stimulus. Competent larvae were tested for behavioral responses to dissolved chemical cues from the adult host algae to address two questions: (i) do larvae have an immediate behavioral response to a waterborne cue of habitat suitability, and (ii) do differences exist in the behavior patterns expressed by larvae of the two development modes? Behavior was quantified using computer-assisted video motion analysis (CAVMA) for the two larval morphs swimming both in the water column and just above the bottom. Bioassays were conducted in parallel with the dissolved cue to determine the minimum dose and exposure time sufficient to induce metamorphosis. Behavior and metamorphosis were quantified in response to sea water collected within and above patches of *Vaucheria longicaulis* in the field and to an aqueous extract of the algae. The results are discussed with regard to both the ecological role of larval behavior during settlement and the challenges imposed by the different life-history strategies of planktotrophy and lecithotrophy in this dimorphic species.

Materials and methods

Collection of organisms and larval culture

Specimens of *Alderia modesta* and patches of *Vaucheria longicaulis* (Abbott and Hollenberg, 1976) were collected from mudflats in the Kendall-Frost Marine Reserve and Northern Wildlife Preserve, San Diego, California, USA. Sea water was obtained from the Scripps Institution of Oceanography experimental aquarium and stored in the dark; prior to use, all sea water was filtered to 0.45 μm (FSW). Adult sea slugs (approximately 100) were placed overnight in Petri dishes to encourage mating. Egg masses of both development modes were harvested the following day and identified as described previously (Krug, 1998). Planktotrophic and lecithotrophic egg masses were separated from each other and were maintained in FSW; the water was changed every other day until hatching. Lecithotrophic larvae were transferred to fresh FSW immediately after hatching and were maintained without food for 24 h; larvae were subsampled for use in experiments as required over the next 48 h. Planktotrophic larvae were hatched and maintained in beakers containing 3 l of FSW at an approximate concentration of 1 larva ml^{-1}. Samples were taken from uni-algal suspensions of the phytoplankton *Rhodomonas* sp., *Isochrysis galbana* and *Pavlova lutheri* and were added to the cultures to give a final concentration of approximately 10^4 phytoplankton cells ml^{-1}. Every 2–3 days, the larvae were sieved through a Tri-Pour beaker with a 52 μm mesh bottom, and the concentrated larvae were transferred to clean FSW; fresh samples of phytoplankton were then added. Each culture was stirred by pipetting water against the bottom twice daily, but cultures were otherwise static. Larvae became competent to metamorphose after 30 days in culture and were then used in experiments. Culturing planktotrophic larvae was technically challenging, and only limited numbers of competent planktotrophic larvae were sporadically available; some experiments were therefore only performed with lecithotrophic larvae.

Larval metamorphosis bioassay

Samples of field-collected sea water or dilutions of algal extract were tested against lecithotrophic and competent planktotrophic larvae for the induction of metamorphosis using

a standard bioassay (Krug and Manzi, 1999). For each experimental treatment, 15 larvae were added to each of three replicate dishes containing 4 ml of the indicated dilution of algal extract or field-collected sea water. Larvae were scored for metamorphosis after 2 days. Each experiment included an FSW-only treatment as a negative control and live *Vaucheria longicaulis* as a positive control. The percentage of metamorphosis for each replicate was arcsine-transformed, and treatments were compared using a one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981). Unplanned comparisons of means were performed using the Scheffé procedure (Day and Quinn, 1989). Metamorphosis data are given as means ±1 S.D. because of the limited number of replicates run for each treatment (*N*=3 assays).

Dose–response curve to boiled Vaucheria extract

An aqueous extract of the alga *Vaucheria longicaulis* was prepared for use in metamorphosis and behavioral bioassays. Algal tissue (1.34 g damp mass) was extracted with 50 ml of boiling water for 10 min to prepare a solution of boiled *Vaucheria longicaulis* extract (BVE), as described previously (Krug and Manzi, 1999). To identify the minimum dose of BVE that would induce the same level of metamorphosis as living algal tissue, dilutions of the concentrated BVE solution were tested on lecithotrophic larvae in the metamorphosis bioassay to generate a dose–response curve. The minimum dose that induced more than 75 % metamorphosis was then used in subsequent assays of larval behavior. The relationship between the dose of BVE ($\log_{10}$-transformed) and percentage metamorphosis (arcsine-transformed) was estimated using a Model 1 regression (Sokal and Rohlf, 1981). Only two different doses of BVE were tested for induction of metamorphosis in planktotrophic larvae because of the limited availability of competent larvae. The mean arcsine-transformed percentage metamorphosis for planktotrophic and lecithotrophic larvae was compared for each dose using an unpaired two-tailed *t*-test.

Field-collected conditioned sea water

Previous studies showed that sea water artificially conditioned by the presence of *Vaucheria longicaulis* in the laboratory induced larval metamorphosis (Krug and Manzi, 1999). To determine whether a natural conditioning process occurs in the field, water was collected from algal patches at the Kendall-Frost field site on two occasions in November 1998. *V. longicaulis* colonizes mudflat surfaces, growing in a spongy mat similar in morphology to terrestrial moss. Patches of the algae are exposed to the air by daily low tides. Trapped seawater pools between algal filaments, and immediately following an ebb tide water is also visible as a slick on the surface of the algal mat. Conditioned sea water (CSW) was collected within patches (hereafter termed 'trapped CSW') or from the layer of water on the surface of recently exposed mats (termed 'surface CSW'). Water was collected by aspiration and transferred into a sterile plastic tube, filtered to 0.45 µm, and frozen at −80 °C until needed for experiments. The collection of trapped CSW was made during a slack low tide when algal mats had been exposed to air for 2 h. This sample of CSW had a salinity of 44 ‰ and, prior to use in assays, was diluted with MilliQ-purified water to 35 ‰, the salinity of Mission Bay water measured during the next high tide. All references to trapped CSW therefore refer to the diluted 35 ‰ salinity solution. Lecithotrophic larvae were added directly to dishes containing 4 ml of trapped CSW or CSW diluted to 50 % or 25 % with FSW and were scored for metamorphosis after 48 h. No planktotrophic larvae were available for experiments when the trapped CSW was collected. Surface CSW was collected on an ebb tide, immediately after patches had been exposed to the air; the salinity of this sample was 35 ‰. Undiluted surface CSW supported a heavy growth of bacteria that proved toxic to the larvae over the course of the 48 h metamorphosis bioassay; metamorphosis data were only obtained for surface CSW diluted to 50 % and 25 % with FSW. Both lecithotrophic and competent planktotrophic larvae were assayed for induction of metamorphosis by these dilutions of surface CSW.

Swimming behavior of larvae suspended in the water

Larval behavior was recorded using a Cohu infrared-sensitive video camera with a Pentax 100 mm macro lens. All video recording was performed in a darkroom using only infrared light; both larval morphs have identical responses to light and dark (P. J. Krug and R. K. Zimmer, in preparation). Assays were performed at 22 °C. Swimming behavior in the water was filmed in a square glass chamber. The field size recorded by the camera was 0.6 cm×0.8 cm, with the long axis parallel to the bottom. All video recordings were made with the middle of the field 1.5 cm above the bottom, with the focal plane in the center of the chamber. Video recordings were processed at 10 frames s^{-1} through a computer-assisted video motion analyzer (Motion Analysis Corp., model VP 320 and ExpertVision software) interfaced with a Sun Microsystem SPARC 2 computer workstation. Mean velocity and angular velocity (measured as the rate of change in direction, RCD) were quantified for all larval paths during each filmed treatment. Differences in mean values for all paths for a given treatment or control were compared using an unpaired two-tailed *t*-test (for comparing two means) or a one-way ANOVA with a *post-hoc* Scheffé test for unplanned comparisons of means when the *F* ratio was significant (Day and Quinn, 1989). Data are given in the text as means ± S.E.M. to reflect the large sample size for each treatment.

Preliminary experiments were conducted to determine the optimal chamber size and larval density for recording swimming behavior. Trials were initially performed in a 5.0 cm×5.0 cm×5.0 cm chamber in 100 ml of FSW at a concentration of 1 larva ml^{-1}. However, both lecithotrophic and competent planktotrophic larvae had a pronounced tendency to swim along or immediately above the chamber bottom; fewer than 2 % of larvae were suspended in the water column at any given time (P. J. Krug and R. K. Zimmer, in preparation). In a large chamber with so few larvae, it was not possible to record sufficient paths for analysis. Behavioral

assays were therefore run at a concentration of approximately 5 larvae ml^{-1} in a chamber measuring 3.0 cm×3.0 cm×3.0 cm. Under these conditions, a sufficient number of larval paths was recorded for treatments and controls to permit statistical analysis. The higher concentration of larvae did not affect the behavioral assays; collisions between swimming larvae were never observed. Likewise, the smaller chamber size did not affect larval swimming or behavioral responses. Wall effects can be ignored when $Y/L>20/Re$, where Y is the distance to the nearest wall, L is the larval characteristic length and Re is the Reynolds number for a swimming larva (Vogel, 1994). For larvae swimming at the mean velocity or faster (approximately 1.4 mm s^{-1} for treatments and controls), wall effects were calculated to be negligible under the filming conditions used. To ensure that slower larvae were not significantly affected by the presence of walls, trials were initially performed in both the larger and smaller chambers, and larval swimming speeds and angular velocities were quantified and compared; no difference was found between conditions for either mean swimming speed (large chamber, 1.3±0.4 mm s^{-1}, N=22; small chamber, 1.2±0.6 mm s^{-1}, N=28; $P>0.25$, unpaired two-tailed t-test) or rate of change in direction (large chamber, 78.1±47.3 ° s^{-1}; small chamber, 63.3±43.6 ° s^{-1}; $P>0.25$, unpaired two-tailed t-test).

Lecithotrophic larvae (50 per replicate) were used for behavioral assays 24 h after hatching; competent planktotrophic larvae (40 per replicate) were used after 32 days in culture. For each treatment and control, 10–12 replicates were run, and all larval paths were recorded and analyzed. Larvae were placed in the recording chamber in 8 ml of FSW and left to acclimate in the dark for 5 min. Control behavior was recorded in the dark for 1 min in FSW. Responses to the aqueous extract of *Vaucheria longicaulis* were recorded by placing larvae in the chamber as above, followed by the addition of 80 µl of concentrated BVE to generate a final concentration of 1 % BVE. After a 5 s delay, behavior was recorded for 1 min. The responses to field-collected conditioned sea water were assayed by adding larvae directly to 8 ml of filtered CSW in the viewing chamber, and their behavior was recorded for 1 min after a 5 s delay. Trapped CSW and surface CSW collections were tested independently on lecithotrophic larvae. Because of the limited availability of larvae, the behavior of planktotrophic larvae was only determined in response to 1 % BVE.

Swimming behavior on the bottom

Behavior on the bottom was determined in a round chamber made of a Plexiglas cylinder with an internal radius of 16 mm. The chamber rested on a clear Plexiglas sheet, and larval behavior was recorded as described above, except with the camera situated beneath the sheet, focused approximately 2 mm above the bottom. Video recordings were processed in the same manner as above. In addition to velocity and RCD, larval retention within the field of view was also quantified as net-to-gross displacement per path. Lecithotrophic larvae (50 per replicate) were placed in 6 ml of FSW and left to acclimate in the dark. After a 5 min acclimation period, 60 µl of FSW (control) or concentrated BVE solution (treatment) was added and, after a 5 s delay, larval behavior was filmed for 1 min. The response to field-collected conditioned sea water was determined by adding lecithotrophic larvae to 6 ml of undiluted surface CSW solution, and filming for 1 min after a 5 s delay. For each treatment and control, 10–12 replicates were run, and all larval paths were recorded and analyzed. Dilutions of surface CSW (1:5, 1:10 and 1:50) were made with FSW to determine the dose–response curves for larval behavior on the bottom; lecithotrophic larvae were added and filmed in the same manner as described for undiluted CSW. Six replicates were run for each dilution. Competent planktotrophic larvae were used to determine the response to 1 % BVE on the bottom. Larvae (20 per replicate) were placed in 4 ml of FSW, 40 µl of FSW (control) or concentrated BVE solution (treatment) was added and, after a 5 s delay, larval behavior was filmed for 1–2 min.

Pulse–chase experiment: metamorphosis following transient cue exposure

A pulse–chase experiment was designed to determine whether larvae would eventually complete metamorphosis in response to a temporary exposure to the chemical cue. Lecithotrophic larvae were exposed to pulses of BVE for varying durations and were then washed and transferred to FSW for the remainder of the metamorphosis bioassay (chase). Small Petri dishes were prepared with 1 % and 4 % solutions of BVE by adding samples of concentrated BVE to 4 ml of FSW. Approximately 50 lecithotrophic larvae were divided into three replicate batches, and each was added to a BVE solution for a precisely timed interval of 1, 5 or 30 min. Larvae recovered from the transfer and resumed swimming within a few seconds; the velum was therefore exposed to the cue solution for the full duration of each time interval. At the end of the timed pulse, larvae were pipetted into dishes containing FSW twice in rapid succession to wash away any traces of BVE; larvae were then transferred to dishes containing fresh FSW. They were maintained in FSW for 48 h, at which time they were scored for metamorphosis. A positive control was run for both concentrations of BVE; three replicate batches of larvae were continuously exposed to the cue for the full 48 h of the assay. Treatments using FSW diluted by 1 % or 4 % with distilled water served as negative controls.

Results

Metamorphic response

Aqueous extract of Vaucheria longicaulis

Lecithotrophic and planktotrophic larvae both metamorphosed in a dose-dependent manner after exposure to boiled *Vaucheria* extract (BVE) (Fig. 1). A dose–response curve spanning three orders of magnitude was constructed for lecithotrophic larvae (Fig. 1A). When plotted against $\log_{10}$-transformed data, variance in the percentage of metamorphosis was almost entirely accounted for by dosage (r^2=0.96). The

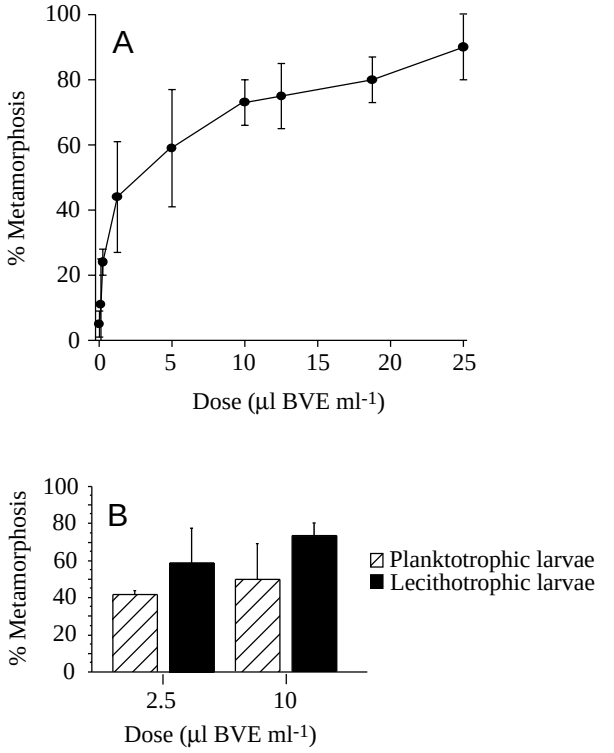

Fig. 1. Metamorphosis of *Alderia modesta* larvae in response to a dissolved chemical cue. Values are mean percentages ± S.D. (*N*=3 replicates for each concentration). (A) Dose–response curve for lecithotrophic larvae exposed to different concentrations of a standard solution of boiled *Vaucheria longicaulis* extract (BVE). The minimum concentration of BVE promoting more than 75% metamorphosis was 10 µl ml^{-1}, the equivalent of a 1% dilution of concentrated BVE. Concentrations higher than 25 µl ml^{-1} all induced more than 90% metamorphosis. (B) Comparison of the metamorphic response of lecithotrophic and competent planktotrophic larvae to two concentrations of BVE. Although lecithotrophic larvae displayed a higher mean response to both doses, the difference between the two larval morphs was not significant at either concentration.

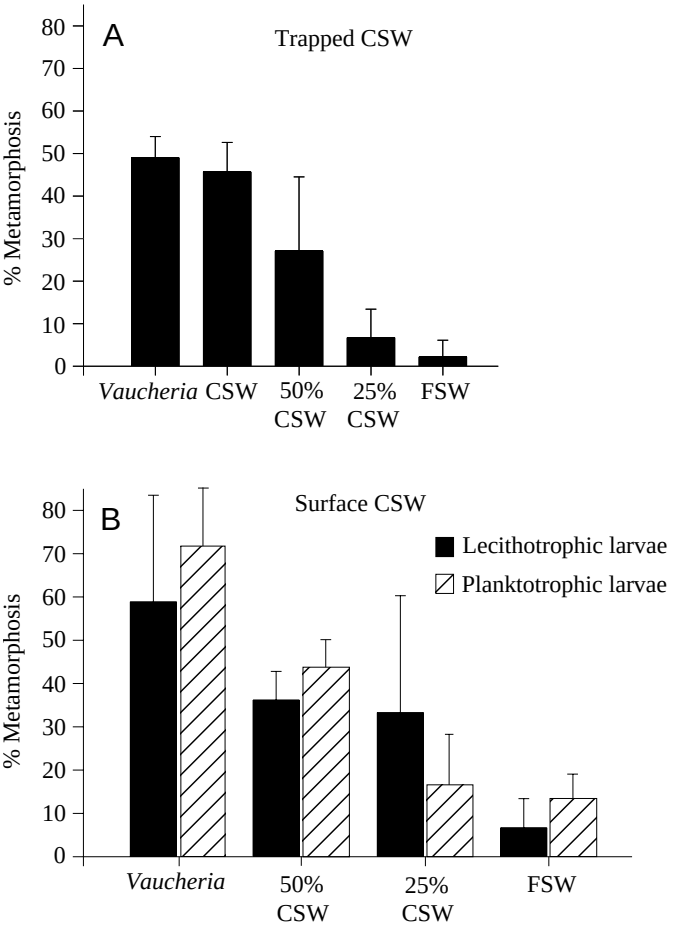

Fig. 2. Metamorphosis of *Alderia modesta* larvae in response to *Vaucheria*-conditioned sea water (CSW) collected from the field. Values are means + S.D. (*N*=3 replicates). Living *Vaucheria longicaulis* tissue was tested as a positive control for metamorphosis, and filtered sea water (FSW) was used as a negative control. (A) Trapped CSW was collected within an algal mat during a low tide and was adjusted to 35‰ salinity prior to the bioassay. Lecithotrophic larvae were assayed for metamorphosis in response to the indicated concentrations of the field-collected CSW. (B) Metamorphosis of lecithotrophic and competent planktotrophic larvae in response to diluted surface CSW. Surface CSW was aspirated from the surface of an algal mat immediately after the tide had receded. Data are presented only for dilutions of 50% and 25% because 100% surface CSW supported a microbial overgrowth that killed the larvae during the bioassay.

curve rapidly became asymptotic at concentrations of BVE higher than 10 µl ml^{-1}. This concentration, a 1% solution of BVE, was the minimum dose that consistently induced the same level of metamorphosis as living algal tissue and was therefore used as the standard concentration of BVE in all subsequent behavioral assays. Competent planktotrophic larvae metamorphosed at a level comparable with lecithotrophic larvae in response to both 1% and 0.25% solutions of BVE (Fig. 1B). The levels of metamorphosis obtained with planktotrophic larvae were slightly lower than those of lecithotrophic larvae, probably because of the presence of a few larvae that had not grown to competence in culture, but the differences were not significant (unpaired two-tailed *t*-test: $P>0.1$ for 1% BVE; $P>0.2$ for 0.25% BVE).

Field-collected conditioned sea water

To determine whether a chemical cue is released into water permeating algal patches under field conditions, samples of *Vaucheria*-conditioned sea water (CSW) were collected both from within and above algal mats and were then bioassayed (Fig. 2). The initial collection of 'trapped CSW' was made by collecting water from within algal mats. When assayed against lecithotrophic larvae, there was no significant difference between the level of metamorphosis induced by trapped CSW, a 50% dilution of trapped CSW and living *V. longicaulis* (Fig. 2A; *post-hoc* Scheffé comparison, $P>0.15$). Dilution to 25% diminished the bioactivity of trapped CSW to the level of negative controls. Water samples were also collected from the surface of damp algal patches immediately upon exposure

to air. This ‘surface CSW’ was diluted with filtered sea water (FSW) to avoid excessive microbial overgrowth during metamorphosis bioassays. Both planktotrophic and lecithotrophic larvae metamorphosed in response to field-collected surface CSW in a dose-dependent manner (Fig. 2B). There was no significant difference between the percentage of metamorphosis induced in planktotrophic and lecithotrophic larvae for any treatment, including live *V. longicaulis* and each dilution of surface CSW. This confirmed the data in Fig. 1B, further demonstrating that competent larvae of both types had approximately the same dose-sensitivity to the chemical inducer, and indicated that both larval types respond to naturally conditioned sea water containing the *Vaucheria*-associated cue.

Behavioral response of larvae to a waterborne chemical cue

Lecithotrophic larvae suspended in the water

Lecithotrophic larvae exhibited pronounced behavioral responses to an aqueous extract of *Vaucheria longicaulis* and to both samples of field-collected CSW when swimming in the water (Fig. 3). Larvae exposed to a 1 % solution of BVE turned significantly more frequently than did controls in filtered sea water (FSW); turning was quantified as a difference in the rate of change in direction (RCD) (Fig. 3A; unpaired two-tailed *t*-test, t=2.01, P<0.05). Control larvae moved in relatively straight horizontal or vertical paths, whereas cue-exposed larvae frequently swam in corkscrew spirals, rapidly reversed direction and tacked back and forth as they swam. The response was even stronger to both trapped and surface CSW, each of which stimulated a highly significant increase in turning rate (Fig. 3B; one-way ANOVA, d.f.=2,424, F=22.3, P<0.0001; *post-hoc* Scheffé test for surface CSW, P<0.0001; Scheffé test for trapped CSW, P<0.005). Both field-collected CSW samples also induced significantly lower larval swimming speeds than seawater controls (Fig. 3C; one-way ANOVA, d.f.=2,424, F=16.7, P<0.0001; *post-hoc* Scheffé test for surface CSW, P<0.0001; Scheffé test for trapped CSW, P<0.01). Both collections of natural CSW induced stronger behavioral responses than did 1 % BVE, indicating that the CSW solutions contained a higher concentration of the dissolved cue.

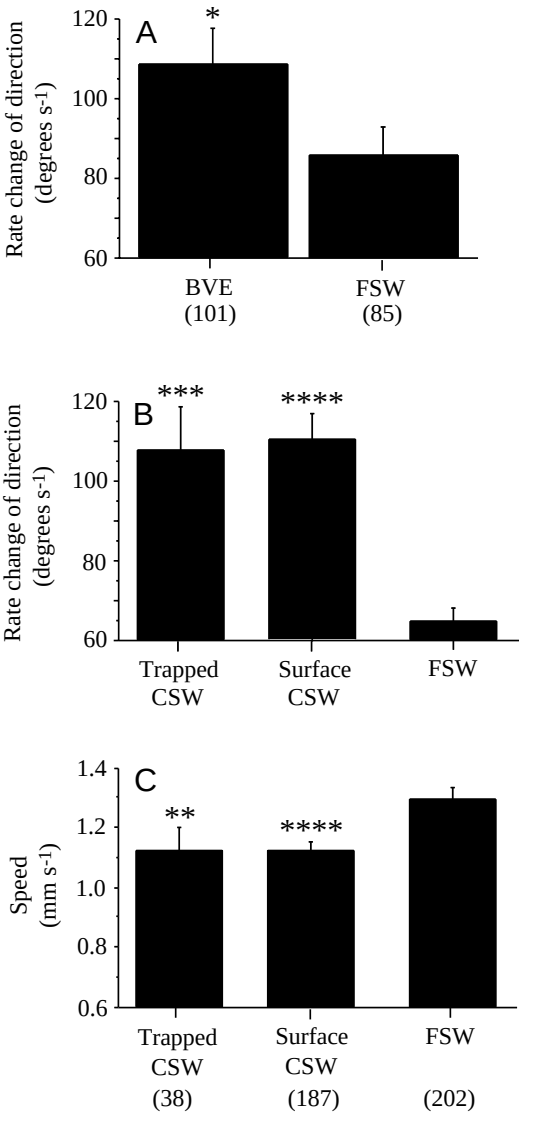

Fig. 3. Behavioral responses of lecithotrophic larvae swimming in the water to the dissolved settlement cue. Paths were recorded from the side for larvae swimming in the water and quantified using computer-assisted video motion analysis. Values are mean + S.E.M.; sample size is given in parentheses. (A) Increased rate of change in direction (RCD) for larvae exposed to 1 % boiled *Vaucheria longicaulis* extract (BVE) compared with those exposed to filtered sea water (FSW). Data were compared using an unpaired two-tailed *t*-test. (B) Increase in RCD for larvae exposed to trapped *Vaucheria*-conditioned sea water (CSW) and undiluted surface CSW. Data were compared using a one-way ANOVA with a Scheffé test for unplanned *post-hoc* comparison of means. (C) Decreased swimming speed for larvae exposed to trapped CSW and undiluted surface CSW. Sample sizes and statistical analysis were the same as in B. *P<0.05; **P<0.01; ***P<0.005; ****P<0.0001.

Planktotrophic larvae suspended in the water

Planktotrophic larvae responded to a 1 % solution of BVE in a similar manner, significantly increasing their turning rate (Fig. 4A; unpaired two-tailed *t*-test, t=2.46, P<0.05). Planktotrophic larvae swam in straight paths with only occasional changes in direction in seawater controls (Fig. 4B), but frequently tacked back and forth or spiraled when exposed to the dissolved cue (Fig. 4C). Limited numbers of competent planktotrophic larvae precluded testing their response to CSW.

Lecithotrophic larvae on the bottom

Larvae swimming along the bottom exhibited even more striking changes in swimming behavior than did those suspended in the water column when exposed to the *Vaucheria*-associated chemical cue. In seawater controls, lecithotrophic larvae swam in long, gradually curving paths or slow arcs (Fig. 5A). Within seconds of exposure to BVE or CSW, however, the larvae dramatically changed their swimming patterns. The paths of cue-treated larvae were usually a series of short rapid hops along the bottom, with

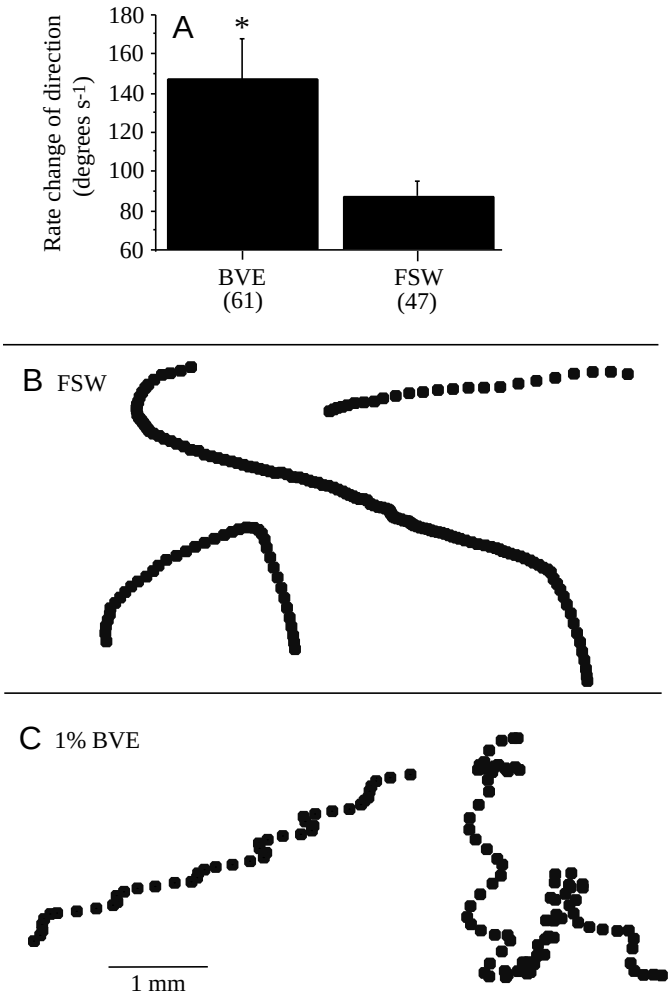

Fig. 4. Behavioral responses of planktotrophic larvae swimming in the water to algal extract. (A) Increased rate of change in direction (RCD) for larvae exposed to 1% boiled *Vaucheria longicaulis* extract (BVE) compared with those exposed to filtered sea water (FSW). Values are mean + S.E.M. and were compared using an unpaired two-tailed *t*-test. **P*<0.05 compared with the control. (B) Representative paths for larvae swimming in FSW. Data are computer-digitized video recordings of horizontal paths as viewed from the side of the chamber. Each path of dots represents the position of a given larva at consecutive one-frame intervals with video data collected at 10 frames s^{-1}. (C) Paths of larvae swimming in a solution of 1% BVE, showing the frequent turns and spirals typical of larvae exposed to the settlement cue.

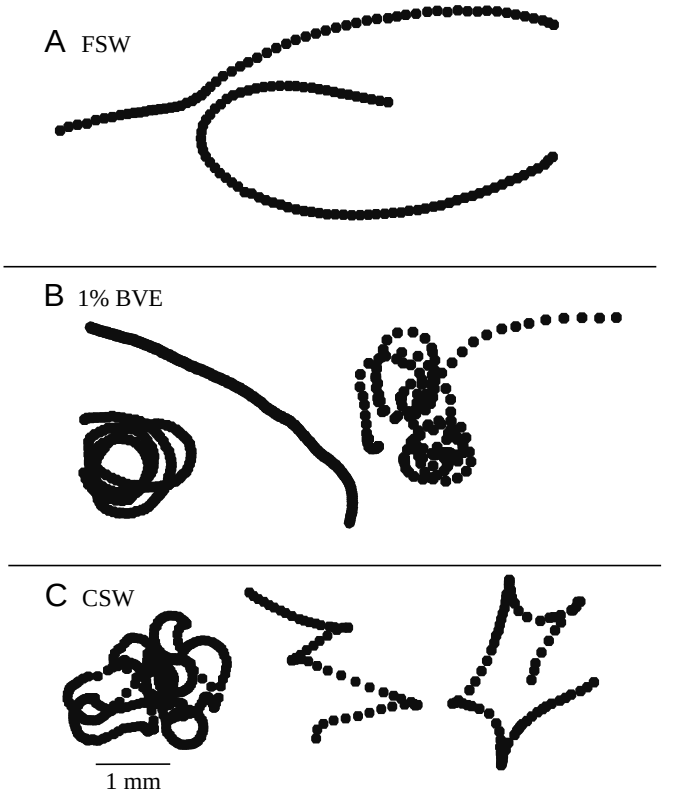

Fig. 5. Typical paths of lecithotrophic larvae swimming along the bottom. Data are computer-digitized video recordings of horizontal paths viewed from below the chamber. (A) Representative paths for larvae in control filtered sea water (FSW). (B) Larval swimming behavior in a solution of 1% boiled *Vaucheria longicaulis* extract (BVE). Paths show the tight spiraling that characterizes cue-stimulated larval swimming. (C) Paths of larvae swimming in surface *Vaucheria*-conditioned sea water (CSW). Larvae were frequently observed to make short, rapid hops across the bottom, touch down and then abruptly change direction, resulting in the zig-zag movements typical of these paths.

frequent touch-downs and direction reversals, or spirals that tended to keep the larvae localized near their starting position (Fig. 5B,C). Comparable behavior patterns were elicited by BVE and surface CSW, both of which triggered a significant increase in turning rate (Fig. 6A; one-way ANOVA, d.f.=2,251, F=18.7, P<0.0001; *post-hoc* Scheffé test for surface CSW, P<0.0001; Scheffé test for 1% BVE, P<0.05). Larvae also swam significantly faster along the bottom in response to CSW, which again elicited a stronger behavioral response than 1% BVE (Fig. 6B; one-way ANOVA, d.f.=2,251, F=3.49, P<0.05; *post-hoc* Scheffé test for surface CSW, P<0.05). The increase in RCD was dose-dependent, diminishing with each dilution of CSW (Fig. 6C; one-way ANOVA, d.f.=4,427, F=19.3, P<0.0001); both undiluted CSW (*post-hoc* Scheffé test, P<0.0001) and a 1:5 dilution of CSW (Scheffé test, P<0.05) induced significantly higher turning rates than seawater controls, and even a 1:10 dilution tended to induce a higher mean RCD than sea water alone.

Planktotrophic larvae on the bottom

Planktotrophic larvae swimming along the bottom also showed a highly significant response to a 1% solution of BVE (Fig. 7A; unpaired two-tailed *t*-test on RCD, t=3.85, P<0.0005). In sea water, planktotrophic larvae moved in slow gently curving paths above the bottom (Fig. 7B). Larvae turned with greatly increased frequency when exposed to the cue treatment (Fig. 7C). No statistical difference was evident in mean swimming speeds for planktotrophic larvae between BVE treatments and controls. Stimulated larvae swam in short, rapid movements but also frequently stopped on the bottom,

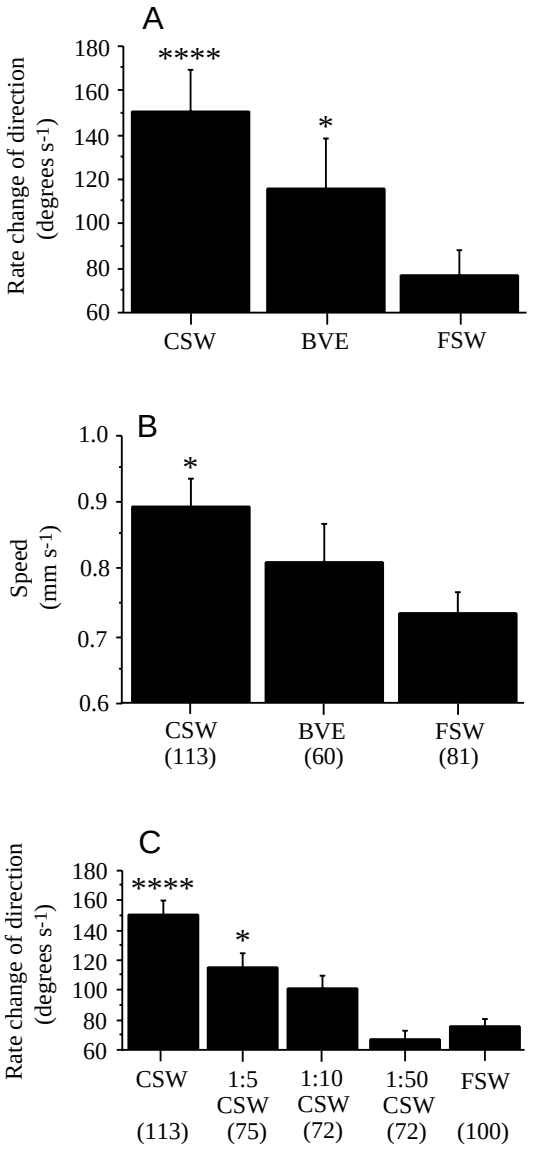

Fig. 6. Analysis of the behavior of lecithotrophic larvae swimming along the bottom. Values are mean + S.E.M.; sample size is given in parentheses. Data were compared using a one-way ANOVA with a Scheffé test for unplanned *post-hoc* comparison of means. (A) Increased rate of change in direction (RCD) for larvae exposed to surface *Vaucheria*-conditioned sea water (CSW) and 1% boiled *Vaucheria* extract (BVE) compared with those exposed to filtered sea water (FSW). (B) Increased swimming speed along the bottom for larvae exposed to surface CSW. Swimming speed also tended to increase in response to the less-active 1% solution of BVE, but the increase was not significant. (C) Dose–response plot for increased RCD in CSW treatments. Dilutions of surface CSW were made to determine the threshold at which increased turning behavior could be detected. **P*<0.05; *****P*<0.0001 compared with the control.

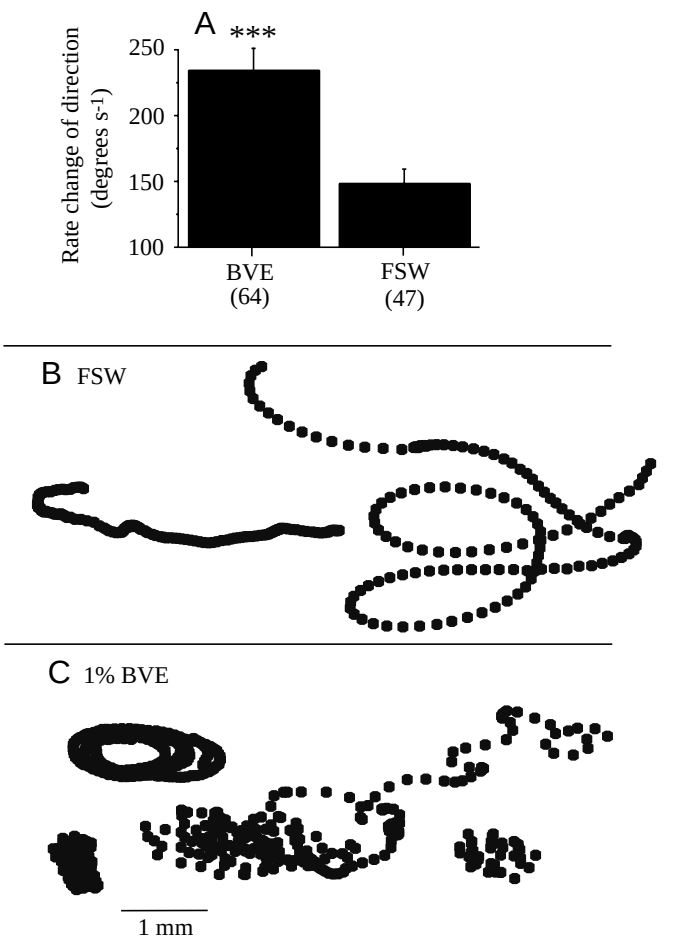

Fig. 7. Behavior of planktotrophic larvae swimming along the bottom. (A) Increased rate of change in direction (RCD) for planktotrophic larvae in response to 1% boiled *Vaucheria* extract (BVE) compared with filtered sea water (FSW). Values are mean + S.E.M.; sample size is given in parentheses. Data were compared using an unpaired two-tailed *t*-test. ****P*<0.0005 compared with the controls. (B) Typical paths of planktotrophic larvae on the bottom in FSW controls. Data are computer-digitized video recordings of horizontal paths viewed from below the chamber showing the gradual arcs and wandering lines typical for swimming behavior in sea water. (C) Paths of larvae exposed to 1% BVE, showing the typical tight, rapid spirals and the tendency of larvae to remain close to their initial position following exposure to the settlement cue.

exploring the substratum, reducing the overall mean speed computed for a given path.

Larval retention on the bottom: net-to-gross displacement

Larval movement across a video field can be expressed as net-to-gross displacement, the ratio of the linear distance from the first to the last point in a given path to the actual distance traveled. A value of zero indicates a complete circle, where the path starts and ends at the same point, whereas 1.0 is the value for a straight line. Cue-exposed larvae had a significant tendency to remain in the video field on the bottom, frequently moving in tight circles rather than straight paths. This was quantified as the difference in net-to-gross displacement, which was significant for all pairs of *Vaucheria* cue treatments and FSW controls (Fig. 8). Lecithotrophic larvae showed a mean decrease in net-to-gross displacement of 0.1 in 1% BVE and CSW treatments compared with seawater controls. Cue-

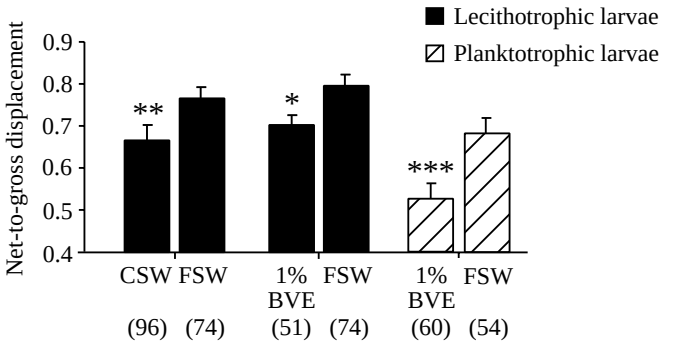

Fig. 8. Net-to-gross displacement for larvae swimming along the bottom in control and cue treatments. Paths of both lecithotrophic and planktotrophic larvae were analyzed for net-to-gross displacement (the ratio of the linear distance from the first to the last point in a given path to the actual distance traveled) as a measure of the tendency for larvae to end up near or far from their point of origin. Data (means + S.E.M.) are presented for lecithotrophic larvae in response to 1 % boiled *Vaucheria* extract (BVE) and surface *Vaucheria*-conditioned sea water (CSW) and for planktotrophic larvae in response to 1 % BVE. Data were compared within each set of treatment and the control (filtered sea water, FSW) using an unpaired two-tailed *t*-test. **P*<0.05; ***P*<0.01; ****P*<0.005 compared with the control.

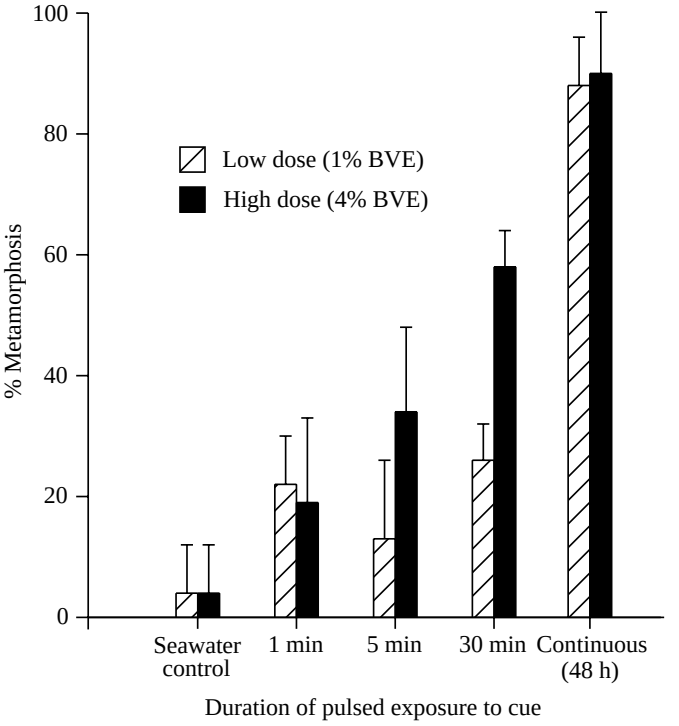

Fig. 9. Pulse–chase experiment with lecithotrophic larvae of *Alderia modesta* transiently exposed to the chemical cue. Values are mean percentages + S.D. (*N*=3 replicates). Larvae were first pulsed with a timed exposure to the concentration of boiled *Vaucheria* extract (BVE) indicated and were then washed twice in filtered sea water (FSW) and transferred to clean FSW (chased) to remove all traces of the chemical cue. Larvae were scored for metamorphosis after 48 h. Two different concentrations were tested separately. Larvae pulsed with FSW were treated in parallel as a negative control, and larvae continuously exposed to the indicated concentration for the full 48 h duration of the experiment were included as a positive control for continuous stimulation. No competent planktotrophic larvae were available for use in this experiment.

stimulated planktotrophic larvae displayed a more pronounced decrease in net-to-gross displacement, with a highly significant mean difference of 0.16 (unpaired two-tailed *t*-test, $t=-3.08$, $P<0.005$). The increased turning rate (RCD) of stimulated larvae therefore resulted in a measurable retention in the area where the cue was initially encountered along the bottom.

Transient exposure to the chemical cue: pulse–chase experiment

Exposure to the waterborne cue triggered immediate changes in behavior, increasing larval retention in areas where the dissolved cue was encountered; prolonged exposure for 48 h induced metamorphosis in most larvae. We therefore sought to determine whether temporary exposure to the cue would be sufficient to induce metamorphosis in at least some larvae. A pulse–chase experiment was designed to test whether short periods of stimulation with algal extract would increase levels of metamorphosis 48 h after the cue treatment. Only lecithotrophic larvae were used in this experiment because of a lack of competent planktotrophic larvae. Transient exposure to the dissolved cue induced significantly varying levels of metamorphosis (Fig. 9; one-way ANOVA, d.f.=9,20, $F=19.9$, $P<0.0001$). The response was sensitive to both the concentration and the duration of the pulse. A 30 min pulse of the higher dose induced significantly more metamorphosis than any other treatment except continuous exposure (*post-hoc* Scheffé comparison, $P<0.05$). A linear regression of exposure time to the 4 % dose against percentage metamorphosis showed that the duration of the pulse accounted for 68 % of the variation in the induced metamorphosis (Model 1 regression, where x is arcsine-transformed percentage metamorphosis and y is timed exposure to 4 % BVE, d.f.=1,10; $y=16.06x+1.46$; $F=21.54$, $P<0.001$, $r^2=0.68$). Even a brief pulse of the chemical cue lasting 1 or 5 min was ultimately sufficient to induce metamorphosis in 20–40 % of exposed larvae, which is higher than the level of spontaneous metamorphosis measured in any seawater controls. Temporary exposure to solutions of the cue thus induced eventual metamorphosis in a subset of larvae.

Discussion

Swimming behavior and metamorphosis induced by a soluble chemical cue

The results of this study demonstrate that larvae of a specialist marine herbivore show immediate and dramatic behavioral responses to naturally occurring chemical cues released by the adult host algae. Algal extract and natural *Vaucheria*-conditioned sea water (CSW) induced metamorphosis and caused significant changes in swimming behavior in larvae of the mollusc *Alderia modesta*.

Lecithotrophic larvae and mature planktotrophic larvae turned significantly more frequently in response to the algal cue, both when suspended in the water and when swimming along the bottom. The highly active field-collected CSW also induced significant changes in larval swimming speed. Larvae significantly reduced their net-to-gross displacement following stimulation with the chemical cue, remaining closer to their point of origin. These findings indicate that waterborne chemical cues are perceived by larvae of *A. modesta* and that larvae change their behavior within seconds of contact with the bioactive molecules. The induced behaviors are consistent with swimming patterns predicted to increase larval retention in areas where a diffusing soluble cue indicates the presence of a suitable juvenile habitat (Turner et al., 1994; Zimmer-Faust and Tamburri, 1994). The marked decrease in net-to-gross displacement shows that cue-exposed larvae will tend to remain close to the point where they encounter a pulse of chemical cue, increasing the odds that stimulated larvae will be retained near, or within, a patch of *Vaucheria longicaulis*. Once inside a patch, larvae will be induced to complete metamorphosis either by trapped water naturally conditioned with molecules released by the algae or through contact with the surface of the algal tissue (Krug and Manzi, 1999).

Larvae of both development modes metamorphosed in a dose-dependent manner in response to extracts of *Vaucheria longicaulis* and to conditioned sea water collected from the field. In most laboratory assays of metamorphosis, larvae are continuously exposed to a chemical inducer for hours or days (Pawlik, 1992, and references therein). However, it is not known how well this mimics field conditions, particularly for water-soluble chemicals. The pulse–chase experiment in the present study demonstrated that transient exposure to a solution of settlement cue lasting only a few minutes tended to induce metamorphosis in a moderate proportion of larvae. In this experiment, the duration of exposure accounted for a significant proportion of the eventual percentage metamorphosis among pulsed larvae. The effect was also dose-sensitive, with a higher concentration of cue producing elevated levels of metamorphosis compared with a lower dose. Our results have implications for metamorphosis in the field, where larvae may only experience high concentrations of waterborne cues for brief periods; turbulent flow conditions may dilute the cue or resuspend larvae into the water column over time. Swimming behaviors such as increased turning rate and decreased net-to-gross displacement will tend to increase larval residence time in areas with high concentrations of the dissolved cue. Patterns of motility such as those exhibited by both larval morphs of *Alderia modesta* will prolong exposure to the molecules that induce metamorphosis. Larvae that are only temporarily exposed to *Vaucheria*-conditioned water in algal patches can therefore actively increase their chance of initiating metamorphosis in response to the algae.

On the basis of the results of this study, predictions can be made about when maximal larval settlement should occur during a tidal cycle in the natural habitat. Mats of *Vaucheria longicaulis* are exposed to air for 5–6 h during daily low tides. Over the course of a low tide, trapped sea water becomes increasingly saturated with carbohydrates released by *V. longicaulis* (P. J. Krug and R. K. Zimmer, in preparation). Our prediction is that, when bay water initially covers the saturated algal mats on a rising tide, the trapped CSW will rapidly leach out into the water column. The flux of this concentrated solution should create a layer of surrounding water enriched in the dissolved settlement cue just above the algae. This release of conditioned sea water during the initial tidal immersion may be sufficient to enhance larval retention and settlement significantly, as a function of larval behavior and hydrodynamics. In the subsequent hours of the high tide, most of the trapped CSW will probably be diluted by turbulent mixing, and larval settlement may then depend primarily upon passive transport of suspended larvae onto patches of algae; during this time, dissolved cues and active larval choice will be less important than local flow conditions and passive distribution (Eckman et al., 1994).

We are currently testing these predictions using carbohydrate markers to trace the release of soluble compounds from *Vaucheria longicaulis* mats, along with behavioral assays of field water samples taken at sequential times during a rising tide and from various places on the mudflat. Initial results confirm that larval response correlates with the distribution of algal exudate in the water column, which varies spatially and temporally (P. J. Krug and R. K. Zimmer, in preparation). The data in the present study do not address the specificity of the observed changes in larval swimming behavior because only the habitat-specific cues produced by *V. longicaulis* were tested. Although non-specific cues from other sources could also potentially induce behavioral responses, preliminary studies indicate that the observed changes in behavior are specific to chemicals released by *V. longicaulis* (P. J. Krug and R. K. Zimmer, unpublished data). The present data demonstrate that waterborne cues are released by *Vaucheria* patches in the field and that larvae exhibit behavior patterns in response to these cues that should increase settlement onto the algae. Prior work has shown that dispersing larvae of *Alderia modesta* complete metamorphosis only in response to *V. longicaulis* and not to any other macroalgae or sediment (Krug, 2000; P. J. Krug and R. K. Zimmer, in preparation); thus, only settlement onto patches of *Vaucheria* will increase recruitment of larvae in the field.

Role of waterborne settlement cues in estuarine systems

Many studies have established that waterborne chemicals induce settlement and metamorphosis of marine larvae (Hadfield and Scheuer, 1985; Chia and Koss, 1988; Pawlik, 1992; Lambert and Todd, 1994). However, only a few studies have quantified changes in larval swimming behavior in response to chemical cues. Dissolved cues were found to affect swimming and settlement rates in larvae of the oyster *Crassostrea virginica* (Turner et al., 1994; Zimmer-Faust and Tamburri, 1994; Tamburri et al., 1996). Larval settlement and changes in swimming behavior were induced by small, basic peptides in adult oyster bath water and by the synthetic peptide

analogue Gly-Gly-L-Arg in both still and flowing water. Oyster larvae suspended in the water column swam significantly more slowly and increased their turning rate in response to the chemical cues, and some stimulated larvae were even observed to swim upstream under slow flow conditions (Tamburri et al., 1996). Oyster larvae swimming less than 1 mm above the bottom also dramatically changed their behavior, increasing their turning rate in cue treatments (Zimmer-Faust et al., 1997). These behavior patterns increased larval delivery to the bottom over a source of dissolved cue.

Waterborne chemical cues were predicted to mediate larval settlement most effectively in estuarine environments similar to those in which oysters settle, which typically have flow velocities below $7\,cm\,s^{-1}$ at 2–10 cm above the bottom (Breitburg et al., 1995; Tamburri et al., 1996). The present study provides some confirmation of this prediction by demonstrating analogous behavior in the larvae of a second estuarine species. Oysters form extensive reefs in areas similar to the mudflat habitat colonized by *Vaucheria longicaulis* and *Alderia modesta*. Oyster larvae settle gregariously in response to peptides produced by adult conspecifics, whereas *A. modesta* larvae settle in response to carbohydrates released by the obligate adult food source (Zimmer-Faust and Tamburri, 1994; Krug and Manzi, 1999). Despite these ecological differences, however, larvae of both species respond to dissolved cues with similar behavior patterns, slowing down in the water column and spiraling with increased frequency both in the water and just above the bottom. This suite of behaviors may be useful in the bottom boundary layers commonly found in estuaries and mudflats, where flow speeds are generally slow and swimming behaviors might significantly increase the rates at which larvae contact the seabed when moving along the bottom.

Developmental dimorphism and chemosensory-mediated behavior

Dimorphic traits are known from many natural animal populations and can be broadly categorized as protective, mating, life-cycle or trophic polymorphisms (Roff, 1996). The alternative development modes expressed by *Alderia modesta* represent a trophic dimorphism, producing feeding and non-feeding larvae, as well as a life-cycle dimorphism, resulting in long-lived and short-lived larvae. In most cases of developmental dimorphism, one morphology has a selective advantage over the other under a given set of environmental conditions, but also carries a fitness cost. For example, spined forms of many animals suffer reduced predation, but also have a reduced fecundity compared with the unprotected form (Roff, 1996). A similar trade-off is found in many cases of dispersal polymorphism. Winged morphs of crickets and aphids can migrate to new food patches, and the terrestrial form of certain salamanders can migrate to new ponds, but the non-dispersing morphs (wingless insects, paedomorphic salamanders) have an increased reproductive potential (Roff, 1986; Semlitsch, 1990). In some species, however, the dispersing morph expresses behavior patterns that increase the odds of colonizing a high-quality habitat, which can partially offset the reduced fecundity of the migratory form (Denno et al., 1980, 1989; Roff, 1996).

For many bottom-dwelling marine species, dispersal polymorphisms can only be expressed in the free-swimming larval stage (Strathmann, 1990). In marine life histories, there is a general trade-off between dispersal ability and survivorship of offspring (Grahame and Branch, 1985; Strathmann, 1985). Planktotrophic species produce large numbers of small, feeding larvae that can disperse over long distances and colonize new habitats (Scheltema, 1962, 1971; Levin and Bridges, 1995). However, such migratory larvae suffer high mortality rates during the prolonged maturation process as a result of predation and transport away from appropriate juvenile habitats (Rumrill, 1990; Pechenik, 1999). Lecithotrophic species produce fewer, larger larvae that do not feed and generally settle soon after hatching (Strathmann, 1978; Palumbi, 1995). Because of their abbreviated planktonic period, non-feeding larvae generally disperse for shorter distances and suffer lower mortality. As *Alderia modesta* produces both planktotrophic and lecithotrophic larvae, it is an excellent model organism for contrasting the two development modes within a single species (Hoagland and Robertson, 1988; Bouchet, 1989; Krug, 1998). Although dispersal polymorphisms have been identified in other marine organisms (Levin, 1984; Gibson and Chia, 1995), previous studies have not examined the effects of trade-offs associated with alternative dispersal strategies on larval behavior and sensory systems. Initially, we expected that planktotrophic larvae might exhibit a stronger behavioral response to habitat cues to offset the risks incurred by a lengthy planktonic phase; lecithotrophic larvae were hypothesized to require a less dramatic response, given that they hatch directly into an appropriate juvenile habitat.

Despite having radically different dispersal potentials, long-lived and short-lived larvae of *Alderia modesta* exhibited a similar suite of behaviors upon contact with environmental signal molecules. In general, there was no major behavioral distinction between the two larval morphs in response to the settlement cue; paths of stimulated larvae were similar regardless of development mode. The expectation that planktotrophic larvae would show a stronger behavioral response was, however, partially supported by the net-to-gross displacement data. Planktotrophic larvae had a more pronounced tendency to remain in place following exposure to *Vaucheria longicaulis* extract than did lecithotrophic larvae; the mean difference in net-to-gross displacement was approximately 60 % higher for planktotrophic larvae. The long-lived planktotrophic larvae were therefore more strongly retained in a given area following stimulation with the settlement cue than the short-lived lecithotrophic larvae. Qualitative observations in the laboratory also suggest that competent planktotrophic larvae tend to adhere firmly to the bottom following exposure to the dissolved cue, a settlement behavior not observed in lecithotrophic larvae. These behaviors may ensure that planktotrophic larvae maximize any fleeting opportunity to settle following a chance encounter with

a patch of *Vaucheria*. The increased responsiveness of settling planktotrophic larvae may partially offset the costs of planktotrophy as a life-history strategy, maximizing the retention of mature larvae in a suitable habitat upon contact with an environmental cue in the water column.

Unlike planktotrophic larvae, which may be carried out of their native estuary into the open ocean, lecithotrophic larvae are unlikely to be carried far from the parental habitat at the present study site (Levin, 1983). Lecithotrophic larvae of *Alderia modesta* generally remain close to the bottom and rarely swim up into the water column (P. J. Krug and R. K. Zimmer, in preparation). However, these short-lived larvae displayed the same behaviors and sensitivity thresholds as planktotrophic larvae in response to the soluble cue. Lecithotrophic larvae increased their turning rate and also showed significant changes in swimming speed following stimulation with *Vaucheria*-conditioned sea water. Thus, despite limited dispersal capacities and a tendency to swim along the bottom, lecithotrophic larvae have retained behavioral responses to waterborne settlement cues. The loss of a prolonged larval stage has not resulted in any co-evolved reduction in chemosensory abilities or diminished response to waterborne cues in this species. This finding suggests that the interaction between larval behavior and dissolved settlement cues may be important in habitat selection even for short-term planktonic organisms.

The results of metamorphosis bioassays indicated that both planktotrophic and lecithotrophic larvae had a comparable threshold for response to the chemical cue, and both larval types showed a similar dose-dependence to algal extract and to *Vaucheria*-conditioned sea water. This may indicate a fundamental physiological constraint common to both larval morphs, where a certain concentration of the environmental signal molecule is necessary to initiate the neuroendocrine cascade leading to metamorphosis. The behavior patterns measured for both larval types may therefore be sufficient to increase delivery to a patch of algae, after which larvae may adhere to blades of *Vaucheria* and metamorphose in response to secreted compounds or surface-associated higher-molecular-mass forms of the chemical cue (Krug and Manzi, 1999).

Concluding remarks

In dimorphic species, differences in development or morphology between alternative forms are often associated with differences in behavior that maintain life-history trade-offs (Roff, 1996). Both long-lived and short-lived larval morphs of *A. modesta* exhibited similar behavior patterns during habitat selection, rapidly increasing their turning rate in response to dissolved chemical cues. However, stimulated planktotrophic larvae showed a more pronounced change in swimming behavior than lecithotrophic larvae, suggesting that longer-lived larvae may have a stronger response to settlement cues. Larvae changed their swimming patterns immediately in response to waterborne cues released by the adult host algae; these behavior patterns may serve to mediate settlement in the field. Similar behavior patterns have been reported previously for another estuarine species, suggesting that larval responses to waterborne cues might commonly occur in habitats characterized by slow water flow. Larval dispersal and settlement are crucial factors affecting the population dynamics, biogeography, gene flow and macroevolution of marine invertebrates (Scheltema, 1962; Hansen, 1983; Vermeij, 1982; Jablonski, 1986; Roughgarden et al., 1988; Underwood and Fairweather, 1989; Gaines and Bertness, 1993). Future experiments conducted in flowing water and in the field will help to expand our understanding of the role dissolved cues play in mediating larval settlement in different hydrodynamic conditions and at various times during daily tidal cycles. Dimorphic species such as *A. modesta* provide the opportunity to compare the effects of life-history trade-offs on sensory systems and behaviors within a single species and should continue to yield insight into the interactions between physiology, behavior and the environment.

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