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Larval vertical migration and hierarchical selectivity of settlement in a brooding marine sponge

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ABSTRACT: Knowledge of larval behaviours of sessile marine invertebrates from release to recruitment and of the role these behaviours play in determining adult distributions is limited. In manipulative experiments using larvae from the Great Barrier Reef sponge *Rhopaloeides odorabile*, we quantified larval behaviours associated with vertical migration, phototaxis and swimming ability. We also measured settlement responses to cues associated with light, settlement surface micro-topography, coral rubble and biofilms. Following an afternoon release, the majority of larvae (72%) migrated vertically to the surface (light) for 6 to 18 h. After 24 h, 55% of active larvae had moved from the surface to the bottom and maintained this position for up to 54 h before settling. Larvae did not display gregarious settlement patterns, or a preference for settlement surface topographies, but did preferentially settle to light-exposed surfaces. Initial settlement to biofilms or coral rubble was higher than in controls with no cue. However, the transition from initial settlement and attachment to metamorphosis was much higher when treatments comprised a combination of biofilm and coral rubble compared to biofilm-only treatments (49 vs. 9%). Overall, this demonstrates that hierarchical cues contribute to selective settlement. Vertical migration to surface waters facilitates passive dispersal via wind-driven surface currents and contributes to wide-scale dispersal, while a subsequent demersal phase, where larvae actively explore the benthos for settlement sites, enables dispersal over fine, micro-geographic spatial scales.

KEY WORDS: Dispersal · Settlement cues · Vertical migration · Metamorphosis · Larvae · Marine invertebrate · Sponge

INTRODUCTION

Larval dispersal in sedentary marine invertebrates has ecological and evolutionary implications providing both adaptive advantages and disadvantages (see review by Pechenik 1999). Larval dispersal can be influenced by both oceanic processes (Sponaugle et al. 2002) and intrinsic biological traits, including larval competency (Nozawa & Harrison 2005), swimming ability (Metaxas 2001), and vertical migration behaviours (Raimondi & Morse 2000, Queiroga et al. 2002). Importantly, the poor swimming abilities observed in many marine invertebrate larvae make directed horizontal dispersal challenging, particularly into opposing currents (Chia et al. 1984, Davis & Butler 1989). Therefore, the ability of larvae to migrate vertically underpins dispersal potential because it positions larvae in bodies of water subject to different flow regimes (Young 1995).

Beyond the processes contributing to wide-ranging dispersal are habitat-related influences that mediate settlement and recruitment, often over fine spatial scales (microhabitats) (Pawlik 1992, Zimmer & Butman 2000). Successful recruitment, in part, relies on larvae identifying favourable habitats to settle. Therefore, for many marine invertebrate larvae, settlement and metamorphosis are often a response to environmental cues signalling favourable habitats. Light, salinity,

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temperature, pressure and gravity all contribute to the settlement process (Young 1995, Underwood & Keough 2000, Maldonado 2006). In addition, cues associated with physical and chemical surfaces affect larval settlement. Surface micro-topography can provide settlement adhesion points (Verran & Boyd 2001) and micro-refuges (Maldonado & Uriz 1998). Chemical cues have also been widely investigated for marine invertebrate larvae and include cues associated with biofilms of micro-organisms (Pawlik 1992, Hadfield & Paul 2001, Heyward & Negri 1999, Huang & Hadfield 2003), conspecifics (Raimondi 1991, Head et al. 2004), and host organisms (Swanson et al. 2004).

Larval dispersal and settlement is well documented for charismatic taxa such as corals, although holistic approaches quantifying the entire pre-settlement phase from larval release to recruitment are rare (Harrison & Wallace 1990, Raimondi & Morse 2000). There are even fewer studies demonstrating these processes in other important benthic taxa including sponges (see review by Maldonado 2006). This is surprising given the remarkable biodiversity of sponges and their global distributions in benthic ecosystems (Hooper & Van Soest 2002). Genetic data suggest limited dispersal abilities for sponge larvae (Duran et al. 2004, Whalan et al. 2005) with relatively few species exhibiting wide-scale dispersal (Lazoski et al. 2001). The duration of pre-settlement stages for sponge larvae is generally short, ranging from 1 h to several days (e.g. Ayling 1980, Maldonado & Young 1999). Furthermore, sponge larvae have poor swimming abilities and crawling is often observed (Bergquist & Sinclair 1968, Woollacott 1993, Maldonado & Young 1996). Therefore, restricted dispersal of many sponge species may be a result of short larval competencies coupled with poor motility.

There have been few studies of settlement behaviours for sponges and the need to develop knowledge in this field has recently been highlighted (Maldonado 2006). Depth regulation (Uriz et al. 1998), phototaxis (Leys & Degnan 2001, Maldonado et al. 2003), and surface micro-refuge characters (Maldonado & Uriz 1998) appear to be important for successful sponge larval settlement. In addition, some species respond to cues associated with micro-organism biofilms before successfully settling (Keough & Raimondi 1995, Woollacott & Hadfield 1996).

Rhopaloeides odorabile (Thompson et al. 1987) is a tropical dictyoceratid sponge common on the central Great Barrier Reef (GBR), Australia (Wilkinson & Cheshire 1989). This species is viviparous and sexually reproductive from September to February and larvae are dribble-spawned over several weeks during January and February (Whalan et al. 2007). *R. odorabile* produces tufted parenchymellae larvae typical for dictyoceratids and is one of 8 larval types known for the Porifera (Maldonado 2006). Importantly, this larval type is restricted to a handful of taxonomic families (9 out of 87) currently considered in the Demospongiae, many of which are characterised by improved mobility in comparison to other larval types in Porifera (Maldonado 2006). The ubiquitous nature of *R. odorabile* within the GBR, coupled with established information on its reproductive biology, makes this sponge an ideal model organism to begin an investigation of larval dispersal ecology. Using manipulative laboratory experiments, this study examined the behavioural characteristics of *R. odorabile* larvae from release to settlement and metamorphosis, to determine both the wide-scale and also finer spatial scale dispersal strategy of this sponge. Specifically, the vertical migration, phototactic responses, and swimming abilities of larvae were determined. In addition, settlement responses to cues associated with physical surface topographies, the degree of light exposure, and surfaces associated with biofilms and coral rubble are quantified.

MATERIALS AND METHODS

Study site and sample collection. Larvae from the sponge *Rhopaloeides odorabile* were collected from the fringing reefs off Pelorus Island (18° 33.48′ S, 146° 29.58′ E) on the central GBR. Experimental manipulations were conducted at Orpheus Island Research Station (OIRS) over 2 spawning seasons in January 2005 and 2006. Larvae were collected using larval traps (see Lindquist et al. 1997), which were placed over sexually mature sponges and checked once in the mid-morning and then 4 to 6 h later in the mid-afternoon. *R. odorabile* is an afternoon dribble-spawner (see 'Results') and afternoon collections of larvae were used in all experiments to provide larvae that could be reliably aged. Following collections, larvae were transported to OIRS to examine pre-settlement larval mobility and settlement behaviour. For the purpose of this study settlement and metamorphosis were assessed and defined separately. A successful settlement event is defined as a larva attached to the substrate by its anterior pole. Therefore, if agitation of the dish did not dislodge the larvae it was recorded as settled. Metamorphosed larvae had undergone clear metamorphic changes characterised by a flattened body plan.

Larval behaviours. The general patterns of larval behaviour were initially qualitatively assessed over 30 h by recording the majority response behaviours (i.e. >50%) of 20 newly released larvae, which were less than 2 h old, in a 100 mm Petri dish filled with 25 µm filtered seawater (FSW). Qualitative measures of be-

haviours were recorded for phototactic responses, mobility (categorized as swimming or corkscrewing), timing of substrate exploration and (or) temporary attachment, settlement, and metamorphosis. Observations were recorded over 2 min intervals, for each behavioural characteristic every hour.

Vertical orientation in the water column over time. Qualitative observations (see previous paragraph) demonstrated that *Rhopaloeides odorabile* larvae are positively phototactic during their pre-settlement phase. Therefore, manipulative experiments were designed to test whether larvae use vertical migration and if they do, whether light mediates larval vertical migration. Experiments were based on methods modified from Uriz et al. (1998). Larvae (n = 50) were placed in an experimental chamber consisting of a 1000 ml graduated glass cylinder (46 × 6.5 cm) filled with FSW. To remove bias associated with the placement of larvae to surface layers and initial static conditions, the tube was gently agitated at the commencement of the experiment. The cylinder was separated into 3 equal zones (top, middle and bottom). The positions of active larvae within each zone were recorded every 6 h until larvae settled or died. To assess whether light influenced the position of larvae within the experimental chamber (i.e. vertical migration) one treatment was exposed to a light cue (n = 6), and one excluded light (n = 6). For both treatments, water temperature was maintained at ambient seawater temperature (≈28°C) by randomly placing the cylinders in 1000 l water baths supplied with flow-through seawater. A filtered red light torch was used to view and count larvae during night observations (for the light cue treatment). For the 'without light cue' treatment, exposure to light was minimal and assumed to not affect larval behaviour for that time period record.

Treatment with a light cue: Larvae were exposed to natural light cues by placing the experimental chambers (and larvae) in an outdoor field laboratory subject to natural photoperiods. To determine the effect of light on larval vertical migration behaviours, comparisons were made to a second treatment group with no light stimulus as described in the following section. If light is important in regulating larval orientations within the water column, then removal of light should result in a random distribution of larvae throughout the water column.

Treatment without a light cue: Experimental chambers were covered with black plastic so no light cue was available to larvae. As with all larval collections, larvae were held in larval traps for up to 2 h before they could be collected and for this treatment an initial exposure to light was an unavoidable artefact. *Rhopaloeides odorabile* did not release larvae in the dark.

Swimming ability. Initial observations showed that larvae did not sustain swimming for more than 24 h. Therefore, quantitative measurements were conducted over 24 h. To quantify swimming speeds larvae were placed in a small glass aquarium (50 cm × 5 cm × 5 cm) with a 1 × 1 cm^2 grid superimposed to measure the swimming rate (cm s^{-1}) for each larva over distances of up to 20 cm. Swimming speeds of individual larvae were recorded at 6, 12, 18 and 24 h post-release (n = 20 larvae for each time period). To avoid repeated measurements monitored larvae were removed following measurements.

Larval buoyancy. Given that buoyancy may contribute to vertical migration, passive buoyancy was tested over time. Larvae (n = 20) were killed at 0, 6, 12 and 24 h post release by placing them into a solution of 10% phosphate buffered formalin followed by placement into a 50 ml graduated cylinder filled with seawater and recording whether they sank or floated.

Settlement and recruitment choices. ***Gregarious settlement:*** Settlement assays using potential cues were undertaken to assess influences on larval settlement. To reduce the risk of misinterpreting results associated with gregarious settlement responses, an experiment was firstly undertaken to determine the influence of aggregative settlement. Larvae were placed into 5 ml Petri dishes containing 25 µm FSW at densities of 1, 2, 5, 10, 25 and 50 larvae (n = 4) and settlement rates were determined every 6 h for 30 h. Larval settlement was not significantly influenced by densities of larvae (repeated measures ANOVA, between subjects, $F_{5,18} = 1.39$, $p > 0.05$). Therefore, settlement assays were conducted with 10 larvae per replicate sample, with any significant effects detected being considered as treatment rather than gregarious effects.

Settlement cues: To determine the behavioural response of larvae to different settlement substrates 2 series of experiments were undertaken. The first tested the settlement responses to shade and light exposed surfaces, surface micro-topography and biofilms. The second investigated the settlement response of larvae in the presence of potential chemical cues.

Settlement in response to light, surface micro-topography and biofilms: PVC tiles (10 × 10 cm) were used as settlement substrata. To assess larval responses to micro-refuge exploitation 3 treatments were investigated: (1) 2 to 3 mm wide × 2 mm deep grooves were cut into the surface of the tile at equal intervals across the tile, and on both surfaces; (2) tiles were roughened with coarse (grade 80) sandpaper — which produces a random pattern with consistent amplitude (Scardino & de Nys 2004) — on both tile surfaces to provide a random roughened surface; and (3) smooth tiles were used. Each surface topography

category was then exposed to either a biofilmed or non-biofilmed surface and a light or shade exposure. Biofilms were developed by placing tiles in a flow-through aquarium for 10 d at OIRS. To test for settlement responses to light (open habitats) or shaded surfaces (cryptic habitats), each tile was supported by a bolt at each corner so it could be suspended mid-water. This provided light to the upper surface whilst the underside surface was shaded. The experiment therefore tested 3 surface topographies in the presence/absence of biofilm, and the degree of light exposure.

Each treatment was replicated 6 times and randomly assigned to a shallow plastic tray (40 × 30 × 4 cm) containing FSW to which 250 larvae were introduced. Periodic observations were not made, in order to eliminate the risk of interrupting larvae settling, and numbers of settled larvae were recorded only once at 48 h. This time period was based on the results of previous experiments which suggested that most larvae had either settled or died by 48 h. After 48 h settlement, tiles were removed and observed with a stereo dissecting microscope, recording numbers of larvae that had settled. The number of larvae that settled on the sides of tiles was also recorded.

Responses to chemical cues: The previous settlement experiments identified biofilms as a settlement inducer. This treatment was therefore tested further in addition to other potential inducers that are found in adult habitats. Initially, larvae were exposed to 2 different treatments containing potential cues associated with (1) biofilmed surfaces on plastic 5 ml Petri dishes, and (2) coral rubble, a common substratum found within the immediate vicinity of adult *Rhopaloeides odorabile* populations.

Biofilm treatments were prepared by placing Petri dishes into a flow-through aquarium for 10 d to allow a biofilm to develop. Prior to experiments biofilmed Petri dishes were rinsed and shaken underwater to remove loose debris that had accumulated in the dish. For the coral rubble treatments (hereafter termed rubble), rubble was collected from the immediate vicinity of adults and maintained in flow-through seawater until required. Small portions (≅0.25 cm^3) of rubble were then placed into Petri dishes. For each group 10 larvae were placed into 5 ml Petri dishes containing FSW and the relevant treatment. A control group was simultaneously run with no treatment. The number of active, settled, metamorphosed and dead larvae were recorded every 6 h until all larvae had settled or died.

The presence of biofilms on both living and inanimate surfaces in the marine environment (including rubble used in this study) prompted an additional examination of the effect of settlement to inanimate surfaces. Of specific interest were the larval settlement responses to coral rubble if the organic biofilm was not present. Rubble was autoclaved to kill any biofilm and small pieces of sterile rubble (≅0.25 cm^3) were then placed in Petri dishes with FSW. In conjunction with this, comparative treatment groups were run with both control (i.e. no rubble) and non-sterile rubble (≅ 0.25 cm^3).

Data analysis. ***Vertical orientation in the water column over time:*** To determine if larval vertical migration behaviour differed between treatments with light, and treatments excluding light, the data were analysed using a 3-factor repeated measures ANOVA. The 6 hourly counts (i.e. time) comprised the within-factor treatment and the position in the experimental chamber (i.e. vertical orientation) and exposure to light the between-factor treatment.

Swimming ability: To assess differences in swimming speed for each time period, a 1-factor ANOVA was performed. To discern differences between swimming speeds from each time period a Tukey's Honest Significant Difference (HSD) post-hoc test was used.

Settlement in response to light, surface microtopography and biofilms: To examine differences in larval settlement responses to biofilmed surfaces, degree of light exposure, and surface topography a 3-factor ANOVA was used with biofilm and degree of light and surface topography all fixed factors. Data were (square root) transformed to satisfy assumptions of normality and homoscedasticity before ANOVA was executed.

Settlement in response to chemical cues: Time was a repeated measure in the chemical cue experiments. These data were analysed using a repeated measures 2-factor ANOVA with time being the within-factor treatment and cue the between-factor treatment. To assess differences of successful transitions from settlement to metamorphosis for each treatment (i.e. biofilm and rubble) *t*-tests were used to compare the metamorphosis for each cue at 48 h.

The difficulties of collecting adequate numbers of larvae, due to the dribble-spawning nature of *Rhopaloeides odorabile*, often did not permit replicate experiments to be run simultaneously on the same day of collection. However, each replicate was run under similar conditions, particularly the time of day the experiment commenced. To account for the potential of results showing 'different day effects' day was used as a blocking factor for all chemical cue experiments. As no significant day effect was detected, the final analysis was carried out using the combined data from all experiments run over different days.

RESULTS

Rhopaloeides odorabile dribble-spawned larvae during the afternoon with each individual releasing 50 to 800 larvae d^{-1}. *R. odorabile* produced typical tufted

parenchymella larvae approximately 250 to 300 µm long, ovoid in shape and white in colour with a dark anterior ring supporting a tuft of cilia, which was used to direct and control movement.

Larval behaviour

As an initial determination of behaviour, larvae responded positively to directed light upon release. This behaviour gradually declined after 12 h with a distinct movement away from a directed light source. Larvae were active swimmers for up to 24 h, during which time continual swimming was occasionally interrupted for exploration of the substrate or surface–water interface. After 24 h, periods of exploration became more frequent and longer. A large proportion of larvae exhibited corkscrewing swimming (i.e. rotational movement whilst positioned vertically) prior to settlement. Settlement occurred by the larvae orientating and attaching themselves to the substrate via their anterior pole. Rapid beating of the posterior cilia maintained this position and provided temporary attachment. Larvae either metamorphosed, or detached and undertook further exploration. Metamorphosis occurred by larvae using pulsating movements of the entire body eventually invaginating. Metamorphosis resulted in a flattened disk shaped form with the pigmented cilia ring occupying the central sector of the disk. Larval metamorphosis occurred on the sides and bottom of the experimental chamber and in some instances at the surface–water interface.

Vertical migration

Treatment with a light cue

The majority of larvae collected during the day and introduced into experiments with exposure to natural photoperiods occupied the surface of the experimental chamber for the first 18 h. At 6 h, 72% (±3.5) (mean ± 1 SE) of larvae were congregated at the surface compared to 6% (±1.7) and 2% (±1.4) at mid-water and the bottom, respectively (Fig. 1a). Numbers of larvae at the surface decreased gradually from 12 to 18 h, and at 24 h there was a majority shift with 42% (±4.4) of larvae migrating to the bottom compared to 20.29% (±1.1) at the surface, 4.57% (±0.7) mid-water, and the remaining 33.1% (±7.5) dead or settled. At 30 h. 42.6% (±6.2) of larvae occurred at the bottom compared to 4.3% (±0.46) at the surface. By 54 h all larvae were aggregated at the bottom. During the experiment less than 5% (±1.7) of larvae were observed at mid-water.

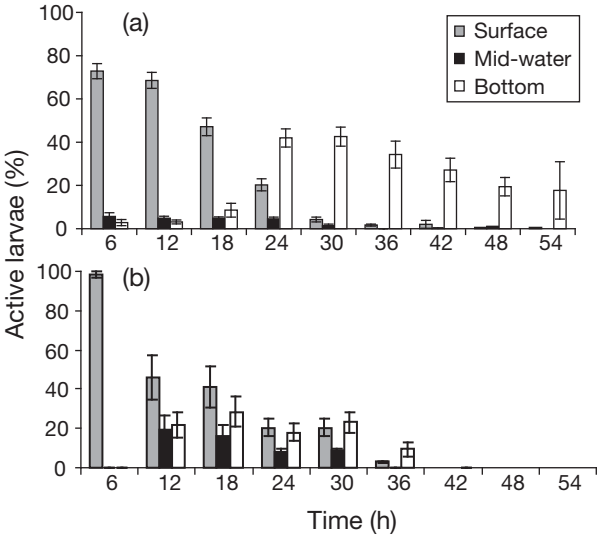

Fig. 1. *Rhopaloeides odorabile*. Mean percentages (±1 SE, n = 6) of larvae and their position within a 1 l experimental chamber over time. Only active larvae were recorded; therefore, the decrease in larval numbers over time reflects larval settlement or death. Experimental chambers (a) with full exposure to natural photoperiod regimes and (b) covered in black plastic and with no stimulus to light during the experiment

Treatment without a light cue

Prior to the experiment commencing larvae were exposed to daylight whilst held in larval traps for up to 2 h before they were collected and exposed to the experimental treatment. Therefore, these larvae were initially exposed to a light cue. When larvae were placed into the treatment in which light was subsequently removed, 98% (±1.6) of larvae occupied the surface at 6 h (Fig. 1b). However, after 12 h larvae were dispersed throughout the water column (i.e. surface, mid-water, bottom) and at 18 h 41.3% (±10.4), 16% (±5.4), and 28% (±7.4) of larvae occupied the surface, mid- and bottom layers, respectively. Similarly, at 30 h, 20.33% (±4.4), and 23% (±5.2) of larvae occupied the surface and bottom layers, respectively.

In contrast, larval migration behaviours were different when there was a light cue (compare Fig. 1a to Fig. 1b). A significant interaction between the position of larvae in the water column and the presence/absence of a light cue confirmed that light is an important determinant of larval migration behaviour (Fig. 1b, Table 1).

Larval buoyancy

Twenty larvae at 0, 6, 12, 24 and 36 h post release were all negatively buoyant and no inferential statistics were required.

Table 1. Summary results of a repeated measures ANOVA examining larval vertical migration behaviours. Time represents the 6 hourly record of larval position in the water column. Light indicates presence/absence of a light cue. Significance values based on the Greenhouse-Geisser corrections

Source	df	MS	*F*	p
Within-subjects				
Time	3.537	50.17	12.85	<0.001
Time × Position	7.1	2382.2	40.81	<0.001
Time × Light	3.53	190.1	3.26	0.02
Time × Position × Light	7.1	399.5	6.84	<0.001
Residual	116.4	58.38		
Between-subjects				
Position	2	5057.3	71.97	<0.001
Light	1	194.1	2.76	0.11
Position × Light	2	479.4	6.82	0.003
Residual	33	70.27		

Larval swimming ability

A maximum swimming speed of 0.4 cm s^{-1} (±0.01) was recorded at the commencement of the experiment, reducing to 0.3 cm s^{-1} (±0.01) at 24 h. Maximum mean swimming times showed a significant decrease over the experimental period (ANOVA: $F_{4,95}$ = 28.78, p < 0.001). Post-hoc tests (Tukey's HSD) identified that swimming speed decreased between 6 and 12 h after which there were no further significant changes in speed.

Larval responses to settlement treatments

Surface complexity, light and biofilm settlement responses

There was no significant interaction among the different treatments. However, larvae preferentially settled to treatments with a biofilm and also to light-exposed treatments (Fig. 2, Table 2). Larvae showed no preference for any of the physical surface treatments. Less than 0.01% of larvae settled on the edges of tiles and were not included in the analysis.

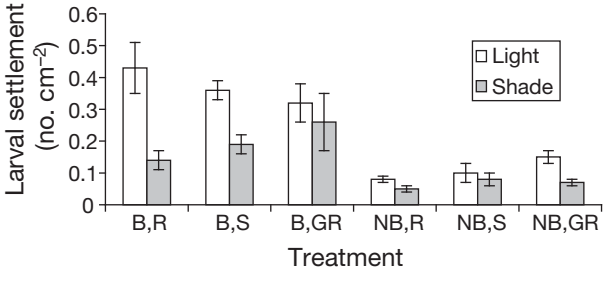

Fig. 2. *Rhopaloeides odorabile.* Mean larval settlement cm^{-2} (±1 SE, n = 6) of larvae in response to choices of biofilmed (B), non-biofilmed (NB), smooth (S), rough (R), or grooved (GR), surfaces, in addition to choices of light or shade for each treatment

Biofilmed and coral rubble settlement responses

Larval settlement response was similar for both biofilm and rubble settlement cues, with larval settlement being significantly higher for both biofilm and rubble in comparison to their respective controls (Figs. 3 & 4, Table 3) Mean larval settlement was 33.9% (±5.2) and 23.3% (±6.3) for biofilm and rubble treatments at 6 h, respectively, increasing to 82.78% (±4.6) and 80% (±5) by 48 h. The mean rates of settlement contrast sharply with the respective controls with 8.24% (±3.2) and 10% (±3.2) at 6 h for biofilm and rubble experiments, respectively, and 39.4% (±6.6) (biofilm) and 40% (±5.1) (rubble) at 48 h.

Whilst settlement rates were similar for biofilm and rubble (which also contains a biofilm) the presence of rubble appears to be required for metamorphosis. Total settlement rates (i.e. combined numbers of

Table 2. Summary results of a 3-factor ANOVA comparing settlement rates when larvae are offered a choice of surfaces including, biofilmed or non-biofilmed, light or shade or different surface textures (i.e. rough, smooth or grooved)

Source	df	MS	*F*	p
Biofilm (B)	1	0.27	22.25	< 0.001
Light (L)	1	920.92	77.58	<0.001
Surface (S)	2	0.008	0.71	0.49
B × L	1	0.19	1.56	0.22
B × S	2	0.009	0.74	0.48
L × S	2	0.006	0.49	0.62
B × S × L	2	0.19	1.57	0.22
Residual	57	0.12		

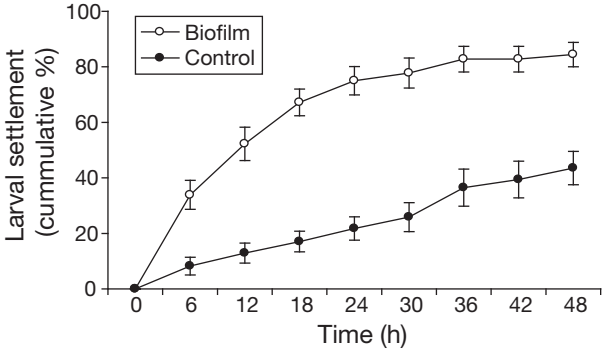

Fig. 3. *Rhopaloeides odorabile.* Cumulative mean percentages (±1 SE, n = 12) of settled larvae when exposed to a biofilmed surface (Petri dish) compared to settlement in control treatments without a biofilm

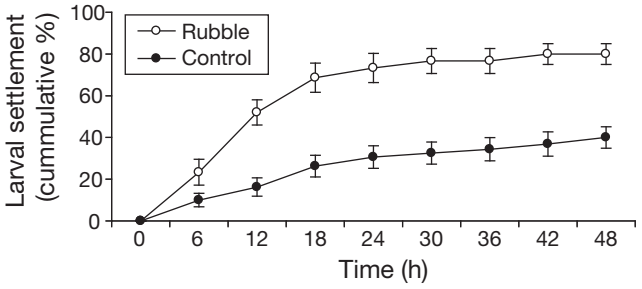

Fig. 4. *Rhopaloeides odorabile.* Cumulative mean percentages (±1 SE, n = 16) of settled larvae when exposed to experimental conditions with rubble compared to settlement in control treatments with no rubble

Table 3. Summary results of 2 repeated measures ANOVAs comparing larval settlement in the presence of each of the cues associated with biofilms and rubble. Significance values based on Greenhouse-Geisser corrections

Source	df	MS	*F*	p
Biofilm				
Within-subjects				
Time	1.9	223.1	32.6	<0.001
Time × Cue	1.9	29.8	4.3	0.02
Residual	38.9	6.9		
Between subjects				
Cue	1	937.6	44.4	<0.001
Residual	21	21.42		
Rubble				
Within subjects				
Time	2.9	92.14	27.16	<0.001
Time × Cue	2.9	9.9	2.93	0.04
Residual	75.5	3.4		
Between subjects				
Cue	1	356.17	14.48	<0.001
Residual	26	24.59		

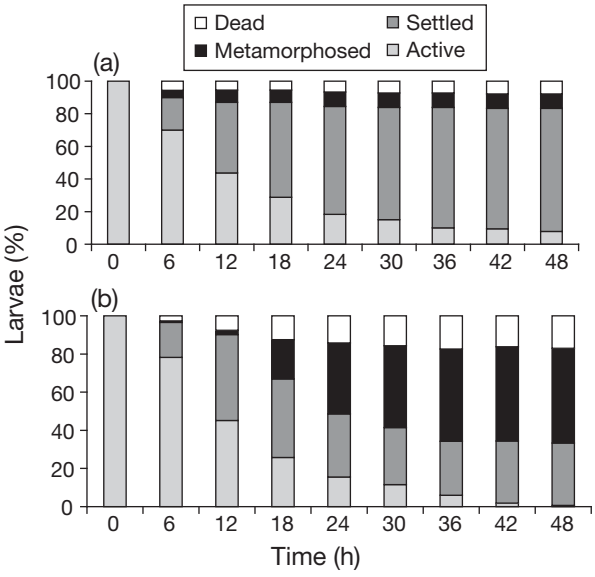

Fig. 5. *Rhopaloeides odorabile.* Percentages of larvae represented as active, settled, metamorphosed or dead when exposed to treatments of (a) biofilmed surfaces over time, and (b) rubble over time

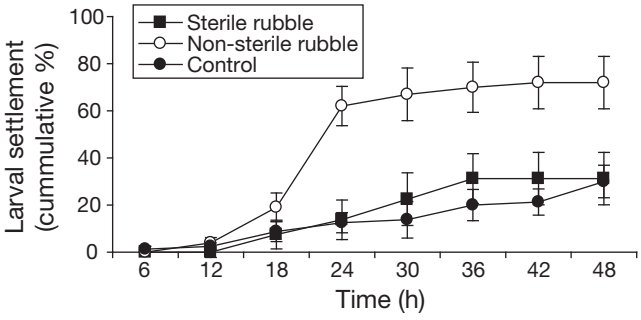

Fig. 6. *Rhopaloeides odorabile.* Cumulative mean percentages (±1 SE, n = 5) of settled larvae when exposed to experimental conditions of non-sterile rubble compared to settlement in sterile rubble and control treatments with no rubble

settled and metamorphosed larvae) were consistent for biofilm and rubble; however, successful metamorphosis was significantly lower in biofilmed treatments (Fig. 5a,b, $t = -4.25$ df = 29, $p < 0.001$). For example, larval metamorphosis reached 8.9% (±2.1) for biofilmed treatments in comparison to 49.4% (±7.1) for rubble treatments. Mortality was quantified at 7.2% (±3.7) for biofilm and 16.9% (±3) for rubble treatments.

Autoclaved rubble presented a sterile, inanimate surface with no biofilm cue. Larval settlement was significantly lower when compared to larval settlement with non-sterile (i.e. biofilmed) rubble (Fig. 6; repeated measures ANOVA, time × cue, $F_{3.6,17.8} = 6.4$, $p < 0.001$).

DISCUSSION

Vertical migration is an important dispersal strategy for many invertebrate larvae (Young 1995) and this is also the case for *Rhopaloeides odorabile*. The swimming abilities of sponge larvae (Maldonado & Young 1996) are unlikely to facilitate independent horizontal dispersal. However, vertical migration positions larvae in bodies of water that provides greater probabilities of passive transport (Willis & Oliver 1990). Vertical migration has been observed in many marine invertebrate larvae, including sponges (Uriz et al. 1998), and is invoked as a process that facilitates dispersal (Willis & Oliver 1990). Although the results of this study are limited to laboratory manipulations the movement of larvae towards surface waters in the field would promote passive dispersal via wind-driven surface currents.

Whilst it is clear *Rhopaloeides odorabile* larvae use vertical migration, a comprehensive understanding of the mechanisms driving this behaviour is less clear. A variety of biological mechanisms can regulate vertical migration behaviours in sponges including phototaxis (+ve and –ve), geotaxis, and changes to buoyancy (see review by Maldonado 2006). Despite no real neural capacity, tufted parenchymella sponge larvae utilize pigment cells in their propelling cilia to respond to light (Leys & Degnan 2001, Maldonado et al. 2003). Indeed, tufted parenchymella larvae, which include *R. odorabile*, are restricted to a handful of taxonomic families (6 out of 87) currently considered in Demospongiae. These families are commonly characterised by improved swimming, sensory and behavioural abilities, in comparison to the other larval types known in Porifera (Maldonado 2006). *R. odorabile* larvae subjected to natural photoperiods moved upwards following release and maintained this position for up to 18 h, suggesting positive phototactic behaviour, after which time they moved, or sank to the bottom. When light was excluded larvae exhibited different movement patterns. Specifically, without light larvae did not show clear congregation patterns and instead dispersed throughout the water column, confirming that light contributed to the direction of vertical migration for *R. odorabile* larvae. However, an initial exposure to light provided a sufficient imprint to direct upward movement despite the light cue being subsequently removed (Fig. 1b). The dispersion of larvae throughout the experimental chamber after 6 h suggests that any initial light imprint is eventually lost.

Larval vertical migration can also be mediated by gravity or buoyancy (see review by Maldonado 2006). Whilst gravity has been implicated in vertical migration in some sponge species (Warburton 1966) there is no evidence of receptors that monitor gravity in sponges (Leys & Degnan 2001). Pre-settlement spicule development may change the centre of gravity in sponge larvae and aid in directed movement (Maldonado et al. 1997), but *Rhopaloeides odorabile* is a dictyoceratid sponge and does not contain spicules. *R. odorabile* is however negatively buoyant for the duration of its larval life, facilitating descent, after which it presumably continues to disperse over finer spatial scales during a demersal phase.

When presented with a range of settlement surfaces *Rhopaloeides odorabile* larvae preferentially settled on plates exposed to light and with a biofilm regardless of the physical surface textures. The preference for larvae to settle on light-exposed surfaces was surprising given the findings for vertical migration for this species, where movement after 18 to 24 h is directed to the bottom (i.e. away from light). Given the larvae's apparent preference to settle to light-exposed surfaces their return to the benthos may be indicative of larvae approaching the end of the settlement phase, at which point they simply sink to the bottom irrespective of their phototactic attitude. The mechanisms explaining this behaviour were not determined, but this pattern has been observed in other sponge larvae, which sink to the benthos irrespective of light conditions (Maldonado 2006). It also supports observations of adult distributions in the field where *R. odorabile* is most commonly found attached to the walls of massive coral structures or directly to benthic surfaces that are exposed to relatively high light levels. The apparent indifference of settling larvae to the level of micro-refuge of the surface was unexpected as micro-refuges minimise the risk of being incidentally grazed by indiscriminate grazers (Maldonado & Uriz 1998).

Biofilms are important in initiating a settlement response for *Rhopaloeides odorabile* larvae. The presence of a biofilm decreases the time to settle, and increases the number of larvae settling. Rubble showed similar results; however, larval settlement to rubble is almost certainly a response to the biofilm associated with the rubble. Higher settlement to treatments containing non-sterile rubble, compared to those containing sterile rubble, shows that settlement to the surface of the rubble is cued by a biofilm.

Although settlement rates were highest in biofilmed treatments, the number of settled larvae successfully undergoing metamorphosis was lower than on rubble. Successful recruitment for *Rhopaloeides odorabile* therefore relies on larvae firstly identifying biofilmed surfaces to induce settlement, with additional cues associated with coral rubble promoting metamorphosis. These may be associated with $CaCO_3$ in the rubble, in particular Ca^{2+} ions as suggested by Anderson (1996) and Guenther & de Nys (2006). Alternatively, additional cues associated with surface structure may be important, as they are for metamorphosis in some coral species (Negri & Heyward 1999).

This study clearly demonstrates that larval behaviour is an important contributing factor to successful dispersal and settlement. The sequential behaviour of vertical migration (phototaxis), swimming, and subsequent preferential selection of habitat cues, provides important information on the processes determining adult distributions of *Rhopaloeides odorabile* and acts as a model for broader investigation of hierarchical cues for sessile marine invertebrates.

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