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RESEARCH ARTICLE

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Settlement of larval blacklip abalone, *Haliotis rubra*, in response to green and red macroalgae

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Abstract Surfaces from the habitat of adult *Haliotis rubra* were tested as inducers of larval settlement to determine the cues that larvae may respond to in the field. Settlement was high on the green algal species *Ulva australis* and *Ulva compressa* (Chlorophyta), the articulated coralline algae *Amphiroa anceps* and *Corallina officinalis*, and encrusting coralline algae (Rhodophyta). Biofilmed abiotic surfaces such as rocks, sand and shells did not induce settlement. *Ulvella lens* was also included as a control. Treatment of *U. australis*, *A. anceps* and *C. officinalis* with antibiotics to reduce bacterial films on the surface did not reduce the settlement response of *H. rubra* larvae. Similarly, treatment of these species and encrusting coralline algae with germanium dioxide to reduce diatom growth did not significantly reduce larval settlement. These results suggest that macroalgae, particularly green algal species, may play an important role in the recruitment of *H. rubra* larvae in the field and can be used to induce larval settlement in hatchery culture.

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Introduction

Many benthic marine invertebrates have a complex life history with a sessile adult phase and a dispersive, planktonic larval phase. Variability in recruitment is one of the major factors that affect the dynamics of benthic populations (Caley et al. 1996). Settling and metamorphosing larvae are highly influenced by chemical, biological and physical cues that are characteristic of a suitable environment for newly recruited individuals. Chemical cues play an important role in determining the selection of habitat by invertebrate larvae (Hadfield and Paul 2001; Steinberg et al. 2001). For example, larvae of the sea urchin *Holopneustes purpurascens* (Swanson et al. 2004; Williamson et al. 2000) and the gastropod *Alderia modesta* (Krug and Manzi 1999) recruit specifically in response to compounds produced by the algal species that provide their juvenile habitat. Chemical cues produced by microbial films associated with habitats are also important to the settlement and metamorphosis of many marine herbivore larvae (reviewed by Holmström and Kjelleberg 2000).

Many species of invertebrate larvae, including abalone, exhibit gregarious settlement, settling in higher numbers in response to high densities of either juvenile or adult conspecifics (Toonen and Pawlick 1994). Encrusting coralline algae also induce a strong settlement response in many species of abalone (Morse et al. 1984; Barlow 1990; Moss 1999; Roberts and Nicholson 1997). This response can be either generally applied to a collection of unidentified encrusting corallines, commonly referred to as CCA (Roberts and Nicholson 1997), or it may be a species–specific settlement response, restricted to a discrete number of encrusting coralline species (Daume et al. 1999a). Such behaviour could account for high numbers of settled larvae in

laboratory studies, and could also explain the distribution of populations in the field (Rodriguez et al. 1993).

Abalone, particularly commercially important species, have been the focus of many studies for settlement cues. Cues from a wide range of sources induce settlement of *Haliotis* larvae, including compounds associated with phycobiliproteins in encrusting coralline algae (Morse et al. 1984), films of diatoms (Takami et al. 1997), mucus trails from grazing adults (Takami et al. 1997), extracts from foliose red algae from the genera *Laurencia, Gigartina* and *Porphrya* (Morse et al. 1984), and artificial inducers such as potassium chloride (Bryan and Qian 1998) and γ-aminobutyric acid (Baxter and Morse 1987). However, macroalgae as a group have yet to be thoroughly investigated as a source of potential settlement cues despite their prevalence in the adult habitat. Also, bacterial films associated with the surfaces of algae are thought to play an important role in inducing settlement of many invertebrate larvae, including the crown-of-thorns starfish (Johnson and Sutton 1994), some hydrozoan and cnidarian species (Leitz 1997), and the coral *Acropora millipora* (Negri et al. 2001). Apart from a very small number of unpublished studies (reviewed by Roberts 2001), the role of bacteria as a source of settlement cues to abalone larvae is unknown.

The Australian blacklip abalone *Haliotis rubra* is a commercially harvested species in Australia worth more than A$300 million annually and our current knowledge of recruitment factors in the field is poor. Existing data suggest that common cues considered responsible for inducing other species of abalone are unlikely to be effective as inducers for *H. rubra* (Daume et al. 1999b, 2000). The settlement response of *H. rubra*, to both encrusting coralline algae and local diatom films is consistently low (Daume et al. 1999b) with a maximum of 25 percent of larvae settling. However, the response to the green alga *Ulvella lens* is significantly higher, reaching up to 52 percent settlement (Daume et al. 2000). Field studies revealed that diatoms did not induce settlement, and a consistently low and species–specific response was observed on encrusting corallines (Daume et al. 1999b). Despite these probing studies, there remains a lack of understanding as to the actual cues to which *H. rubra* larvae respond in the field.

To investigate the response of *H. rubra* to local red, green and brown algae, and biofilmed abiotic surfaces, and the source of settlement cues (plant, bacterial or diatom), we conducted a series of settlement experiments. For comparison *Ulvella lens* was included in some settlement assays even though it was not present at any of our sampling sites.

Materials and methods

Study sites and organisms

Settlement assays were done using local algae at two locations in New South Wales: Tomaree Head (New South Wales Fisheries) and at the University of New South Wales, (UNSW) Sydney. Algae used in the settlement assays at Tomaree were collected from Shoal Bay (152°10′00″E, 32°43′05″S), Anna Bay (152°5′00″E, 32°49′12″S) and Fingal Bay (152°10′00″E, 32°45′30″S). Algae and other substrata used in settlement assays at UNSW were collected from Shark Bay in Sydney Harbour (33°51′09″S, 151°16′00″E), Bare Island in Botany Bay (33°59′38″S, 151°14′00″E), and Clovelly, Sydney (33°54′00″S, 151°16′00″E), subject to weather conditions. These sites are representative of common subtidal habitats found along the eastern coast of New South Wales; for a detailed description see Wright and Steinberg (2001). For comparison, the green alga *Ulvella lens*, known to induce high rates of settlement in *H. rubra* larvae (Daume et al. 2000) was also tested. *U. lens* was not found in the field, instead it was grown on plastic plates (Melbourne University, Australia), transported to Sydney in seawater and held at the UNSW in a 30,000 l recirculating seawater system for several weeks at 19°C with a 14:10 h light:dark cycle.

Haliotis rubra larvae were reared at the NSW Fisheries abalone hatchery at Tomaree Head. Batches of 7- to 8-day-old competent larvae (i.e., those with the third tubule on the cephalic tentacle present and exhibiting crawling and columning behaviour (Hahn 1989) were used in each experiment. Each assay used larvae from a single batch and no batch was used for more than one assay.

Protocol for settlement assays

The initial two macroalgal assays were conducted at the abalone hatchery at Tomaree Head. Following the completion of these two assays, all subsequent assays were conducted at UNSW. Competent larvae were transported from the hatchery in sterile seawater, and allowed to acclimatise at 19°C for 24 h. We have defined the term ‘settlement’ to describe the permanent attachment of larvae to the substrate after shedding the velum to complete metamorphosis.

Pieces of algae (0.05–0.1 g wet weight) were presented in each macroalgal assay, with the exception of *Ulvella lens* that was grown on plastic plates and presented in 1 cm^2 pieces, and of crustose coralline algae (CCA), ‘lumpy and encrusting’ (Daume et al. 1999a), presented as a minimum of 95% cover on small rocks with upper surface approximately 1 cm^2 (following Roberts and Nicholson 1997). To ensure independence, each replicate piece of alga or square of plate was selected from a new plant or plate.

Initial shell growth observed under light microscopy was used to identify newly metamorphosed individuals (Hahn 1989). Assays included ten replicate treatments and a sterile seawater control, and additional controls described for each experiment. Seawater was sterilised in 10 l containers by autoclaving (SSW). Algal pieces were added to sterile 5 mL Petri dishes containing 4 mL SSW.

Ten competent larvae were added to each dish, except for the assay testing for gregarious settlement, as outlined below. After 48 h and 96 h the number of metamorphosed individuals per dish was counted using a dissecting microscope. All results presented refer to 96 h counts.

Gregarious settlement

To determine if competent larvae respond gregariously to conspecifics, abalone larvae were added to dishes at densities of 1, 5, 10 and 50 larvae per dish. SSW, along with positive controls, based on Daume et al. (1999b, 2000), of the coralline alga *Corallina officinalis* were used for each density treatment.

Settlement in response to macroalgae

Two macroalgal assays were done to determine the settlement response of larvae to the dominant local marine flora. The first assay tested settlement in response to the red algae *Delisea pulchra, Amphiroa anceps, Solieria robusta, Jaynia micrarthrodia* and pebbles coated with encrusting coralline algae (CCA); the green algae *Codium fragile* and *Ulva australis*; and the brown algae *Ecklonia radiata, Sargassum vestitum* and *S. linearlifolium*. The second assay aimed to confirm the high settlement response shown by larvae to the green alga *U. australis* in the previous assay, and tested settlement in response to the coralline red algae *Corallina officinalis*, CCA and *J. micrarthrodia*; the green alga *U. australis*; and the brown algae *E. radiata* and *S. vestitum*.

Settlement in response to green algae

Larval settlement in response to several green algal species was compared. Species used in the assay were *Ulva australis, Ulvella lens, Codium harveyii, C. lucasii, Caulerpa filiformis* and *U. compressa*. *U. lens* was included in the assay as it has previously been reported as a high settlement inducer, inducing up to 52% of *H. rubra* larvae (Daume et al. 2000).

Settlement in response to rocks, sand, shells and shell-grit

The settlement response to common substrata from the adult abalone habitat was tested to assess biofilmed abiotic surfaces as settlement cues for *Haliotis rubra*. Substrates were rocks, shell, shell-grit and sand. Seawater collected from 1 m depth was also included as a control, and based on the results from the macroalgal assays, the coralline alga *Amphiroa anceps* was used as a positive control. Shell and rock pieces used in the assay were approximately 1 cm^2, and both shell-grit and sand were added to Petri dishes so that a complete coverage of the bottom of the dish was achieved, to a depth of approximately 0.5 mm. The surface area of the bottom of the dishes was approximately 3.5 cm^2.

Settlement in response to algal extracts

To determine if larvae respond to natural products from algae, *Amphiroa anceps* and *Ulva australis*, two algae that induce high rates of settlement, were extracted. Algae were extracted in methanol (AR) (MeOH) for 24 h. The MeOH was removed, and the algae subsequently re-extracted twice with MeOH for 3 h. All three MeOH fractions were retained. The MeOH was reduced in vacuo and the resultant crude extract separated into polar and non-polar fractions by partitioning between dichloromethane (DCM) and MilliQ water (MQ). Each fraction was reduced in vacuo and re-dissolved in small volumes of either DMSO (non-polar) or MQ (polar) for addition to bioassays. Crude extracts were presented to larvae in a settlement assay at two concentrations. One concentration level was equal to the extract of 0.1 g algae (calculated from the weight of algae extracted, and the weight of extract produced). This was doubled for the higher concentration treatment. Extracts were added to SSW and the larval settlement response compared to the response to control plants.

Settlement in response to the presence/absence of biofilms

The high settlement response to a number of red and green algae in this study provides the rationale to investigate if *Haliotis rubra* larvae are responding to a bacterial cue that is shared by all of these algae. To determine if the cue was associated with bacteria, we treated three representative algal species with antibiotics and presented them to competent *H. rubra* larvae. Assays were conducted using the three algal species *Ulva australis, Amphiroa anceps* and *Corallina officinalis* that induced a high settlement response (see results), and included the following five treatments.

1. *Antibiotic treatment*: Algal pieces were soaked in 10% Betadine solution, diluted in SSW, for 5 min, rinsed in SSW and soaked in a 2 μm filter sterilised antibiotic solution containing streptomycin sulphate (20 mg/L), penicillin G (10 mg/L), neomycin (2 mg/L) and kanamycin (10 mg/L) for 48 h (modified from Johnson and Sutton 1994; Xue-wu and Gordon 1987). Algal pieces were then rinsed again in SSW before being added to assay dishes.
2. *Antibiotic treatment with agar wipe*: Identical to (1) with the addition of wiping algal pieces over the surface of sterile agar both before soaking in Betadine and after soaking in the antibiotic solution. This treatment was included in order to facilitate the physical removal of the biofilm from the surface of the algae. After wiping over agar, algal pieces were gently rinsed in SSW.

3. *Control treatment for the agar wipe process*: The plant was wiped over the surface of sterile agar and gently rinsed in SSW.
4. *Control treatment for soaking*: Algal pieces were soaked in SSW for 48 h.
5. *Unmanipulated control* piece of algae.

To determine the success of the above treatments in removing bacteria from the algal surfaces, the number and diversity of bacteria living on each of the algal treatments was assessed for *C. officinalis* and *A. anceps* using culturing methods. Triplicate pieces of algae from each treatment were added to 1 mL SSW. Samples were vortexed for 5 min, repeated four times, to remove bacteria from the surface of the plants. Hundred microliters of the resulting solution was added to 900 µl of SSW, and this solution was vortexed for 30 s, then a further three dilutions were serially prepared in the same manner. Thus the resulting dilution series contained the dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} of the original bacterial solution. Hundred microliters of each dilution was added to Marine Agar plates and spread evenly over the surface of the plate. Plates were observed daily for 2 weeks, and the number and diversity of colonies per plate counted. Bacterial colonies were distinguished on the basis of their colour, lustre, margin, elevation, form and agarolytic status (Prescott et al. 1995).

Settlement in response to diatoms

Four algal species—*Corallina officinalis, Amphiroa anceps*, CCA and *Ulva australis*—were high inducers in settlement assays (see results). The response of abalone larvae to these algae treated with germanium dioxide, which reduces diatom growth (Mansilla et al. 2002), was assessed. Algal pieces were held in an aerated 1 mL/L germanium dioxide solution for 3 weeks, with changes of solution every 3–4 days, at 19°C with a 14:10h light:dark cycle. Control algal pieces were held in aerated SSW in the same conditions and for the same duration. Fresh algae were collected on the morning of the assay as controls.

To assess the effectiveness of the germanium dioxide treatments in reducing diatom cover for *C. officinalis* and *A. anceps*, diatom counts were conducted for 10 haphazardly selected sections of each piece of alga. Each section was photographed using a compound microscope, with 40× objective lens, and camera, and the total cover of diatom patches present on algal pieces (area mm^2/mm algal perimeter) was measured. Since initial trials of this method proved unreliable for *U. australis* and CCA, no formal assessment of diatom cover was made for these species.

Data analysis

Statistical analyses were carried out using SYSTAT 7.0. Percentage of larvae metamorphosed after 96 h, abundance and diversity of bacterial isolates and diatom cover were analysed. Assumption of normality and homogeneity of variance was checked graphically for each data set prior to analysis, and data were transformed where necessary. For gregarious settlement, single larva data were excluded and data was arcsin-transformed. One-way Analysis of Variances (ANOVA) with Tukey's HSD post hoc tests were used for all analyses.

Results

Gregarious settlement

There was no evidence of gregarious settlement by *Haliotis rubra* larvae. Larval settlement did not increase with increasing density (one-factor ANOVA, $F_{5,54}$ 51.326, $p < 0.05$, data arcsin transformed) and at the highest density, larval settlement was decreased slightly (31.4%, $n = 50$ larvae) in comparison to lower density treatments (50, 45 and 49%; $n = 1$, 5 and 10 larvae respectively). In sterile seawater alone, no settlement occurred, even when high densities of larvae were present.

Settlement in response to macroalgae

Larvae metamorphosed at varying rates in the presence of macroalgal species.

In the first macroalgae settlement assay significantly higher settlement occurred on *U. australis* (25.8 ± 2.9%), *J. micrarthrodia* (23.9 ± 1.9%), encrusting coralline algae (20.7 ± 2.0%) and *C. officinalis* (19.1 ± 1.8%) than on co-occurring macroalgae and SSW controls (one-factor ANOVA, $F_{10,98}$3.770, $p < 0.05$, Fig. 1). A low settlement response was observed on the brown algae *E. radiata* (1.8 ± 2.25%) and *S. vestitum* (1.5 ± 2.1%). In the second macroalgal settlement assay, significantly higher settlement occurred on encrusting coralline algae (33.5 ± 3.4%), *J. micrarthrodia* (27.1 ± 2.5%), *A. anceps* (13.5 ± 1.5%) and *U. australis* (13.1 ± 1.3%) than on co-occurring macroalgae and seawater controls (one-factor ANOVA, $F_{6,\ 63}$20.582, $p < 0.05$, Fig. 2). A low settlement response was observed to the brown algae *E. radiata* (5.9 ± 0.6%), *S. vestitum* (6.7 ± 0.6%) and *S. linearfolium* (4.2 ± 0.5%), the fleshy red algae *D. pulchra* (3.6 ± 0.4%) and *S. robusta* (7.5 ± 0.8%) and the green alga *C. fragile* (1.1 ± 0.1%). These macroalgae did not induce settlement at rates significantly higher than control SSW treatments (1.0 ± 0.1%). Larvae exhibited normal swimming behaviour and appeared healthy throughout the 96 h assays despite small water volumes and close proximity to damaged algae.

Settlement in response to green algae

To further investigate the importance of green algae as settlement inducers, a settlement assay including six

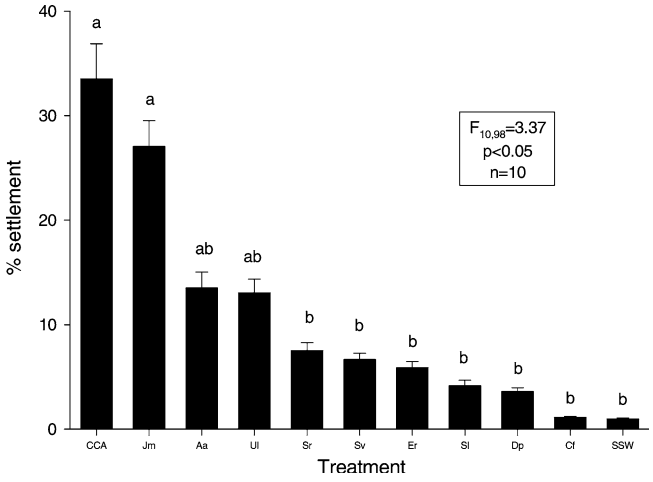

Fig. 1 *Haliotis rubra* larval settlement after 96 in response to local algal species. Data are mean percent settlement ± SE, $n = 10$. *CCA*: encrusting coralline algae, *Jm*: *Jaynia micrarthrodia*, *Aa*: *Amphiroa anceps*, *Ul*: *Ulva australis*, *Sr*: *Soleria robusta*, *Sv*: *Sargassum vestitum*, *Er*: *Ecklonia radiata*, *Sl*: *Sargassum linearifolium*, *Dp*: *Delisea pulchra*, *Cf*: *Codium fragile*

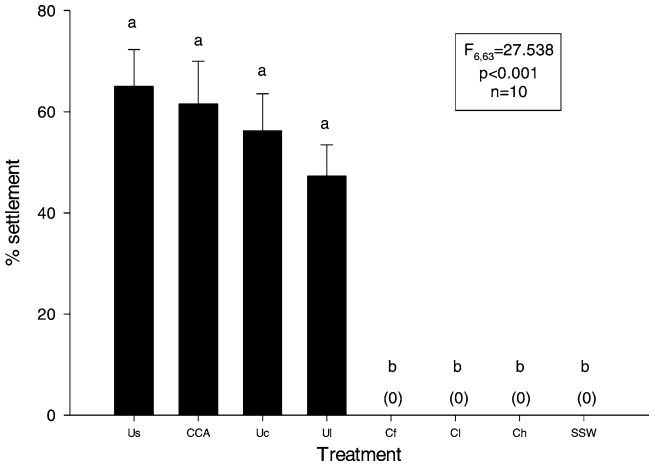

Fig. 3 *Haliotis rubra* larval settlement after 96 in response to local green algal species. Data are mean percent settlement ± SE, $n = 10$. *Ul*: *Ulva australis*, *CCA*: encrusting coralline algae, *Us*: *Ulvella lens*, *Uc*: *Ulva compressa*, *Cf*: *Caulerpa filiformis*, *Cl*: *Codium lucasii*, *Ch*: *Codium harveyii*

green algal species was conducted. *U. compressa* (56.2 ± 7.3%) and *U. australis* (47.7 ± 6.2%) induced settlement at similar rates to the highly inducing green alga *U. lens* (65.0 ± 7.3%) (Fig. 3). In comparison, *C. fragile, C. filiformis* and *C. harveyii* did not induce any settlement.

Settlement in response to rocks, sand, shells and shell-grit

There was no settlement in response to any of the biofilmed abiotic surfaces presented to larvae. All larvae in treatments containing rocks, sand, shells and shell-grit remained unmetamorphosed after 96 h. The positive controls *A. anceps* (62 ± 12%) and *C. officinalis* (30 ± 9%), both induced settlement indicating that larvae were competent, but chose not to settle when given no choice other than a biofilmed abiotic surface.

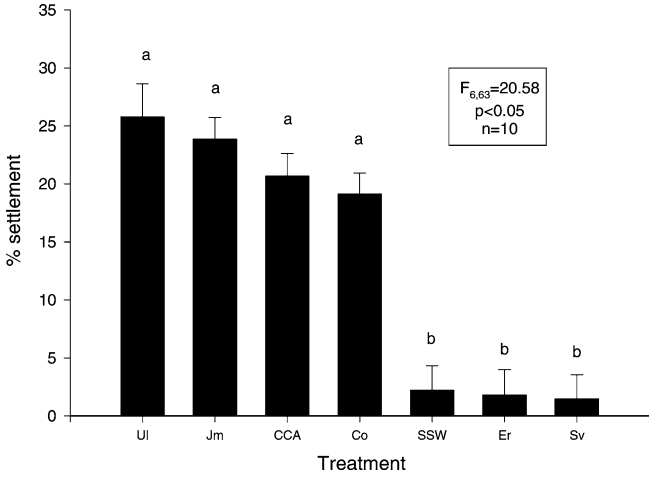

Fig. 2 *Haliotis rubra* larval settlement after 96 in response to local algal species. Data are mean percent settlement ± SE, $n = 10$. *Ul*: *Ulva australis*, *Jm*: *Jaynia micrarthrodia*, *CCA*: encrusting coralline algae, *Co*: *Corallina officinalis*, *Er*: *Ecklonia radiata*, *Sv*: *Sargassum vestitum*

Settlement response to algal extracts

There was no effect of any of the crude algal extracts. Neither the polar nor the non-polar fractions induced settlement of abalone larvae, despite the high response to both the *A. anceps* (88 ± 6%) and *U. australis* (72 ± 11%) control plants, indicating larval competence. No attachment was observed for treatments with algal extracts, and larvae appeared healthy and exhibited normal swimming behaviour throughout the assay, despite the addition of potentially toxic crude extracts.

Settlement response to bacterial biofilms

Soaking algae in antibiotics, significantly increased the numbers of bacteria on the surface of *A. anceps* with respect to all other treatments (one-factor ANOVA, $F_{4,10}7.278$, $p < 0.01$). For *C. officinalis*, abundance was also increased with antibiotic treatments, but this increase was not significant (one-factor ANOVA, $F_{4,10}$ 1.240, $p = 0.335$). Even though the antibiotic treatments did not remove the bacterial biofilm, or reduce bacterial abundance, they significantly reduced the diversity of the microbial community on *A. anceps* with respect to controls plants (one-factor ANOVA, $F_{4,10}$ 15.195, $p < 0.01$). Again, this trend was similar for *C. officinalis*. This reduced diversity did not result in a corresponding reduction in larval settlement, although the settlement response to *U. australis* treated with antibiotics was slightly reduced. (*A. anceps:* one-factor ANOVA,

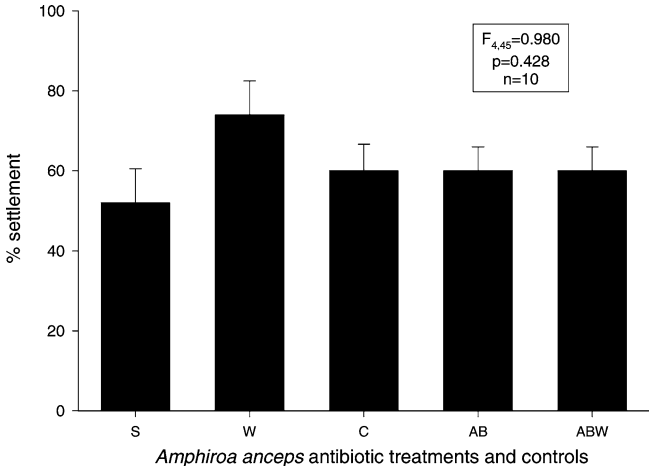

Fig. 4 *Haliotis rubra* larval settlement after 96 in response to *Amphiroa anceps* treated with antibiotics. *S*: soaking control, *W*: agar wipe control, *C*: unmanipulated control, *AB*: antibiotic treatment, *ABW*: antibiotic and agar wipe treatment. Data are mean percent settlement ± SE, $n = 10$.

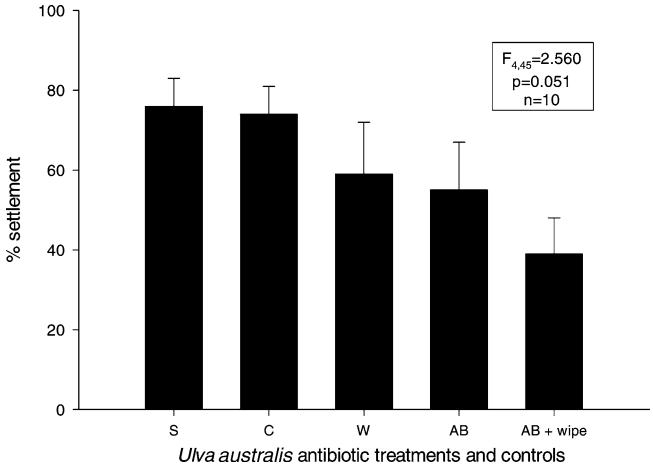

Fig. 6 *Haliotis rubra* larval settlement after 96 in response to *Ulva australis* treated with antibiotics. *S*: soaking control, *W*: agar wipe control, *C*: unmanipulated control, *AB*: antibiotic treatment, *ABW*: antibiotic and agar wipe treatment. Data are mean percent settlement ± SE, $n = 10$

$F_{4,45}$0.97959, $p = 0.428$, Fig. 4; *C. officinalis:* one-factor ANOVA, $F_{4,45}$ 2.1800, $p = 0.086$, Fig. 5; *U. australis* $F_{4,45}$ 2.5620, $p = 0.051$, Fig. 6). Despite a drop in the diversity of bacteria, and an increase in the abundance, overall, the larval settlement response was unaffected by antibiotic treatments. Larvae across all treatments, including sterile seawater alone, appeared healthy and exhibited normal swimming behaviour.

Settlement response to diatoms

For *C. officinalis*, germanium dioxide significantly reduced diatom cover (0.0054 ± 0.0005 diatom cover: area mm^2/mm perimeter) in comparison to the soaking control (0.0238 ± 0.0043 diatom cover: area mm^2/mm perimeter), but neither differed from the fresh control plant (0.0138 ± 0.0008 diatom cover: area mm^2/mm perimeter; one-factor ANOVA, $F_{5,54} p < 0.01$, Fig. 7). For *A. anceps* both the soaking control (0.0067 ± 0.0006 diatom cover: area mm^2/mm perimeter) and the germanium dioxide treatment (0.0127 ± 0.0007 diatom cover: area mm^2/mm perimeter) had significantly reduced diatom cover with respect to fresh control plants (0.0038 ± 0.0004 diatom cover: area mm^2/mm perimeter; one-factor ANOVA, $F_{5,54}$ $p < 0.01$, Fig. 7). However, despite significant differences in diatom cover, no differences were observed in the corresponding larval settlement assay. Within any given algal species, the settlement response between

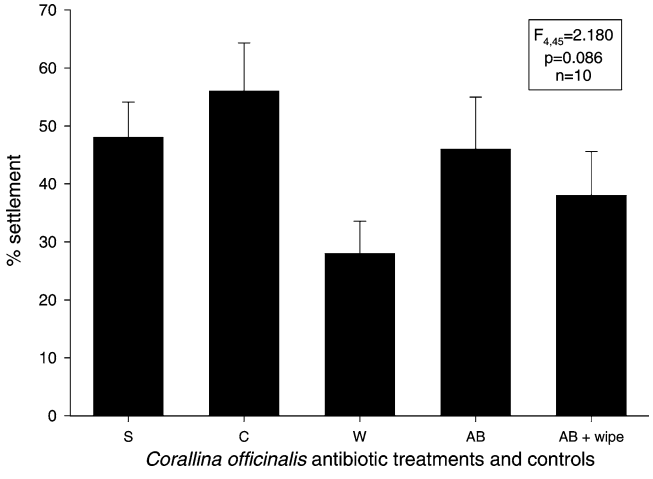

Fig. 5 *Haliotis rubra* larval settlement after 96 in response to *Corallina officinalis* treated with antibiotics. *S*: soaking control, *W*: agar wipe control, *C*: unmanipulated control, *AB*: antibiotic treatment, *ABW*: antibiotic and agar wipe treatment. Data are mean percent settlement ± SE, $n = 10$

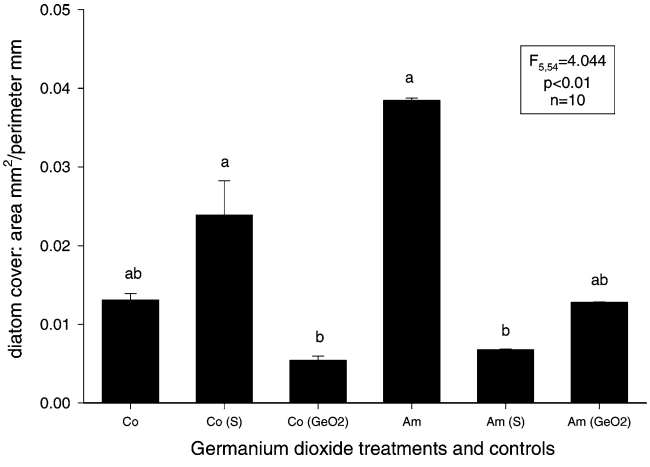

Fig. 7 Diatom cover (area mm^2/perimeter mm) of *Am*: *Amphiroa anceps* and *Co*: *Corallina officinalis* treated with germanium dioxide (*G*), soaking control (*S*), or untreated. Data are means ± SE, $n = 10$

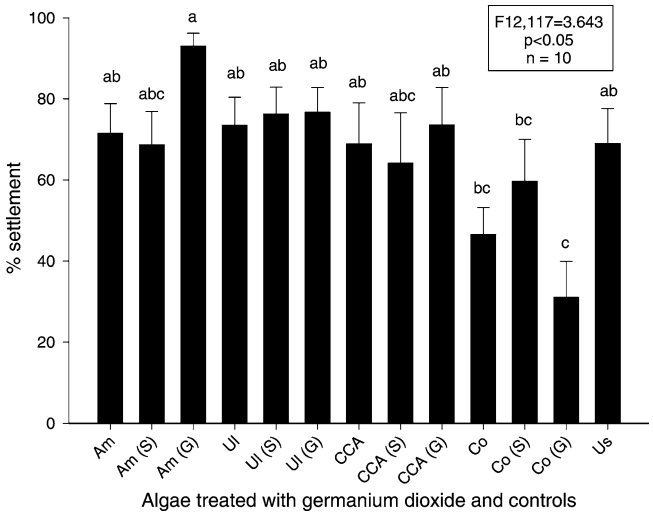

Fig. 8 *Haliotis rubra* larval settlement in response to *Am*: *Amphiroa anceps*, *Co*: *Corallina officinalis Ul*: *Ulva australis* and CCA either treated with germanium dioxide (*G*), soaking control (*S*), or untreated. *Us*: *Ulvella lens* was also presented as a control comparison. Data are mean percent settlement ± SE, $n = 10$

treatments did not differ, although the coralline soak treatment resulted in a significantly lower settlement response compared to all other species of algae (one-factor ANOVA, $F_{12,117}$ $p < 0.01$, Fig. 8).

Discussion

Haliotis spp are well known to settle in the presence of coralline algae, but *H. rubra* had previously been an exception. Daume et al. (2000) found that local corallines induced a low settlement response by *H. rubra* larvae, but that the commercially cultivated encrusting green alga *Ulvella lens* induced significantly higher settlement in hatchery cultures. Here, we find for the first time, that *H. rubra* larvae exhibit a strong settlement response to both encrusting and articulated coralline algae from local subtidal assemblages, and also to the green algae *Ulva australis* and *U. compressa*. Due to differences between batch settlement rates, overall settlement levels in our assays varied, but settlement was consistently highest on CCA, *U. australis* and articulated coralline algae between replicates and across all assays. The settlement response was not reduced when algae were treated to reduce the bacterial biofilm or the diatom film on the surface of plants. Neither polar nor the non-polar algal extracts induced settlement, suggesting that the cue is not water soluble, but instead may be surface associated. Larvae did not settle gregariously.

Coralline algae have been implicated as a source of settlement cues for marine invertebrate larvae from a wide range of phyla including molluscs, echinoderms and cnidarians. The settlement response of larvae has been attributed to compounds associated with the alga (phycobiliproteins) (Morse et al. 1984) and also with bacteria associated with the surface of the alga (Johnson and Babcock 1994; Negri et al. 2001). In our experiments CCA induced high rates of settlement. Encrusting corallines occur throughout the adult range of *H. rubra*, and therefore could represent a suitable habitat for settlement. Our results suggest that neither the bacteria nor the diatoms present on the surface of CCA are instrumental in inducing settlement. Moreover, Daume et al. (1999b) found that the encrusting coralline alga *Phymatolithon repandum* induced reasonable settlement rates but still low, at around 25%, while local diatom species did not induce settlement at all. Although variable, our results generally show a stronger settlement response to CCA than those of Daume et al. (1999b). We collected CCA from local New South Wales sites and species in New South Wales may differ with those in Victoria, or a mixture of encrusting coralline species presented together may induce a settlement response where a single species, such as that presented by Daume et al. (1999b) induces only low settlement.

An unexpected result of this study was that *H. rubra* larvae exhibit a strong settlement response to the green macroalgal species *Ulva australis* and *U. compressa*. Seki (1997) reported that *H. discus hannai* settle on several macroalgae, including *U. pertusa*. Ours is therefore the second time that a species of non-encrusting green macroalgae has been linked with settlement of abalone larvae. Another green alga, the microalga *Ulvella lens* also induces high rates of *H. rubra* larval settlement (Daume et al. 2000). These results suggest that green algae may play a previously unrealised but important role in the recruitment of abalone larvae to benthic populations. These algae may share a common property that causes the larval settlement response, such as the chemical compounds they produce or their surface texture and topography.

The Ulvales are relatively rare in subtidal habitats along the New South Wales coast. Herbivory plays a significant role in this regard, and the removal of herbivores from rocky substrates often facilitates the rapid recruitment of *Ulva* spp. (Sousa 1979; Underwood and Jernakoff 1981). When herbivores are added again, *Ulva* spp. are preferentially consumed before others (Janke 1990). As such, early colonising algal species such as *U. australis* are unlikely to be present in great numbers in the subtidal habitat for adult *H. rubra*. For this study, several large (approx. 20 cm in height) *U. australis* plants were collected subtidally from Shoal Bay, but were never seen at other sites. We predict that the strong settlement response to an early algal settler may be a competitive advantage for juvenile abalone settling in subtidal habitats since the presence of these species may indicate habitats containing reduced densities of grazing herbivores, thus decreasing the risk of 'accidental' predation from herbivorous grazers. This has important implications for the enhancement of abalone stocks in the wild. One local herbivore that is also commercially harvested—the sea urchin *Centrostephanus rodgersii*—is strongly associated with the decline of stocks of *H. rubra* along the southern Australian coast (Andrew and

Underwood 1992). When *C. rodgersii* are removed from experimental sites, recruitment of *H. rubra* into the sites increases in comparison to untreated sites (Andrew et al. 1998). Potentially, abalone re-seeding sites could be manipulated by removing such herbivores thus, allowing *U. australis* to establish. This predicted increase in *U. australis* may, in turn, facilitate enhanced settlement rates of *H. rubra* larvae.

Interestingly, the other green alga that induces settlement, *U. lens*, has the opposite implication as it is common on heavily grazed surfaces, such as abalone tanks (Roberts 2001). It is potentially advantageous for larvae to be able to respond to algae that become abundant under different grazing pressures, but still indicate a suitable shallow reef on which to settle.

There are few studies that have addressed the importance of diatom films for *Haliotis* settlement in the field. Many *Haliotis* species attach and metamorphose in response to diatom films (Gallardo and Buen 2003; Takami et al. 1997). This response is often variable, and may depend on the age and morphology of the diatom film (Daume et al. 1999b). Diatom films are used worldwide as settlement surfaces for abalone larvae in hatcheries and commercial operations. In most cases, no attempt is made to identify the diatom species that are important for settlement. However, diatom species are not an important source of settlement cues for *H. rubra*. Daume et al. (1999b) tested the settlement response of *H. rubra* to several local diatom strains and found only small numbers attached and metamorphosed. Our treatments were successful in reducing the cover of diatoms, either by simply soaking in SSW, or with the addition of germanium dioxide to the SSW solution. The presence of diatoms did not effect larval settlement, suggesting that the settlement response was linked to the plants themselves, rather than the diatom film. Furthermore, the lack of settlement on common substrates such as rocks, sand and shells, all of which are naturally filmed with both bacteria and diatoms, suggests that the settlement cues that larvae detect is associated with the plants themselves, and not the biofilms on their surfaces. Despite the significant reduction in diatom cover, abalone larval settlement was not reduced in response to any of the foliose or encrusting algal species tested. Therefore, in natural populations diatoms do not appear to be an important source of settlement cues for *H. rubra* larvae.

Bacteria induce settlement in a range of marine invertebrate larvae. However, their role in the settlement and recruitment of abalone species is unknown. The few studies that have acknowledged the potential interaction between larvae and bacteria are reviewed by Roberts (2001). Of the low number of *Haliotis* species tested none has responded to the presence of single strain biofilms or mixed natural films (Roberts 2001). However, most studies that test the response of larvae to mucus films, diatoms or algae make no attempt to identify the relative importance of the bacterial biofilm in the interaction between cue and larva. We successfully reduced the diversity of culturable bacteria associated with inductive algal species and using molecular techniques, we can distinguish a shift in the bacterial community composition on both plant surfaces in response to our treatments (Huggett, in preparation). However, changes in the bacterial community diversity and composition did not correspond to a reduction in larval settlement. A consistently high metamorphic response was found across all treatments, despite the change in bacterial community. The method we used to reduce the bacterial community on the surface of the plants did not render these surfaces axenic, so it is possible that bacteria are important, and that the inducing species are among the few strains that were resistant to our treatments. However, the settlement response of larvae to biofilmed abiotic surfaces such as rocks, sand and shell-grit was insignificant, and we would expect these surfaces to all harbour marine bacterial biofilms, and if biofilms were important then these surfaces should also have induced significant rates of settlement. Bacterial biofilms are not a primary source of settlement cues for *H. rubra*.

The results of this study also have important implications for the aquaculture of *H. rubra* and other species of abalone. Settlement of *Haliotis* species is generally low and a limiting step in the production of aquaculture stocks (Roberts 2001). In hatcheries and farms, *U. australis* can be grown quickly and simply (Neori et al. 2000), in contrast to the slower growing CCA and other macroalgal species, and presented to larvae as a settlement surface, facilitating increased settlement rates. In a commercial hatchery, this method has been tried using *U. lens*, and is successful for the settlement of *H. rubra* larvae (Daume et al. 2004). However, *U. lens* alone was not nutritionally adequate for growth of juveniles. Although *Ulva* spp. are not an appropriate juvenile diet for abalone in comparison to other macroalgae and formulated feeds, it can provide a good diet when high nitrate or ammonium enrichments are used in the media or seawater (Boarder and Shpigel 2001) and may be able to provide both a settlement cue for larvae and the diet of juvenile abalone in hatcheries.

In conclusion, it appears that bacterial and diatom biofilms are not important for *H. rubra* larval settlement in the wild. As for many species of abalone larvae, encrusting coralline algae induced high rates of settlement in laboratory assays. *H. rubra* larvae showed an unexpectedly high settlement response to the geniculated coralline algae *A. anceps* and *C. officinalis* and the green algae *U. australis* and *U. compressa*. Daume et al. (1999b) also came to the same conclusion—that settlement induction is primarily related to the alga; bacteria and diatoms on coralline algae may contribute, but are not the main factor that drives settlement. Understanding the role of green algae as natural inducers of abalone larvae may increase our understanding of the demography of abalone in relation to other herbivores. The settlement response to green algae also has potential commercial implications as they are easy to culture and are fast growing. Further research may reveal a suite of

algae that provide strong settlement cues for this species with the potential for application to both enhance wild populations and increase production of hatcheries.

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