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Settlement of crown-of-thorns starfish: role of bacteria on surfaces of coralline algae and a hypothesis for deepwater recruitment

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ABSTRACT: Settlement trials conducted with larvae of crown-of-thorns starfish *Acanthaster planci* revealed a moderate degree of substratum specificity. Highest rates of settlement and metamorphosis occurred on coral rubble and the crustose coralline alga (CCA) *Lithothamnium pseudosorum*, but rates were more variable on the coralline. Interpretation of settlement on the rubble is difficult because rubble always supported some CCA. Settlement was significantly lower on other CCA (*Porolithon onkodes* and *Neogoniolithon foslei*), non-calcareous crustose red algae (*Peyssonellia* sp.), and fouled ceramic tiles. These results were consistent irrespective of whether larvae were offered a choice of substrata or not. When larvae were separated from *L. pseudosorum* by mesh, settlement was highly variable but sometimes occurred at high rates, suggesting that contact with the algae is not obligatory for induction. Larvae were not induced to settle by GABA (γ-amino butyric acid), elevated K^+ concentrations, or coral blocks fouled for 9 d, and settlement rates were virtually zero in controls without a known added inducer. Treatment of highly inductive shards of *L. pseudosorum* with antibiotics reduced their inductive activity to low levels, suggesting that induction of settlement and metamorphosis of *A. planci* by *L. pseudosorum* may be mediated by epiphytic bacteria. Other results were consistent with the notion of bacteria-mediated induction. The inductive ability of different regions on individual *L. pseudosorum* plants varied greatly, as did densities of bacteria on the plant surface. Larvae always settled on sections of thallus having high densities of bacteria, but never on adjacent areas where epiphytic bacteria were sparse. The inductive stimulus is likely to be chemical since it was inactivated by boiling or autoclaving, and may be a relatively large molecule since it was not detected in water, ethanol or chloroform extracts of inductive algae or coral rubble, and was retained by dialysis tubing of pore size 10 000 Daltons. The spatial distribution of coral rubble and *L. pseudosorum* on and around GBR midshelf reefs, the location of hydrodynamic retention cells around reefs, and the pattern of outbreaks on the GBR, suggest that mass settlements of *A. planci* are more likely to occur in deep than in shallow water. This would explain the paradox that outbreaks of *A. planci* on the GBR are not heralded by increases in abundances of juveniles in shallow water, but are first observed as adult starfish ascending from deepwater. Preliminary deep water videotransects off Davies Reef showed that rubble and CCA were abundant in deep water (30 to 65 m) adjacent to the area where aggregations of adult starfish were first seen moving up from deep water, but the substratum in deep water off other sections of the reef was sand.

INTRODUCTION

A singular feature of outbreaks of the coral-eating crown-of-thorns starfish *Acanthaster planci* on the Great Barrier Reef (GBR) in Australia is that initial outbreaks trigger a series of secondary outbreaks that propogate southward at a velocity equivalent to the average velocity of the southward East Australian current (Reichelt 1990). These waves of outbreaks cause extensive destruction to large numbers of reefs in a relatively short period. In the most recent, 65 % of reefs in the central section of the GBR have been affected seriously (Endean & Cameron 1985, Moran 1986, Johnson et al. 1988, Moran et al. 1988).

All evidence suggests that secondary outbreaks are the result of mass settlements of *Acanthaster planci* such as has been observed in Fiji (Zann et al. 1987) and not of accumulations of individuals from many separate recruitments; size frequency-distributions of most out-

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break populations on the GBR are unimodal initially (Moran et al. 1985, see also Moran 1986) and transport of *A. planci* larvae has emerged as a key parameter in modelling of outbreaks on the GBR (James et al. 1990, Reichelt et al. 1990). The large number of secondary outbreaks that characterise the 'wave' phenomenon on the GBR indicates that mass recruitments are not erratic and infrequent as reported for *A. planci* in other systems (Zann et al. 1987). However, despite considerable and focussed effort, relatively few juvenile *A. planci* have been found on the GBR (Pearson & Endean 1969, Endean & Stablum 1973, Moran et al. 1985, Moran 1986, 1989, Doherty & Davidson 1988). Moreover, those that have been found were in their second year post metamorphosis (= 1+; the starfish which Pearson & Endean (1969) and Endean & Stablum (1973) believed were only a few months old were 1+ individuals; see Lucas 1984 and Zann et al. 1987). In the rare cases where moderate densities of 1+ starfish have been found, they are thought to be the progeny of outbreak populations of adults at those sites (Endean & Stablum 1973, Moran et al. 1985, Doherty & Davidson 1988).

These observations raise important questions about processes of settlement and recruitment of *Acanthaster planci* on the GBR, viz. (1) Do larvae manifest substratum-specific settlement and metamorphosis? and if so, (2) What is the nature of cues that induce these processes? (3) Are the cues spatially concentrated in particular microhabitats on reefs that constitute key nursery sites? and (4) What are the implications of answers to these questions for patterns of starfish recruitment? Laboratory experiments (usually unreplicated and without controls) have indicated that *A. planci* larvae will not settle on clean glass surfaces (Henderson & Lucas 1971) or bleached crustose coralline algae (Yamaguchi 1973a), but that beyond this they are not highly specific in substratum requirements for settlement. Settlement has been reported on both crustose coralline algae (CCA) and noncalcareous crustose algae (Henderson & Lucas 1971, Yamaguchi 1973a, Lucas 1975), but also on sand, serpulid tubes, filamentous green algae, live coral (*Pocillopora*), dead and bleached coral, and tube feet and spines of adult *A. planci* (Henderson & Lucas 1971, Ormond & Campbell 1974). From laboratory observations, Lucas (1975) suggested that *A. planci* larvae require only a 'biological film' of bacteria and encrusting algae for settlement. If so, given the enormous reproductive capacity of crown-of-thorns (Moran 1986), the scale of outbreaks on the GBR and that 'biological films' are ubiquitous in the marine environment, it is surprising that so few juveniles are found.

Against this background, we identified several specific aims for the present study, viz. (1) to examine the ability of various natural substrata, including CCA, to induce settlement of *Acanthaster planci*, (2) to determine whether the morphogens associated with highly inductive substrata might be bacterial in origin, (3) if there was evidence of a role of bacteria, to isolate and identify particular strains that induce settlement, and finally, (4) to attempt to relate the spatial distribution of inducers on reefs to both patterns in *A. planci* outbreaks on the GBR and to the paucity of juvenile starfish in shallow water.

METHODS

Field and laboratory experiments were undertaken at Lizard Is. (14°40.5' S, 145°27.6' E) during the *Acanthaster planci* spawning period (November to January) in 1986 and 1987 to examine substratum preferences of larvae and the effect of extracts from crustose coralline algae (CCA) and certain known chemicals on settlement rates. A second series of laboratory experiments were conducted at the Australian Institute of Marine Science (AIMS) during November 1987 to January 1988 to investigate the role of bacteria from the coralline algae *Lithothamnium pseudosorum* in inducing *A. planci* settlement and metamorphosis, and to corroborate some of the earlier preference experiments. A summary of experiments is given in Table 1.

Culturing of *Acanthaster planci* larvae. Larvae used in experiments at Lizard Is. were grown from gametes obtained by excising gonads. Eggs were fertilised and maintained in the laboratory for 2 d following the methods of Lucas (1982). Larvae were moved after 2 d to 3 l containers at a density of ca 300 l^{-1} in an in situ culturing apparatus (details in Olson 1985) moored at 3 m depth inside the edge of the reef lagoon. Larvae developed uniformly, and all settlement experiments used 14 d old larvae, at which time their starfish primordia were developed fully and they were judged competent to settle; we refer to these larvae as 'competent'.

Larvae used in experiments at AIMS, which includes all experiments conducted with bacteria, were grown in the laboratory. Adults with ripe gonads were induced to spawn by injecting with 8 to 10 ml 10^{-3} M 1-methyladenine, and fertilised eggs transferred either to 5 l cylindrical aquaria with mechanical stirrers at a density$_{max}$ of ca 600 larvae l^{-1}, or to a 400 l aquarium with bubble agitators at a density$_{max}$ of ca 1200 larvae l^{-1}. Larvae were cultured in 1 µm filtered fresh seawater at 28 °C on a mixed diet of microalgae (*Dunaliella* sp., *Phaeodactylum* sp., *Isochrysis* sp., *Chaetoceros gracilis* and *Tetraselmis chuii*) in equal proportions at a final concentration of ca 8×10^6 cells l^{-1}. Larvae grown in 5 l containers were transferred to clean containers

Table 1. Summary of experiments (and location of their results). Values in parentheses refer to number of non-independent 'replicates' nested within each true replicate; na: not applicable

Location	Temp. (°C)	Incubation period (d)	No. of replicates	No. of larvae per replicate	Location of results
1. Substratum selection					
Settlement on various substrata; no choice offered					
Lizard Is.	Ambient	2	3(3)	125	Fig. 1A
Settlement preferences; choice of substrata offered					
Lizard Is.	Ambient	2	4	125	Fig. 1B
2. Bioassays with CCA and extracts of *Lithothamnium pseudosorum*					
Settlement on CCA shards variously treated and on coral blocks perfused with extracts of CCA (use of mesh barriers, boiled shards, dialysis tubing etc.)					
Lizard Is.	25–27	2	3	40–50	Table 2
Settlement on scratched and unscratched *L. pseudosorum*					
AIMS	25–27	4	5	10	Fig. 1C
Within-plant variation in inductive ability of *L. pseudosorum*					
AIMS	25–27	4	5	10	Fig. 1D
Microhabitat selection on *L. pseudosorum*					
AIMS	25–27	3	n.a.	n.a.	Table 3
3. Bioassays with fouled coral blocks and extracts from coral rubble					
Settlement on coral blocks fouled for 9 d and on coral blocks perfused with extracts from coral rubble					
Lizard Is.	25–27	2	3–6	40	Table 2
4. Chemical induction					
Inductive ability of GABA and K^+					
Lizard Is.	25–27	2	3	40	Table 2
5. Bacterial induction					
Effects of antibiotics; comparison of inductive ability of untreated and antibiotic-treated *L. pseudosorum*					
AIMS	25–27	2	10	20	Fig. 2A
Effect of antibiotics on consistency of inductive ability of *L. pseudosorum* shards; shards moderately to highly inductive					
AIMS	25–27	2	6	20	Fig. 2B
Effect of antibiotics on consistency of inductive ability of *L. pseudosorum* shards; shards of a range of inductive ability					
AIMS	25–27	2	10	20	Fig. 2C
6. Bioassays with epiphytic bacteria isolated from *L. pseudosorum*					
Inductive ability of individual and mixed pure strains of bacteria coated onto glass slides and pre-sterilised shards of *L. pseudosorum*					
AIMS	25–27	3	5–10	10	Table 4

every 2 d, and water in all culture vessels was changed every 2 d. Some cultures were treated once with antibiotic (1 ppm erythromycin) when larvae were at mid-brachiolaria stage. Because rates of larval development varied, larvae were screened using a dissecting microscope and only late brachiolaria with fully developed larval arms, primordia, and sensory papillae on the pre-oral brachiolar arms were deemed competent and selected for experiments.

Substratum selection.

Coral rubble vs crustose algae: Two experiments were conducted at Lizard Is. to assess preferences of *Acanthaster planci* larvae for settlement onto coral rubble vs calcareous and non-calcareous crustose algae (all Rhodophyta). The rubble and crustose algae were collected locally at 6 to 9 m depth, and fresh material was used in experiments. We were unable to find pieces of rubble that did not support some epilithic CCA. Algal shards were placed in experimental chambers with their live algal surface facing downward, since preliminary experiments indicated that larvae prefer to settle on undersurfaces. Settlement was scored as the number of newly metamorphosed juveniles and settled post-larvae. Post-larvae are those that have attached to the substratum with their adhesive disc and have commenced metamorphosis, as evidenced by the absorption of larval structures (see Henderson & Lucas 1971, Yamaguchi 1973a). Larvae crawling on substrata were not counted as settled individuals. Thus, we use the term 'settlement' to indicate larvae that have undergone both the behavioural transition from neutrally buoyant swimming plankton to negatively buoyant crawling larvae, and that have at least commenced the physiological transition of metamorphosis. In scoring settlement on algal fragments, only larvae that had settled directly onto pink surfaces of live algal tissue were counted.

In first experiment, larvae were offered a single sur-

face on which to settle. Three fragments (ca 1 cm^2) of either coral rubble, or CCA (*Porolithon onkodes, Neogoniolithon fosleii,* and *Lithothamnium pseudosorum*), or fleshy crustose algae (*Peyssonellia* sp.) were placed at the bottom of each of 3 replicate 3 l plastic flow-through chambers (100 µm mesh windows). All 3 fragments within any one chamber were taken from the same plant or piece of rubble. After adding 125 competent 14 d old larvae to each chamber, the 15 chambers were mounted on culture tables in the field at 3 m depth. The chambers were collected after 2 d, the fragments examined using a dissecting microscope, and the number of metamorphosed juveniles and settled post-larvae recorded.

In the second experiment, larvae within each of 4 replicate 20 l flow-through containers (100 µm mesh windows) were given a choice of 7 different substrata, viz. 3 species of CCA (*Lithothamnium pseudosorum, Neogoniolithon fosleii,* and *Porolithon onkodes*), 2 *Peyssonellia* species, coral rubble, and porcelain tiles fouled for several weeks in aquaria. Each chamber contained a single piece of each type of substratum. Chambers were mounted on culture tables in the field after adding 125 competent 14 d old larvae to each. The substrata were recovered after 2 d and examined as described above.

Neither experiment included a control treatment in which potential inducing substrata were absent. However, in raising several thousand larvae over several seasons using the in situ system, spontaneous settlement has never been observed in 14 d old larvae.

Bioassays with CCA and Lithothamnium pseudosorum extracts: Our initial substratum selection experiments indicated that the crustose coralline *Lithothamnium pseudosorum* induces settlement of *Acanthaster planci* larvae at relatively high rates. Experiments were conducted at Lizard Is. to elucidate the nature of the cue associated with this alga, and to compare its inducing ability with *Porolithon onkodes*. Settlement of competent *A. planci* larvae was examined on each of the following substrata in separate assay vessels (i.e. no choice offerred): (1) fresh *L. pseudosorum* shards, (2) fresh *L. pseudosorum* shards separated from larvae at a distance of 15 to 30 mm by 100 µm mesh, (3) mesh only = control for treatment (2), (4) *L. pseudosorum* shards boiled for 15 min, (5) dialysis tubing (10 000 Daltons pore size) containing *L. pseudosorum* shards, (6) dried coral (*Porites* sp.) blocks perfused with ethanol or chloroform or aqueous (distilled water) extracts of *L. pseudosorum*, and (7) and (8) shards of *P. onkodes* from sunny and shaded habitats respectively. Bioassays vessels (3 replicates per treatment) were 300 ml plexiglas flow-through chambers (100 µm mesh windows) mounted on culture tables in the field. To each replicate chamber were added 40 or 50 competent 14 d old larvae, and after 2 d substrata were removed and examined microscopically for juveniles and settled post-larvae. Larvae settled on both pink and white algal surfaces, were counted in these experiments. The surface area (epithallus) of each algal shard was ca 1 cm^2, and pieces of dialysis tubing offered a similar area for settlement. Separating larvae from *L. pseudosorum* by mesh tested for the requirement of larval contact with the alga for metamorphosis.

For solvent extractions, 10 g of algae was soaked overnight in 50 ml solvent. For ethanol and chloroform extractions solvent was removed by rotary evaporation under vacuum at 40 °C, the residue resuspended in ca 1 ml of pure solvent, then transferred equally to 3 coral blocks (1 cm × 1 cm × 0.3 cm) and allowed to evaporate at room temperature (ca 25 °C). The aqueous (distilled water) extract was freeze-dried, the residue redissolved in ca 1 ml distilled water and transferred to coral blocks as described above. All coral blocks were then freeze-dried overnight to remove traces of solvent.

To corroborate results of experiments at Lizard Is., further experiments were conducted at AIMS using *Lithothamnium pseudosorum* collected at 6 to 9 m depth at Davies Reef (18°50' S, 147°39' E). Settlement bioassays were conducted in 80 ml evaporating dishes containing 40 ml of 0.45 µm filtered seawater (FSW) and the test substratum. Ten competent larvae were incubated in each of 5 replicate settlement dishes per treatment. In each experiment a control treatment (FSW without CCA) was included to control for spontaneous settlement, which can occur at low levels when larvae are reared in the laboratory (C. R. Johnson pers. obs.).

In the first experiment, settlement was compared among 3 treatments, viz. undamaged plants of *Lithothamnium pseudosorum*, plants that had been damaged by scratching their surfaces with a sterile scalpel blade 48 h prior to settlement trials, and controls. Plants were scratched to encourage growth of bacteria, after the evidence of Johnson et al. (unpubl.) that bacteria colonise damaged regions of crust. A second experiment examined the degree of variability in inducing ability within individual plants. Settlement was compared among 2 *L. pseudosorum* plants (n = 5 shards from each plant) and controls. In both experiments, settlement was scored after 4 d.

The final experiment investigated selection of settlement sites on pieces of *Lithothamnium pseudosorum*. Microhabitats in which a total of 607 larvae settled and commenced metamorphosis were scored in 9 separate settlement trials. Trials were in vessels containing 3 pieces of *L. pseudosorum* in 250 ml 1 µm filtered seawater. Settlement was scored after ca 3 d, and only settled individuals that were still adhered and incompletely metamorphosed (i.e. non-mobile) were

counted, since completely metamophosed individuals (juveniles) may have moved from their initial site of attachment. A total of 6 microhabitats were delineated on fragments of the alga: (1) unbranched live surface, (2) branched live surface, (3) dead crust surface, (4) exposed subsurface regions (i.e. perithallus and hypothallus) showing development of an organic film, and (5) heavily fouled exposed subsurface regions. The sixth microhabitat was a small area of exposed mollusc shell surrounded by *L. pseudosorum* which was overgrowing the shell. Microhabitats were further classified as being on an upper or lower (= underneath) surface. Not all microhabitats were present in any one trial. For each trial the percentage surface area of each microhabitat on upper and lower surfaces was estimated to the nearest 10%. Because all coralline pieces in all trials were of similar size (ca 6 to 7 cm^2 live coralline surface), it was possible to estimate the total percentage cover of each microhabitat for all trials combined, and therefore to calculate expected settlement based on the relative areas of each microhabitat assuming no selectivity in settlement.

Bioassays with fouled coral blocks and extracts from coral rubble: Because initial experiments showed that settlement rates of *Acanthaster planci* were relatively high on coral rubble, additional experiments were undertaken to examine the nature of inducers associated with rubble. Aqueous, ethanol and chloroform extracts from pieces of coral rubble (10 g per extract) were prepared and perfused into coral blocks for use in bioassays as described above for extracts from *Lithothamnium pseudosorum*.

To test for differences in larval settlement on newly fouled surfaces from different habitats, 6 coral blocks (1 × 1 × 0.3 cm) cut from dried *Porites* sp. colonies and mounted onto equivalent sized plexiglass bases were set out at each of 6 different sites. The 6 sites were in 3 habitat types (shaded, beneath a coral ledge; unshaded, among coral heads; on sand 10 m from the nearest reef) in both the lagoon and on the fore-reef at Lizard Is. Blocks were allowed to foul for 9 d before transferring to separate 3 l flow-through chambers (100 µm mesh windows) in the field where they were incubated with 40 competent 14 d old larvae. Settlement of post larvae was scored after 2 d in the manner outlined above.

Chemical induction. GABA (γ-amino butyric acid) and elevated K^+ concentration were tested for ability to induce settlement and metamorphosis of competent *Acanthaster planci* larvae. Both GABA (e.g. Morse et al. 1979, Rumrill & Cameron 1983, Pearce & Scheibling 1988) and elevated K^+ levels (Yool et al. 1986) have been shown to induce settlement and metamorphosis of some marine invertebrate larvae. Five concentrations of GABA (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M in 1 µm filtered seawater; Sigma) and 4 of KCl (10, 20, 30 and 40 mM above background; AR grade in 1 µm filtered seawater) were tested. Bioassays were in 250 ml glass evaporating dishes (3 replicates per treatment concentration). In each dish 40 competent 14 d old larvae were incubated with ca 150 ml solution. Glass surfaces were examined microscopically for settled post-larvae after 2 d.

Bacterial induction.

Experiments with antibiotics: To test the hypothesis that bacteria on the surface of *Lithothamnium pseudosorum* play a role in inducing settlement of *Acanthaster planci*, settlement bioassays were conducted after treating *L. pseudosorum* with a mixture of equal amounts of penicillin G, streptomycin sulphate and tetracycline, to a final concentration of 100 ppm in sterile seawater (SSW = autoclaved 0.45 µm filtered seawater). This concentration was a compromise chosen to maximise the effect of antibiotics on bacteria but to avoid possible deleterious effects to the alga. Pilot tests using plate-viable isolates from surfaces of antibiotic-treated *L. pseudosorum* showed that ca an order of magnitude fewer colonies grew at 100 ppm than at 50 ppm antibiotics, but only ca 1.6 times more colonies grew at 100 ppm that at 150 ppm. After treatment with antibiotics, plants were washed vigorously with several changes of SSW to remove the antibiotics and lysed cells of antibiotic-sensitive bacteria.

In the first experiment 20 pieces of *Lithothamnium pseudosorum* (each of surface area ca 2 to 3 cm^2) were assigned randomly to 2 treatments (i.e. 10 replicates per treatment), one in which plants were exposed to the antibiotics for 24 h, and the other treated only with SSW (control). All 20 pieces were then washed with several changes of SSW, and each piece transferred to an 80 ml evaporating dish containing 40 ml SSW and 20 competent larvae. The plant surfaces were examined microscopically after 2 d for settled post-larvae and newly metamorphosed juveniles.

The pattern of settlement in the control treatment of the experiment outlined above was similar to that in previous experiments with *Lithothamnium pseudosorum* in that settlement rates were extremely variable among replicates. Thus, a second experiment was designed to control for among replicate/within treatment variability in settlement. The procedure was similar to that of the experiment just described (i.e. control and '+ antibiotics' treatments, 10 replicates per treatment, 20 larvae per settlement dish per settlement trial), except that 2 settlement trials were conducted, one before and one after treatment with antibiotics. In the first trial, settlement of post-larvae on each piece of *L. pseudosorum* was recorded after 2 d, then all settled and metamorphosed starfish were removed from the coralline pieces prior to treating one group with anti-

biotics, and the control group with SSW, for 24 h. Both the antibiotic-treated and untreated corallines were then washed thoroughly several times with SSW before adding new larvae and scoring settlement (after 2 d) in a second trial. This procedure examined both (1) the consistency of individual coralline pieces in their ability to induce settlement in separate trials, and (2) the effect of antibiotics on plants initially identified as having high inducing ability.

This experiment was repeated, but in the repeat run, only pieces of coralline that had already been identified as having moderate to high inducing ability were used. In this experiment, there were n = 6 independent replicates per treatment.

Isolation and identification of bacteria from surfaces of coralline algae: We isolated bacteria from surfaces of CCA for testing in bioassays with *Acanthaster planci* larvae, comparing bacteria isolated from surfaces of *Lithothamnium pseudosorum* with those from several other corallines, including *Porolithon onkodes*. Coralline algae were collected in separate plastic bags from depths of 5, 10, 15, 20 and 25 m on the fore-reef slope of Davies Reef on 14 November 1988, and processed immediately on return to the surface. The surface of each plant was rinsed vigorously with sterile artificial seawater (= ASW; see below), and three 1 cm^2 areas swabbed with sterile cotton-wool swabs. Swab inocula were spread uniformly over the surface of agar plates, or applied in a concentrated area (ca 1 cm^2) on the edge of a fresh plate, from which the inoculum was streaked to effect a dilution series. Isolation plates contained 4 % agar, but were otherwise identical to other growth media (see below). Corallines from which swabs were taken were frozen and later identified according to the keys of Adey et al. (1982) and Steneck et al. (unpubl.).

After incubation of isolation plates at 25 °C for 48 h, colonies representing the major morphological phenotypes were subcultured to purity and maintained at 16 °C by monthly subculture. Thus, sampling of the isolation plates was qualitative, but this method ensured that all common plate-viable colony morphologies were represented. Solid growth medium (SGM) was ASW containing 10 g l^{-1} NH_4Cl, 0.025 g l^{-1} ammonium ferric citrate, 1.0 g l^{-1} K_2HPO_4, 1.0 g l^{-1} Bacto peptone, 2.0 g l^{-1} Difco yeast extract, 2.0 g l^{-1} Bacto agar, and 10 ml l^{-1} 1M MOPS buffer pH 7.5; ASW consisted of distilled water containing 17.6 g l^{-1} NaCl, 5.1 g l^{-1} $MgCl_2 \cdot 6H_2O$, 0.15 g l^{-1} $CaCl_2 \cdot 2H_2O$, 0.29 g l^{-1} Na_2SO_4, and 0.75 g l^{-1} KCl. Agar media were sterilised by autoclaving at 110 KPa for 15 min at 121 °C.

Bacterial isolates were identified to genus level on the basis of morphological and biochemical criteria (Baumann et al. 1972, Reichelt & Baumann 1973, Krieg & Holt 1986, Cropp & Garland 1988). The tests employed were: colony morphology and pigmentation; gram stain; motility; cell morphology; sodium requirement; formation of poly-β-hydroxybutyrate; oxidation/fermentation of glucose (growth and acid production); ability to de-nitrify; production of oxidase and catalase; number, type and insertion point of flagella; and production of the extracellular enzymes amylase, gelatinase, lipase and DNase. Methods used were those of Stanier et al. (1966) modified for heterotrophic bacteria (Baumann et al. 1971).

In addition to isolating bacteria from *Lithothamnium pseudosorum* and other CCA immediately after collection, several fragments and whole plants of *L. pseudosorum* from 6 to 9 m on Davies Reef were assayed with *Acanthaster planci* larvae to identify pieces with high inducing ability. Bacteria from highly inductive pieces were isolated and maintained in pure culture in the manner described above. No attempt was made to identify bacteria isolated from these plants.

Bioassays with coralline bacteria: Bioassays for bacterial induction of settlement of competent *Acanthaster planci* larvae were conducted with either single pure strains of bacteria or mixtures of pure strains, and with both (1) known genera of bacteria isolated from surfaces of *Lithothamnium* sp., and (2) unidentified isolates taken from surfaces of shards of *L. pseudosorum* known to be highly inductive of settlement. Bioassays were in 80 ml evaporating dishes, each containing 40 ml 0.45 μm filtered seawater (FSW), the treatment 'substratum', and 10 competent *A. planci* larvae. Numbers of attached post-larvae and newly metamorphosed juveniles were scored after ca 3 d. Bioassays were conducted in several runs and each included a control treatment free of bacteria (see below). An '*L. pseudosorum* control' was included in some runs to affirm that larvae selected for bioassays were competent to settle. Because results of all runs were consistent, they were pooled for presentation.

A. Bioassays with single strains of bacteria: Bioassays used bacteria coated onto acid-washed and presterilised ground-glass slides (24 × 38 mm). Pure isolates were subcultured onto agar plates (SGM, see above) and grown at 25 °C for 24 h. Next, bacteria were suspended in 25 ml sterile ASW by agitating with a sterile glass rod, and the suspension decanted and bacterial density adjusted with sterile ASW to at least 10^6 ml^{-1} (determined spectrophotometrically at A_{660} nm). A sterile glass slide was incubated in the suspension for 6 h to facilitate attachment of bacteria, rinsed in sterile ASW to remove unattached cells, and transferred to an assay dish. Bacteria-free control treatments to test for spontaneous settlement of larvae and possible inducing substances from the bacterial growth medium were included. Glass slides used as control substrata were

prepared as described above, except that agar plates were not inoculated with bacteria. For each bacterial isolate and control treatment, 5 replicate slides were prepared independently and each transferred to separate assay dishes.

B. Bioassays with mixtures of several strains of bacteria: Bioassays were undertaken with mixtures of pure isolates of bacteria coated onto glass slides (i.e. as for individual isolates) and presterilised pieces of *Lithothamnium pseudosorum*. Mixtures of bacteria were used to investigate the possibility of synergistic effects in bacteria mediating settlement. Sterilised coralline chips were included to test the possibility that both bacteria and a particular surface texture are required to induce high rates of settlement. They were prepared by bleaching in chlorine, then rinsing for at least 12 h in running freshwater, followed by extensive rinsing in distilled water and airdrying before autoclaving. Suspensions of individual strains ($> 10^6$ ml^{-1}, prepared as described above) were mixed and coated onto glass slides and sterile chips of *L. pseudosorum* by the methods outlined above. Control per treatments were included as described earlier. Two categories of mixtures were used, viz. (1) mixtures of identified bacteria isolated from *L. pseudosorum* immediately upon its collection at Davies Reef, and (2) mixtures of unknown isolates obtained from *L. pseudosorum* shards shown to have high inducing ability. For all experiments using mixtures of bacteria, n = 10 replicates per treatment were prepared independently and transferred to individual assay vessels.

Scanning electron microscopy (SEM). The distribution of surface bacteria on *Lithothamnium pseudosorum* collected at Davies Reef at 6 to 9 m depth was examined by SEM. Categories examined were (1) undamaged plants with no evidence of grazing scars, (2) damaged plants (see below), (3) pieces identified in bioassays to have low and (4) high inducing ability, and (5) plants on which newly metamorphosed starfish were still attached. Plants in the first 2 categories were fixed immediately on return to the surface. Plants designated as 'damaged' were those whose surfaces were scratched in situ with a stainless steel scribe. Prior to scratching, these plants bore no visible signs of grazing or other trauma. Scratched plants were collected and fixed at intervals 24, 51, 75 and 99 h after they were damaged. The time series was examined for evidence of bacterial colonisation of damaged cells as indicated by Johnson et al. (1991).

All corallines prepared for SEM were rinsed in sterile ASW prior to fixing for 48 h in 2.5 % glutaraldehyde in 0.1 % cacodylate buffer at 4 °C. After fixing, samples were desalinated and dehydrated (after the methods of Johnson et al. 1991), then critical-point dried, mounted onto stubs, and coated with gold paladium prior to viewing.

Data analysis and statistics. In the experiment at Lizard Is. in which larvae were offered a choice of substrata, settlement rates, and therefore expected frequencies, were too low to apply a replicated goodness of fit test (G-statistic) of the null hypothesis of equal settlement on all substrata (ANOVA cannot be used as the different substrata are not independent in each replicate). Thus, the 2 species of *Peyssonellia* were pooled into a single class and data summed across replicates within substratum classes, which permitted testing these data against H_0 using the G test (adjusted using William's correction for Type I error; see Sokal & Rohlf 1981, p. 704).

In analysing results of experiments with antibiotics in which 2 separate settlement trials were conducted with the same pieces of *Lithothamnium pseudosorum*, the response variable was the difference in the proportion settled in the first and second trials (Trial 2 – Trial 1). This procedure controlled for variability in the settlement rates among the 2 batches of larvae (i.e. for the first and second trials) independent of the effect of treatment with antibiotics.

Where necessary, data were transformed to stabilise variances. Transformations are expressed in terms of the untransformed variate, Y (e.g. $\ln(Y + 1)$). In the one case when a suitable transformation could not be found, a nonparametric procedure (χ^2 approximation of Kruskal-Wallis test) was used in lieu of parametric ANOVA. Analysis of variance procedures, Tukey's multiple range test, and the regression analysis were conducted using the SAS software package. Comparison of slopes of regression lines was undertaken after the method of Zar (1984, p. 292).

RESULTS

Behaviour of larvae of *Acanthaster planci* was similar in all settlement trials and bioassays. Competent late brachiolaria were negatively buoyant and, on contacting the bottom of the assay vessel or the experimental substratum, commenced a characteristic searching behaviour. Larvae contacted the substratum with the papillae on their pre-oral (anterior) brachiolar arms and using these arms could 'crawl' short distances. Larvae would often alight then move to another area, either making loose contact with the substratum with their pre-oral arms or swimming clear of it. None of the experimental substrata, chemicals, or other prepared treatments appeared toxic to the larvae. In all experiments, all unsettled larvae, and settled and metamorphosed forms, were alive and normal at the time of final scoring of settlement.

Substratum selection

Experiments with natural substrata at Lizard Is. revealed a moderate degree of substratum selectivity, and results of settlement trials were similar irrespective of whether larvae were offered a choice of substrata or not. Highest rates of settlement and metamorphosis were on coral rubble and the crustose coralline alga (CCA) *Lithothamnium pseudosorum*, but were highly variable on the CCA (Fig. 1A, B). Settlement was poorest on the CCA *Porolithon onkodes*. In the one (laboratory) trial in which settlement on *P. onkodes* was observed, settlement rates did not differ significantly from zero (Table 2). Settlement on crustose fleshy red algae (*Peyssonellia* sp.) was uniformly low in both the 'choice' and 'no choice' trials, and was similar to that on fouled ceramic tiles in the experiment in which a choice of substrata was offered (Fig. 1A, B). When there was no choice, settlement on the CCA *Neogoniolithon foslei* was similar to that on *L. pseudosorum*, but when larvae could choose among several substrata, settlement on *L. pseudosorum* was greater than on *N. foslei* (Fig. 1A, B). However, because settlement in the experiment offering larvae a choice of substrata was (unexplainably) low, detailed interpretation of these results should be regarded cautiously.

In addition to high settlement on rubble and *Lithothamnium pseudosorum*, relatively dense settlement was observed on a small area (< 2 cm^2) of mollusc shell surrounded by *L. pseudosorum* (which was over-

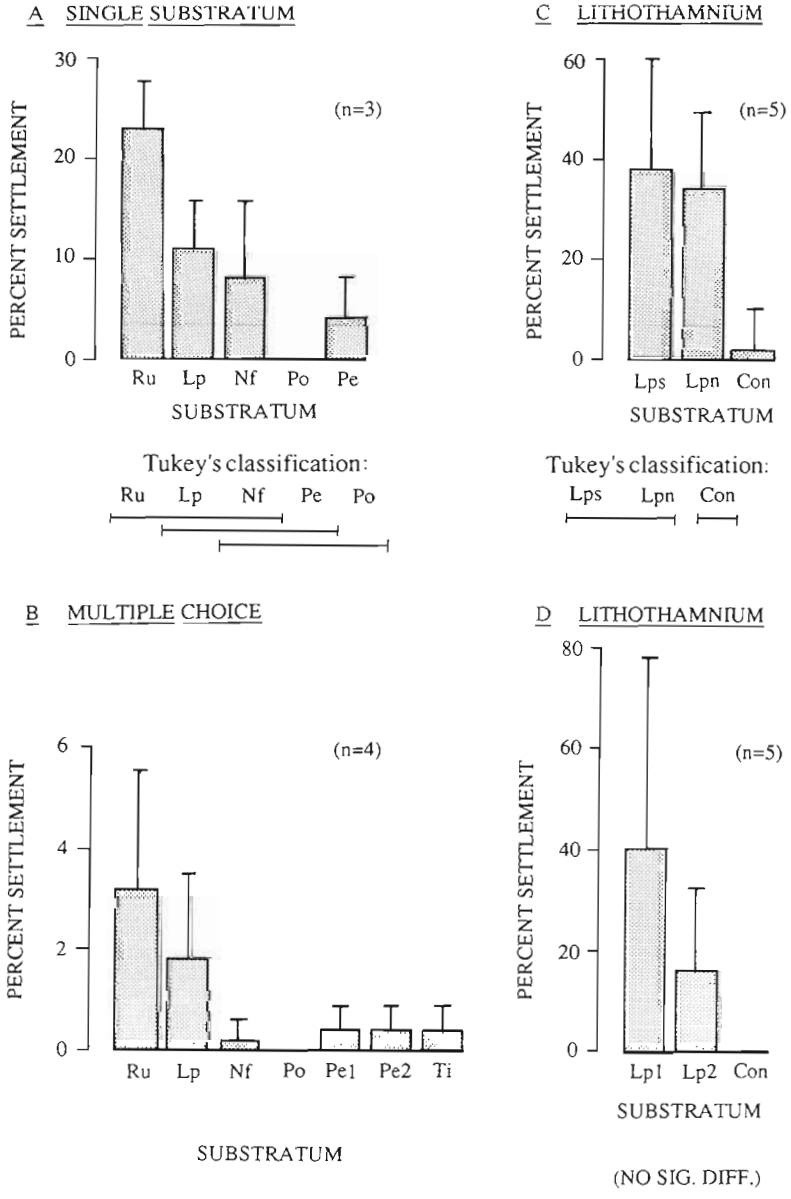

Fig. 1. *Acanthaster planci*. Settlement responses of larvae incubated with several substrata in experiments conducted at Lizard Is. and AIMS. Data are mean percentage settlement, bars are SD; homogeneous subsets according to Tukey's classification are given where ANOVAs indicate significant differences (α = 0.05, transformations as for ANOVA). (A) Settlement on coral rubble and crustose algae, no choice offered, showing highest settlement rates on rubble and 2 species of CCA (Lp and Nf); differences among substrata are significant (nested ANOVA, transformation = arcsin ($\sqrt{(Y/100) + 0.01}$), substratum effect $F_{[4,30]} = 7.80$, $p < 0.001$, chamber within substratum effect $F_{[10,30]} = 0.69$, $p = 0.73$). (B) Settlement when offered a choice of substrata, identifying coral rubble and *Lithothamnium pseudosorum* as preferred substrata; settlement was dependent on substratum type ($G_{adj[5]} = 33.77$, $p \ll 0.001$; substrata Pe1 and Pe2 pooled into single class, data pooled across all replicates). (C) Settlement on undamaged and scratched plants of *L. pseudosorum* and in controls, demonstrating high inducing ability of this alga but no differences among scratched and undamaged plants; differences are significant (ANOVA, transformation = $\ln((Y/100) + 0.1)$, $F_{[2,12]} = 22.47$, $p < 0.001$). (D) Settlement on 2 different *L. pseudosorum* plants, in which replicates were shards of individual plants, showing high degree of within-plant variability in ability of different sections of thallus to induce settlement; differences not significant (Kruskal-Wallis, $\chi^2_{[2]} = 4.82$, $p = 0.090$). n: no. independent replicates per treatment; Con: control (1 µm filtered seawater only); Lp: *L. pseudosorum* (Lp1 and Lp2 indicate 2 different plants); Lps: scratched *L. pseudosorum*; Lpn: undamaged *L. pseudosorum*; Nf: *Neogoniolithon foslei*; Pe: *Peyssonellia* sp. (Pe1 and Pe2 indicate 2 different species); Po: *Porolithon onkodes*; Ru: coral rubble; Ti: ceramic tile

Table 2. *Acanthaster planci*. Settlement response of larvae incubated for 2 d with preparations of coralline algae, coral rubble, and known chemicals, in experiments at Lizard Is. Data are means (SD in parentheses) of n = 3 independent replicates except for the fouled coral blocks where n = 6; 'No. larvae' indicates number of larvae in each replicate; GABA concentrations are final in assay vessel; K^+ concentrations are above background; significance levels in *t*-tests of null hypothesis that mean settlement = 0 are: ^{ns} $p > 0.05$, $^{••}$ $0.01 < p < 0.001$

Treatment	Settlement surface	No. larvae	Percent settlement	
Coralline algae				
Lithothamnium pseudosorum				
Fragments	Alga	50	44.7	$(7.6)^{••}$
Fragments beneath mesh	Mesh	40	26.7	$(32.2)^{ns}$
Plexiglas beneath mesh (control)	Mesh	40	0	
Boiled fragments	Alga	40	0	
In dialysis tubing	Tubing	40	0	
Aqueous extract	Coral block	40	0	
Ethanol extract	Coral block	40	0	
Chloroform extract	Coral block	40	0	
Porolithon onkodes				
Fragments (sun morph)	Alga	50	5.3	$(4.2)^{ns}$
Fragments (shade morph)	Alga	50	14.7	$(11.7)^{ns}$
Coral blocks and rubble				
Coral blocks fouled for 9 d	Coral block	40	0	
Aqueous extract of rubble	Coral block	40	0	
Ethanol extract of rubble	Coral block	40	0	
Chloroform extract of rubble	Coral block	40	0	
Known chemicals				
GABA (10^{-3}, 10^{-4}... 10^{-7}M)	Glass bowl	125	0	
K^+ (10, 20, 30 & 40 mM)	Glass bowl	125	0	

growing the shell) in a single unreplicated laboratory trial at AIMS (Table 2).

Settlement on *Lithothamnium pseudosorum*

The result of initial settlement trials, demonstrating that settlement of *Acanthaster planci* larvae was enhanced by *Lithothamnium pseudosorum*, was substantiated in further experiments at Lizards Is. and AIMS (Table 2, Fig. 1C, D), but settlement on *L. pseudosorum* was notably higher in experiments conducted at AIMS (cf. Fig. 1A, B with 1C, D). High variance in settlement on *L. pseudosorum* was characteristic of all trials with this alga, regardless of whether replicates were obtained from the same plant (Fig. 1A, B, D; Table 2) or from different plants (Fig. 1C). Settlement varied as much as 0 to 80 % on different shards of the same plant.

Although the inducing stimulus associated with *Lithothamnium pseudosorum* was not distributed uniformly on its surface, it was not restricted to a specific microhabitat on the alga. Larvae of *Acanthaster planci* attached to and metamorphosed in large numbers on both the pink growth surfaces and also on the white edges of fragments (i.e. exposed perithallus and hypothallus that were fouled lightly by exposure to seawater in aquaria; Table 3). Pooling all microhabitats, it was clear that significantly more larvae settled on undersides than on upper surfaces of fragments (Table 3).

Nature of inductive stimulus associated with *Lithothamnium pseudosorum*

Several observations indicated that the inductive agent associated with *Lithothamnium pseudosorum* is chemical, and that it may originate from, or be associated with, bacteria on the plant's surface. No settlement occurred on boiled (Table 2) or autoclaved (Table 4) fragments, suggesting that the stimulus is unrelated to physical characteristics of the crust surface, but is a chemical that is denatured at high temperatures. Chemical induction may also be indicated by the finding that larvae did not require contact with the surface of *L. pseudosorum* for induction to occur. Relatively high settlement rates sometimes occurred when larvae were separated from *L. pseudosorum* fragments by mesh (although variances were high and replication low so that mean settlement did not differ significantly from zero; Table 2). However, settlement was never observed in the presence of the mesh alone, suggesting that the inducing activity is associated with the coralline and can be released from it.

There was no evidence that the inducer was soluble

Table 3. *Acanthaster planci*. Settlement preferences of larvae for different microhabitats on shards of *Lithothamnium pseudosorum* in 9 separate settlement trials. Larvae showed marked preference for undersurfaces ($\chi^2_{[1]} = 146.9$, $p \ll 0.001$), and attached and metamorphosed in large numbers on both pink and exposed white surface on algal fragments. Note that not all microhabitats were available in each trial

Microhabitat	Upper surface			Under surface		
	No. settled:		% of total surface	No. settled:		% of total surface
	Observed	Expected[b]	area[a]	Observed	Expected[b]	area[a]
Lithothamnium pseudosorum						
Unbranched surface	3	47.2	7.8	132	94.4	15.6
Branched surface	11	94.4	15.6	0	33.7	5.6
Dead crust (surface)	10	10.1	1.6	37	13.5	2.2
Broken edges, sub surface[c]	66	91.1	15.0	247	134.9	22.2
Heavily fouled, sub surface	21	47.2	7.8	0	20.2	3.3
Mollusc shell[d]	36	6.7	1.1	44	13.5	2.2
Totals	147	296.7	48.9	460	310.2	51.1

[a] Estimated as percentage of all substrata in all trials
[b] Expected number of larvae calculated on basis of proportion of total surface area assuming no preferences
[c] 'Broken edges' were exposed regions of perithallus and hypothallus on fragments of plants (fragments were not freshly broken and thus were fouled lightly with an organic film)
[d] Around which *L. pseudosorum* was growing

in water, ethanol or chloroform, and it was retained by large diameter (10 000 Daltons) dialysis tubing, which suggests that it is a relatively large molecule (Table 2). However, since extraction may affect the inducer in some way, and amounts of concentrated extracts perfused into coral blocks may be unsuitable for induction (concentrations would decrease with time), these results should be regarded as tentative.

Effect of antibiotics

Results of all 3 experiments in which *Lithothamnium pseudosorum* was treated with antibiotics demonstrated clearly that settlement of *Acanthaster planci* on plants pre-treated with antibiotics was significantly lower than on untreated controls (Fig. 2A to C). In the first experiment, mean settlement on treated plants was ca 5 times lower than on plants untreated with antibiotics. The 2 experiments that examined both the effect of antibiotics on induction by CCA and the consistency with which individual shards were able to induce settlement, gave similar results. In the experiment conducted with fragments known to have high inducing ability, settlement on both the control and antibiotic treatment groups was similar in the first trial prior to administering antibiotics (mean settlement = 42.2 % and 40.7 % respectively, not significantly different, ANOVA, $F_{[1,10]} = 0.03$, $p = 0.87$). In the second trial, settlement on the control plants (55.0 %) was slightly higher than in the first, so that the difference in settlement (Trial 2 – Trial 1) was positive (Fig. 2B). In marked contrast, settlement on plants pretreated with antibiotics was reduced greatly, so that in the paired comparison, the difference in settlement (Trial 2 – Trial 1) was negative and significantly less than in the control group (Fig. 2B).

This result was confirmed in a third experiment, which also showed that settlement on highly inductive shards (in the first trial) was reduced significantly in the second trial after treatment with antibiotics (Fig. 2C). In contrast, settlement on control plants was similar in both trials, so that the inductive ability of individual pieces in the first trial correlated highly with their inductive ability in the second (Fig. 2C). In the control treatment, larvae were observed to settle on approximately the same sites on individual shards in both trials.

Bioassays with bacteria from CCA

There was no evidence that the common plate viable bacteria isolated from surfaces of *Lithothamnium pseudosorum* play a role in inducing settlement or metamorphosis of crown-of-thorns larvae (Table 4). In most assays with bacteria, whether single strains or mixtures of bacteria coated onto ground-glass slides or sterile fragments of *L. pseudosorum*, all larvae either continued normal planktonic swimming, or sank and commenced crawling on the bottom of assay dishes. Those that sank did not attach to the sides of the dishes and/or commence metamorphosis. Low levels of settlement recorded in some treatments containing bacteria can be attributed to 'spontaneous settlement', such as was observed in some of the control treatments consisting of SSW plus a glass slide without added bacteria (Table 4). Larvae used in these experiments were competent to settle, since high levels of settlement occurred

Table 4. *Acanthaster planci*. Settlement response of larvae incubated with bacteria isolated from surfaces of *Lithothamnium pseudosorum*. Controls for spontaneous settlement (= SSW + glass slide) were run with each set of bioassays (i.e. with each batch of larvae); controls with coralline algal fragments demonstrate competency of larvae. Bacteria from plants A to C were isolated immediately on collecting the alga; those from plants D to G were isolated from shards known to induce high rates of settlement of *A. planci*. Data are means (SD in parentheses); n = number of replicates, each containing 10 larvae; SSW = 0.45 µm filtered seawater; ni: not identified; significance levels in *t*-tests of null hypothesis that mean settlement = 0 are: ^{ns} $p>0.05$, $^{••}$ $0.01<p<0.001$, $^{•••}$ $p<0.001$ For mixtures of bacteria, upper case letters (A) identify different plants, lower case letters (a) refer to particular bacterial strains; mix identifications are Mix 1 = Strains Ab, Bd, Bf, Cg; Mix 2 = Strains Aa–Ad, Bd; Mix 3 = Strains Ae, Bc, Be, Bf; Mix 4 = Strains Ba–Bb, Ca–Cc; Mix 5 = Strains Cd, Ce, Cg, Ch; Mix 6 = Strains Da–De; Mix 7 = Strains Ea–Ee; Mix 8 = Strains Fa–Fe; Mix 9 = Strains Df, Ff, Ga–Ge

Treatment	n	Percent settlement	
Controls			
SSW + glass slide (4 sets)	All 5	All 0	
SSW + glass slide	5	2.0	$(4.5)^{ns}$
SSW + glass slide	5	8.0	$(8.4)^{ns}$
L. pseudosorum fragments	5	72.0	$(24.9)^{••}$
L. pseudosorum fragments	10	60.0	$(30.9)^{•••}$
Sterilised *L. pseudosorum* fragments	10	0	
Bacteria: individual strains coated onto slides			
From *L. pseudosorum* plant A; 10 m depth			
Strain b = *Vibrio*	5	6.0	$(8.9)^{ns}$
Strain d = *Vibrio*	5	0	
Strain e = *Alteromonas/Pseudomonas*	10	0	
From *L. pseudosorum* plant B; 15 m depth			
Strains a, b (ni)	Both 5	Both 0	
Strain d = *Vibrio*	5	8.0	$(13.0)^{ns}$
Strain e = *Vibrio*	5	4.0	$(5.5)^{ns}$
From *L. pseudosorum* plant C; 15 m depth			
Strain a = *Vibrio*	5	0	
Strain g = *Vibrio*	5	14.0	$(13.4)^{ns}$
Strain b, d, e = *Alteromonas/Pseudomonas*	5, 10, 5	All 0	
Strain c = (ni)	5	0	
From *L. pseudosorum* shards shown to have high inducing ability			
Plant D; 8 m depth			
Strains a, e, f (ni)	All 5	All 2.0	$(4.5)^{ns}$
Plant E; 8 m depth			
Strains a–e (ni)	All 5	All 0	
Bacteria: mixtures of strains coated onto glass slides			
Identified strains from *L. pseudosorum*			
Mix 1	10	1.0	$(3.2)^{ns}$
Mixes 2–5	All 10	All 0	
From *L. pseudosorum* shards shown to have high inducing ability			
Mix 6	10	1.0	$(3.2)^{ns}$
Mixes 7, 8	Both 10	Both 0	
Mix 9	10	2.0	$(3.2)^{ns}$
Bacteria: mixtures of strains coated onto sterilised *L. pseudosorum* fragments			
Identified strains from *L. pseudosorum*			
Mixes 1–5	All 10	All 0	
From *L. pseudosorum* shards shown to have high inducing ability			
Mixes 6–9	All 10	All 0	

in controls containing *L. pseudosorum*. Larval behaviour indicated no adverse effects in treatments containing added bacteria.

Common bacterial genera on tropical CCA

The plate-viable isolates identified from all 6 species of CCA were all commonly occurring genera of gram-negative marine bacteria (Table 5). There was no evidence that crust surfaces of these coralline species were dominated by genera that occur rarely elsewhere in the marine environment, such as has been reported for temperate CCA (Garland et al. 1985). All 49 positively identified isolates belonged to one of 6 genera (or genus groupings), viz. *Aeromonas* (1 isolate), *Alcaligenes* (2), *Alteromonas/Pseudomonas* (15), *Flavobac-*

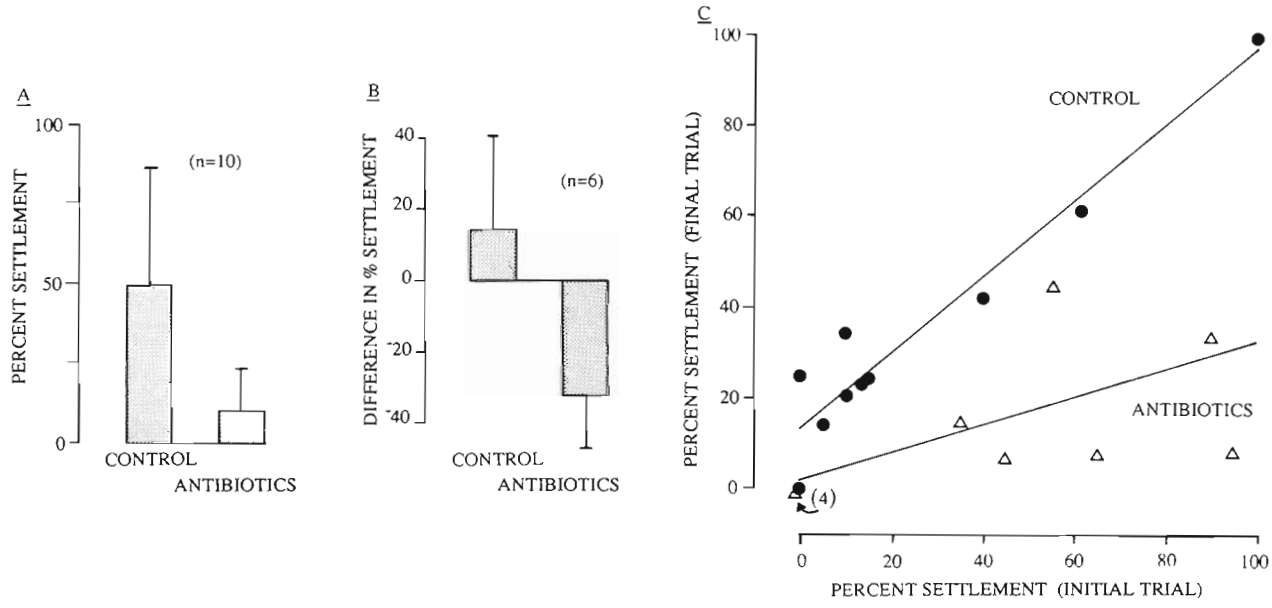

Fig. 2. *Acanthaster planci*. Effect of treatment of *Lithothamnium pseudosorum* with antibiotics on its ability to induce settlement of larvae (n = no. of independent replicates). For bar graphs data are means, bars are SD. (A) Settlement on plants treated and untreated with antibiotics, showing that treatment with antibiotics reduces settlement; difference is significant (ANOVA, $F_{[1,18]} = 9.55$, $p = 0.006$). (B) Difference in settlement between 2 consecutive settlement trials (Trial 2 – Trial 1; the same plants used in both trials), in which one group of plants was treated with antibiotics prior to the second trial and the second (control) group was not; all plants known to have high inducing ability; difference is significant (ANOVA, transformation = arcsin ($\sqrt{Y + 0.44}$), $F_{[1,10]} = 11.75$, $p = 0.007$). (C) Relationship of settlement in 2 consecutive settlement trials; data are for 2 groups of algal fragments (same individual fragments used in both trials), in which one was treated with antibiotics prior to the second trial and the second (control) was not, showing that inducing ability of individual fragments is consistent in consecutive trials except when fragments are treated with antibiotics, in which case inducing ability is reduced greatly; line of best fit for control group has slope = 0.84, which differs significantly from 0 ($t_{[8]} = 9.99$, $p < 0.001$), $R^2 = 0.92$ (adjusted for df and no. of parameters); line of best fit for treatment group has slope = 0.25, which does not differ significantly from 0 ($t_{[8]} = 2.18$, $p = 0.06$), $R^2_{adjusted} = 0.29$; difference in slopes of the 2 lines is significant ($t_{(2)[16]} = 3.92$, $0.001 < p < 0.002$); mean difference in settlement rate (initial-final) is significantly smaller in control group than in treatment group (ANOVA, transformation = $\ln(Y + 0.3)$, $F_{[1,18]} = 14.57$, $p = 0.001$)

terium (2), *Photobacterium* (3) and *Vibrio* (26). There was no indication that *Lithothamnium pseudosorum* supported characteristic bacteria not found on the other species of CCA; the 2 genera (*Vibrio* and *Alteromonas/Pseudomonas*) identified from 19 distinct phenotypes isolated from *L. pseudosorum* occurred on all of the other 5 species of CCA examined, including *Porolithon onkodes*.

Spatial distribution of bacteria on CCA

Examination (SEM) of the distribution of bacteria on surfaces of *Lithothamnium pseudosorum* revealed that bacterial colonisation was highly variable both within and among plants (cf. Fig. 3b to d). Because surface cells are sloughed (Fig. 3a), the degree of fouling varied from zero on newly exposed regions (Fig. 3b), to dense colonisation by bacteria and/or microalgae on other sections of crust (Fig. 3c, d).

Exposed cell walls appeared to be covered with a smooth film (Fig. 3b, d), and were unlike the rough naked calcium carbonate found on some other CCA species (Garland et al. 1985, Johnson & Mann 1986, Johnson et al. unpubl.). At low to moderate densities, epiphytic bacteria aggregated on the exposed polysaccharide-rich middle lamellae, and were notably less dense toward the centre (primary pit connection) of exposed walls (Fig. 3d, e). Damaging the algal surface by scratching it with a metal scribe did not facilitate colonisation by bacteria. At 24, 51, 75 and 99 h (Fig. 3e to 3h respectively) after damaging, colonisation of scratches was usually extremely low despite being only micrometers from a ready inoculum on the adjacent unscratched crust, although small dense clusters of rod-shaped bacteria were observed rarely in localised regions of scratches (Fig. 3i). Scratching the surface in this manner appeared to smear and seal the surface rather than exposing cell contents.

Densities of bacteria on areas of *Lithothamnium*

Table 5. Identification of bacteria (common morphological phenotypes only) isolated from surfaces of crustose coralline algae collected on the fore-reef slope of Davies Reef. ui: unidentified isolate having no requirement for sodium; missing isolates are those that died before all tests required for identification were completed; •• isolates that died before final tests to distinguish among several genera could be completed

Crustose coralline alga	Depth (m)	Isolate	Genus of isolate
Porolithon onkodes	5	a, b	*Vibrio*
		c	*Alteromonas/Pseudomonas*
		e	*Alcaligenes*
Paragoniolithon conicum (plant A)	5	a, b, d, e, g, h, j	*Vibrio*
		c, f, k	*Alteromonas/Pseudomonas*
		m	*Alcaligenes*
Neogoniolithon foslei	10	d, h	*Vibrio*
		g	*Flavobacterium*
		j	*Alteromonas/Pseudomonas*
Lithothamnium pseudosorum (plant A)	10	b, c, d	*Vibrio*
		a, e	*Alteromonas/Pseudomonas*
Lithothamnium pseudosorum (plant B)	15	c, d, e, f, g	*Vibrio*
		a, b	ui
Lithothamnium pseudosorum (plant C)	15	a, g, h	*Vibrio*
		b, d, e, f	*Alteromonas/Pseudomonas*
Mesophyllum syrphetodes	20	c	*Vibrio*
		a	*Alteromonas/Pseudomonas*
		b	*Aeromonas*
Paragoniolithon conicum (plant B)	20	a, b	*Alteromonas/Pseudomonas*
		c, d••	*Alteromonas/Pseudomonas/Alcaligenes*
Paragoniolithon conicum (plant C)	25	a	*Vibrio*
		b, c, d	*Photobacterium*
Mesophyllum purpurescens	25	a, c	*Vibrio*
		b	*Flavobacterium*
		d	*Alteromonas/Pseudomonas*

pseudosorum crust on which starfish were settling were higher (Fig. 4a) than on areas where settlement was not observed (Fig. 4b). Newly metamorphosed starfish were not always observed on regions of dense colonisation by bacteria, but were never observed on areas where bacteria were sparse. Threads of mucus on the crust surface were normally observed around starfish > ca 2 d post settlement, and were sometimes colonised by bacteria (Fig. 4c, d). It is likely that this mucus is produced by the starfish.

No induction by GABA or K^+

Larvae were not induced to settle or metamorphose when incubated with GABA at final concentrations of 10^{-7} to 10^{-3} M, or with K^+ ion concentrations elevated to between 10 and 40 mM above background (Table 2). In nearly all cases larvae continued their normal planktonic swimming behaviour and there was no evidence of deleterious effects or toxicity of any of the treatments. The only exception was at 40 mM K^+ above background, which initially caused larvae to sink to the bottom of the assay vessel and to retract their larval arms and contract in size. However, after several hours they re-extended their arms and resumed normal swimming behaviour in the water column.

DISCUSSION

Specificity of substratum selection

The specificity of substratum selection by settling larvae potentially influences patterns of recruitment. Our results show that larvae of *Acanthaster planci* require a cue for high rates of settlement and metamorphosis and that larvae manifest a moderate degree of selectivity in choosing among surfaces available in their natural habitat. They settle and metamorphose on a variety of biologically active substrata, but do so at significantly higher rates on coral rubble and the crustose coralline alga (CCA) *Lithothamnium pseudosorum* than on other CCA (*Porolithon onkodes* and *Neogoniolithon foslei*), non-calcareous crustose algae, and fouled ceramic tiles. Our single observation (Table 3) of high settlement on a small area of exposed mollusc shell (that was otherwise covered with *L. pseudosorum*) cannot be compared meaningfully with results of the

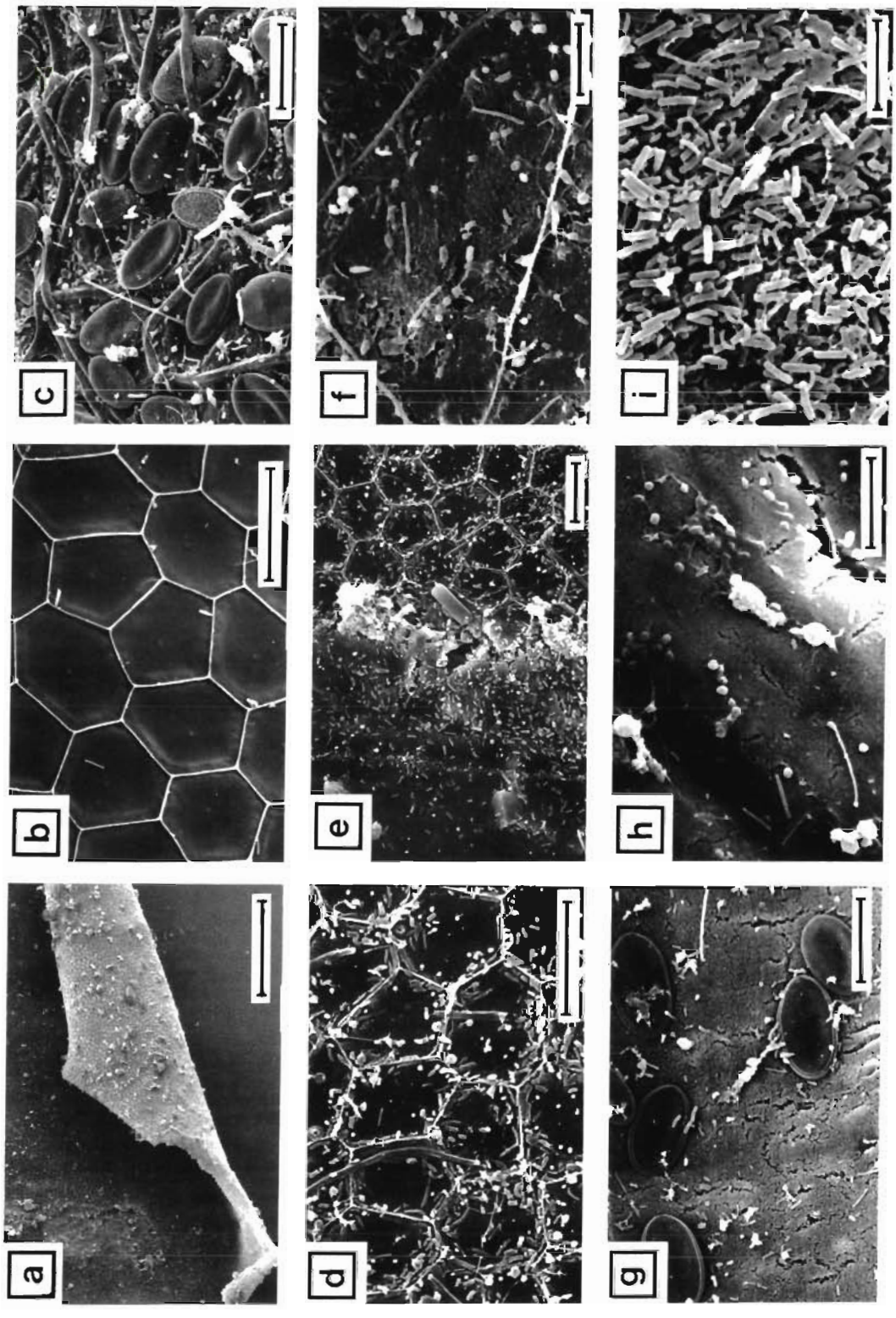

Fig. 3. *Lithothamnium pseudosorum*. Scanning electron micrographs of surfaces revealed that the nature and degree of fouling of the surface varies greatly both within and among plants. When surface cells were sloughed (a) they exposed new cells without epiphytes (b) (note primary pit connections), but other areas of the same crust showed greater amounts of fouling by bacteria and microalgae (c). Bacteria at moderate densities were often aggregated on the middle lamella at boundaries of cells (d), (e). Damaging plants by scratching them with a metal scribe rarely induced dense colonisation by bacteria, even after 24 h (e), 51 h (f), 75 h (g) or 99 h (h), but occasionally aggregations of rod-shaped bacteria at densities higher than on adjacent undamaged thallus occurred in small patches in a scratch. Scale bars are (a): 200 µm; (b) to (e) and (g): 10 µm; (f), (h) and (i): 5 µm

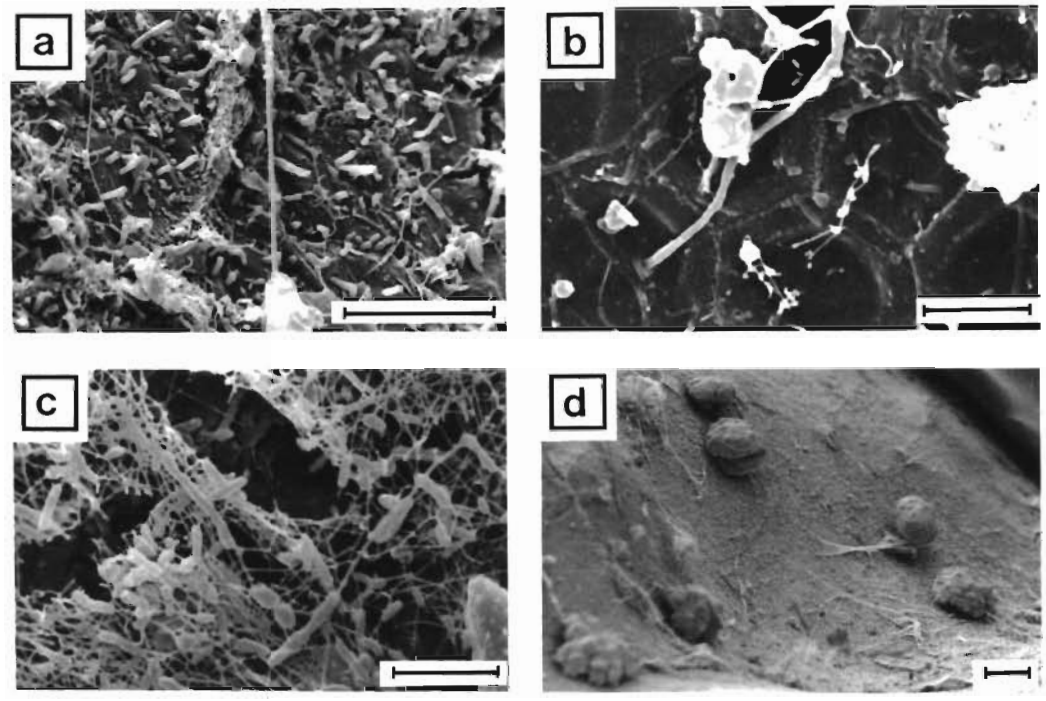

Fig. 4. *Lithothamnium pseudosorum*. Scanning electron micrographs of surfaces adjacent newly settled and metamorphosed *Acanthaster planci* showed 2 features; first, that densities of bacteria on crust surfaces onto which *A. planci* were settling (a) were greater than on other regions of the algal surface where settlement was not observed (b), although regions could be found where bacterial densities were high but where no settlement was observed. Second, mucus threads, sometimes colonised by bacteria (c), were always observed on the substratum around juveniles > ca 2 d post-settlement, and large stands of mucus over the crust were visible at low magnification in situations where several larvae had metamorphosed on a highly inducing section of crust (d). Scale bars are (a): 10 μm; (b) and (c): 5 μm; (d): 200 μm

selectivity experiments, but suggests that high settlement rates may not be specific to rubble and *L. pseudosorum*. Although rates of settlement and metamorphosis were more consistent on rubble than on *L. pseudosorum*, interpreting this result is difficult because all coral rubble supported some epilthic CCA. Our results are broadly consistent with those of several earlier studies indicating that a biologically active stimulus is required and that CCA can induce settlement and metamorphosis (Henderson & Lucas 1971, Yamaguchi 1973a, Lucas 1975), but early methods were not sufficient to resolve much about the cues, and do not allow detailed comparison with the present study.

The stimulus for settlement does not appear to be tactile or from a non-specific bacterial film. Larval induction apparently requires more than a non-specific fouling film since settlement on ceramic tiles allowed to foul for several weeks and on coral blocks fouled for 9 d was zero or negligible. A tactile stimulus is unlikely because larvae will settle at high rates on live *Lithothamnium pseudosorum* but not on (physically similar) boiled, bleached or autoclaved fragments. Larvae do not settle and metamorphose, or only at negligible rates, on the roughly texured surface of coral (*Porites*) blocks or on smooth plastic or glass surfaces. Others have also reported that bleached CCA (Yamaguchi 1973a) and clean glass surfaces (Henderson & Lucas 1971, Ormond & Campbell 1974) are not inductive. In contrast, Ormond & Campbell (1974) observed that larvae settled in the calices of bleached coral of one species (*Acropora hyacinthus*) but not others, and concluded that a tactile response to surface rugosity may be important in settlement.

It must be emphasized that finding small (< 1 +) juveniles of *Acanthaster planci* feeding on or associated with *Porolithon onkodes* (Zann et al. 1987) or unidentified CCA (Yogochi & Ogura 1987) in the field does not indicate that these substrata induce larvae to settle and metamorphose, or that they are a preferred food. *P. onkodes* is by far the most abundant crustose coralline growing in shallow (0 to 3 m) water in the tropical Pacific (Adey et al. 1982, Steneck et al. unpubl.) and therefore is the species most likely to be encountered by coralline-feeding juveniles in shallow water irrespective of settlement or food preferences.

Nature of inducer of *Acanthaster planci* associated with coralline algae

It is widely recognised that CCA induce settlement and metamorphosis of a great diversity of marine invertebrate larvae other than *Acanthaster planci* (e.g. soft octocorals: Sebens 1983; scleractinian corals: Morse et al. 1988; polychaetes: Gee 1965; abalone: Morse & Morse 1984, Shepherd & Turner 1985, Prince et al. 1987, McShane & Smith 1988; limpets: Steneck 1982; chitons: Barnes & Gonor 1973, Rumrill & Cameron 1983; sea urchins: Pearce & Scheibling 1988), but the exact nature and origin of inducers associated with CCA is still unclear (see Johnson et al. 1991). The present work is the first in which bacteria have been implicated in induction by CCA.

The inducer of *Acanthaster planci* larvae associated with the CCA *Lithothamnium pseudosorum* appears to be a chemical either originating from, or associated with, bacteria epiphytic on the alga, since inductive activity is inactivated by boiling or autoclaving, and reduced greatly by treating the alga with antibiotics. We assume that vigorous rinsing of the alga with sterile seawater ensured that larvae were not exposed to antibiotics, and that the moderate concentrations of antibiotic did not affect the alga itself. Low levels of settlement on plants treated with antibiotics is explained by reduced densities of inductive bacteria and/or 'spontaneous' settlement in the absence of a known inducer (note that many marine bacteria are not sensitive to antibiotics; our pilot tests showed that the antibiotic treatment did not render the algal surface axenic).

Other of our results are consistent with induction mediated by bacteria. Large differences in inductive ability of different regions of a single crust surface indicates that the activity and/or distribution of the inducer is not uniform over the plant surface. This correlates qualitatively with densities of bacteria on the crust surface, which range from virtually no bacteria on patches recently exposed by sloughing to areas heavily fouled by both bacteria and diatoms (Fig. 3). Furthermore, the crust adjacent to newly metamophosed starfish always supported high densities of bacteria, but starfish were never observed to settle on sections of crust where bacteria were sparse. Epiphytic bacteria on other species of CCA are also distributed patchily (Johnson et al. unpubl.; see also Garland 1985, Johnson & Mann 1986). High variability in settlement cannot be ascribed to chance selection of larvae at different stages of development because all larvae used in experiments at AIMS were selected after careful microscopic examination. Moreover, individual shards were consistent in their inductive ability, i.e. poorly (or highly) morphogenic pieces were consistently poorly (or highly) morphogenic in successive trials with independent batches of larvae. The consistency in ability of individual pieces of crust to induce settlement is an important result. It suggests a concentration-dependent response of larvae to inducer, which may relate to densities of inductive bacteria. It is unlikely to derive from first-settling starfish conditioning the surface in some way (e.g. by release of mucus) since this cannot account for inductive CCA collected in areas where juveniles were never observed and unlikely to occur. Also, the action of antibiotics in reducing induction would not be expected if the inducer was in a compound released by juvenile starfish.

The inability of bacteria isolated from inductive CCA to induce settlement cannot be taken to indicate that bacteria play no role in the morphogenic process. Since it is highly likely that $< 1\,\%$, and almost certainly $< 5\,\%$, of epiphytic bacteria on CCA are plate-viable (see Kaneko et al. 1978, Davis et al. 1983, Hobbie 1988), the vast majority of strains epiphytic on *Lithothamnium pseudosorum* could not be tested for ability to induce settlement.

Our experiments with extracts of *Lithothamnium pseudosorum* perfused into coral blocks suggest tentatively that the alga-associated inducer is insoluble in water, ethanol and chloroform. Alternative explanations are that the inducer is soluble but was not present at concentrations optimal for settlement, or that it was affected by the experimental procedure. If the inducer is insoluble in water, this does not contradict our observation that larval contact with *L. pseudosorum* is not obligatory for induction, but suggests that the inducer can be dislodged from the algal surface and detected in suspension. Bacteria are clearly a candidate for a morphogen that is dislodged readily.

In sum, our evidence suggests that induction of *Acanthaster planci* on surfaces of *Lithothamnium pseudosorum* may be mediated by bacteria. If so, then *A. planci* joins several other marine invertebrate species whose larvae are induced to settle by particular bacteria or bacterially-produced compounds (scyphozoans: Hofmann et al. 1978, Neumann 1979, Wolk et al. 1985, Fitt et al. 1987, Hofmann & Brand 1987; polychaetes: Gray 1966, 1967, Kirchman et al. 1982; oysters: Weiner et al. 1985, Fitt et al. 1990) or by an unspecified 'bacterial film' (polychaetes: Meadows & Williams 1963, Kirchmann et al. 1982; sea urchins: Cameron & Hinegardner 1974; bryozoans: Mihm et al. 1981, Brancato & Woollacott 1982; general reviews: Crisp 1974, Scheltema 1976).

Specificity of epiphytic bacteria to *Lithothamnium pseudosorum*

If *Acanthaster planci* larvae are induced by bacteria, this raises the question of the specificity of their settle-

ment on *Lithothamnium pseudosorum* and coral rubble. Are inductive bacteria more likely to occur on some substrata than on others, or are marine bacteria in general inductive and settlement rates depend only on bacterial densities? The latter seems unlikely since bacteria are abundant both in water on coral reefs (Moriarty 1979) and on solid substrata (that lack antifouling mechanisms) in the marine environment in general (e.g. Seiburth et al. 1975). Thus, substratum selection by *A. planci* larvae is likely to depend on the nature of bacteria present.

This argument is consistent with findings that bacterial populations on different species of CCA are distinct from each other and from those in other marine microhabitats (Lewis et al. 1985, Johnson et al. 1991). Evidence of Johnson et al. (unpubl.) indicates that bacteria-CCA associations may result from some bacteria utilising nutritional substrates that are specific to particular species of CCA. The spatial distribution of bacteria on *Lithothamnium pseudosorum* also suggests that they derive nutrition from the alga. At medium densities microbes aggregated around the polysaccharide-rich middle lamellae (Fig. 3d, e), but bacterial colonisation of scratches, which provided a large area for attachment but appeared to seal the surface rather than expose fresh cellular material, was low despite a ready source of inocula adjacent to the scratch (Fig. 3f to h).

Our methods did not enable us to determine whether bacterial populations on *Lithothamnium pseudosorum* are in any way characteristic. Isolates identified from surfaces of *L. pseudosorum* and poorly inductive CCA (*Porolithon onkodes* and *Neogoniolithon foslei*) were all commonly occuring marine genera (*Vibrio* and *Alteromonas/Pseudomonas*). However, since so few bacteria are plate-viable, that identification was to genus level only and that a comprehensive analysis of physiological properties was not attempted, it cannot be concluded that bacteria associated with *L. pseudosorum* are unspecific.

Deep water recruitment hypothesis

Given the unique feature of large numbers of secondary outbreaks of *Acanthaster planci* on the GBR and evidence to indicate that they are the result of mass settlement events (see 'Introduction'), the question arises, Why aren't high densities of juveniles readily observed on the GBR? Erratic and infrequent mass recruitments, such as recorded for *A. planci* in Fiji (Zann et al. 1987) and for other echinoderms with similar planktonic life cycles (Ebert 1983), cannot account for the paucity of juveniles found on the GBR because the number of outbreaks is so large. However, it may explain why so few juvenile *A. planci* have been found elsewhere in the Indo-Pacific (Yamaguchi 1973b, 1975, Birkeland & Lucas 1990 and references therein).

Our result that larvae of *Acanthaster planci* manifest clear preferences for settlement and metamorphosis on coral rubble and *Lithothamnium pseudosorum* supports the hypothesis that mass recruitment of *A. planci* is more likely to occur in deep than in shallow water. Species of *Lithothamnium*, including *L. pseudosorum*, while occurring in shaded and cryptic microhabitats in shallow water, are most abundant in deep water and dominate the CCA flora below 50 to 60 m where they may form extensive beds of free-living maerl or rhodoliths (Adey et al. 1982, Steneck et al. unpubl.). Similarly, greatest accumulations of coral rubble (inevitably supporting epilithic CCA) occur at the base of reef slopes in deep water. Advantages to larvae settling on CCA include an immediate food source, since they feed exclusively on CCA for at least the first 7 to 10 mo of life (Yamaguchi 1973a, Lucas 1984, Zann et al. 1987), and protection from predators in the plants' interstices. In and among rhodoliths, which are widely reported in deep water (see Littler 1973, Scoffin et al. 1985 and references therein), interstices are large enough to shelter juveniles until they reach the coral-eating phase. Accumulations of rubble also provide interstices and a ready supply of CCA.

Observations of the pattern of outbreaks also supports the notion of deep-water recruitment. Among GBR reefs that were monitored continuously prior to outbreaks (i.e. ensuring that outbreaks were discovered in their initial stages), increased abundances of *Acanthaster planci* were always first observed as aggregations of adults (estimated at 2 to 3 yr old) ascending the reef from deepwater (Table 6). On a larger number of reefs that were visited less frequently, it was often observed that adult starfish were aggregated at high densities at depth (> 20 to 40 m), and that dead coral and starfish feeding scars were clearly visible below the aggregations, but there was no evidence of predation of coral above the starfish (Endean & Stablum 1973, Moran 1986, P. J. Doherty pers. comm., P. J. Moran pers. comm.). This pattern has been interpreted to indicate the onset of an outbreak with starfish infesting the reef from depth.

Black & Moran (1991) simulated the local hydrodynamics of 6 of the reefs listed in Table 6, and in every case identified a retention cell in deep water off the reef but adjacent to the location where starfish were first detected as adults ascending from depth. We conducted preliminary deep water (ca 30 to 65 m) video transects off the northern end of Davies Reef and found that, close to the site of initial outbreak, rubble and CCA occurred abundantly to ca 60 to 65 m (Fig. 5). This abundance of coralline algae and rubble in deep water

Table 6. Reefs of the GBR system on which outbreaks of *Acanthaster planci* were detected in their initial stages. In all cases outbreaks were first observed as aggregations of adult starfish ascending the reef from deepwater (only reefs which were surveyed several times each year and which supported no or only low numbers of starfish when first visited were considered, thus ensuring that outbreaks were observed at their beginning; unpublished data courtesy P. Moran)

Reef	Date	Location of outbreak
Lodestone	25 Mar 83	Northeast corner
John Brewer	14 May 83	Southern slope
Keeper	21 Oct 83	Southern slope
Glow	10 Mar 84	Northeast corner
Dip	12 Mar 84	Northeast corner
Faraday	12 Mar 84	Northern corner
Hopkinson	13 May 84	Northeast slope
Helix	13 May 84	Northern slope
Yankee	24 Jun 84	Northwest slope
Wheeler	18 Jul 84	Southeast slope (adjacent cay)
Bowden	21 Oct 86	Southwest corner
Davies	23 Oct 86	Northern slope

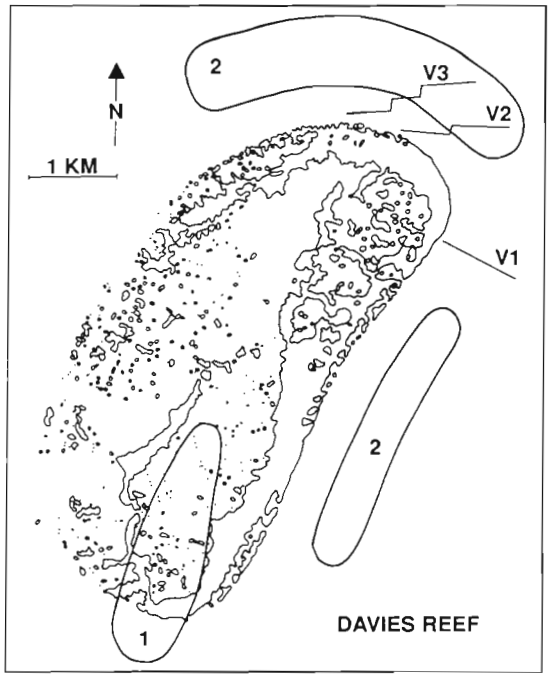

Fig. 5. Davies Reef showing position of (i) 3 deep water (30 to 65 m) videotransects (V1 to V3), (ii) sites where *Acanthaster planci* outbreak was first observed as adult starfish ascending the reef from deep water (shaded area), and (iii) primary (1) and secondary (2) hydrodynamic retention cells (after Black & Moran 1991). Coralline algae and coral rubble occurred abundantly along the length of V2 and V3, but V1 was over a sandy bottom

coincides with one of the hydrodynamic retention cells identified by Black & Moran (1991). In contrast, on Transect V1 (Fig. 5), away from the area of the initial outbreak, the substratum was sand over a similar depth range.

The total of these observations leads us to propose the hypothesis that settlement and recruitment of *Acanthaster planci* in high densities on the GBR is more likely to occur in deep water by the following mechanism: (1) subsequent to mass spawning (Moran 1986), parcels of water containing high densities of neutrally or positively buoyant larvae are transported by water currents, and the probability of 'fallout' of negatively buoyant late brachiolaria (buoyancy observations this study) is greatest where hydrodynamic retention cells occur; (2) if inductive substrata (rubble and CCA) coincide with areas of highest likelihood of sinking then high rates of settlement and metamorphosis occur; (3) starfish remain in these deepwater nursery areas until reaching their coral-eating phase, at which time they become increasingly mobile and begin to ascend the reef slope. On every reef modelled by Black & Moran (1991), they identified retention cells adjacent to areas where outbreaks did not occur initially. The absence of initial outbreaks adjacent to some retention cells may reflect lack of suitable substrata for induction of settlement, as suggested by Videotransect V1 (Fig. 5). This hypothesis does not posit that mass recruitment does not occur in shallow water on the GBR (clearly it does in other areas; see Zann et al. 1987), only that it is more likely to occur in deep water. We suggest strongly that the hypothesis of deepwater recruitment warrants further examination.

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