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REPORT

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Natural inducers for coral larval metamorphosis

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Abstract Coral gametes from *Acropora millepora* (Ehrenberg, 1834) and from multi-species spawning slicks provided larvae for use in metamorphosis assays with a selection of naturally occurring inducer chemicals. Four species of crustose coralline algae, one non-coralline crustose alga and two branching coralline algae induced larval metamorphosis. However, one additional species of branching coralline algae did not produce a larval response. Metamorphosis was also observed when larvae were exposed to skeleton from the massive coral *Goniastrea retiformis* (Lamarck, 1816) and to calcified reef rubble, demonstrating metamorphosis is possible in the absence of encrusting algae. Chemical extracts from these algae and the coral skeleton, obtained using either decalcification or simple methanol extraction procedures, also contained active inducers. These results extend the number of crustose algal species known to induce coral metamorphosis, suggest that some inducers may not necessarily be strongly associated with the calcified algal cell walls, and indicate that inducer sources in reef habitats may be more diverse than previously reported.

Key words Coral · Larvae · Metamorphosis Recruitment · Chemical · Inducer

Introduction

A majority of sclerectinian corals reproduce by broadcast spawning, with many species ejecting gametes into the water column simultaneously at annual spawning events (Richmond and Hunter 1990). The planula larvae of some broadcast spawning corals can be competent to metamorphose as early as 2–3 days post-fertilization (e.g. Heyward et al. 1987; Harrison and Wallace 1990), but early metamorphosis on the fourth and fifth days is reported for many species in seawater temperatures around 27 °C (e.g. Babcock and Heyward 1986). As the energy reserves from the oocyte (Arai et al. 1993) are used up during larval development, cilia develop and the sensory and secretory cells of the epidermis differentiate. The onset of larval competency coincides with decreasing larval buoyancy, increased motility and further development of secretory cells and sensory capability at the aboral end, which is likely to allow the larvae to sample the substrate and then adhere to it (Heyward 1987). Competent coral larvae maintained *in vitro* appear to probe or crawl over available surfaces and are thought to actively select a suitable substrate upon which to attach and metamorphose (Fadlallah 1983; Harrison and Wallace 1990).

While substrate orientation and rugosity are well known to influence the site of metamorphosis in larvae of several coral species, more recent investigations have revealed that the process of metamorphosis itself can be triggered by biochemical inducers (Morse et al. 1988; 1994; 1996). Larvae of the temperate soft coral *Alcyonium siderium* (Sebens 1983) and the agariciid corals, *Agaricia tenuifolia*, *A. agaricites humilis* and *A. agaricites danai* (Morse et al. 1988) were reported to prefer crustose coralline algae (CCA) as a substrate for settlement and metamorphosis. Morse et al. (1988) showed that *A. humilis* larvae were triggered to metamorphose by a chemical component of CCA and the authors suggested a chemical cue might be required for metamorphosis in this species. Morse et al. (1996) went on to describe a "common chemosensory mechanism" for the larvae of several Pacific acroporid and favid coral species. This inducer was not water-soluble but appeared to be associated with the cell-wall fraction of the CCA *Hydrolithon boergesenii* and was released by decalcification (Morse et al. 1994). Although the structure of the

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inducer has not yet been fully elucidated, the molecule appears to be a large polysaccharide (6000–8000 Da) (Morse and Morse 1991; Morse et al. 1994).

Several studies of larval development, recruitment and subsequent survival of juvenile corals (e.g. Heyward 1987; Babcock 1992; Babcock and Mundy 1996; Wilson and Harrison 1998) have achieved settlement using seawater conditioned substrates without acknowledging the presence of coralline algae. This may simply be a matter of lack of detection or observation, but the role that CCA may play in these types of studies is yet to be routinely considered as a key variable in field or laboratory studies of coral recruitment. While there is mounting data describing the important role of CCA in coral settlement and metamorphosis, larvae may also respond to environmental triggers from a wider range of substrates than previously reported. In this study, we confirm settlement induction in Indo-Pacific reef-building scleractinian corals in response to several species of calcareous red algae (CRA), branching coralline algae (BCA) and extracts of CRA. We examine other potential settlement substrates such as coral rubble and coral skeleton for settlement inducers to further elaborate the range of inducer sources on coral reefs, and we use CRA to induce settlement of wild-caught larvae from two multi-species cohorts generated on nights of coral spawning.

Methods

Larval culture

Based on previous work that demonstrated common morphogenic response to crustose algae among several Pacific acroporids (Morse et al. 1996), *Acropora millepora*, a widespread and common Indo-Pacific coral species was selected as an indicative organism to test the various inducer sources for morphogenic activity. Experiments were conducted following coral spawning events at Lizard Island on the Great Barrier Reef (LI, latitude 14 °40′S; longitude 145 °26′E) and Coral Bay on Ningaloo Reef (CB, latitude 23 °09′S; longitude 113 °46′E). Live pieces of the coral *A. millepora* measuring approximately 30 × 30 cm were collected from reefs crests and maintained in outdoor seawater tanks at the laboratory (LI) or in clear shallow water near the shore (CB). Prior to sunset on the nights of spawning; 29 November, 1996 (LI), 31 March, 1997 (CB) and 19 March, 1998 (CB), the individual colony pieces were isolated in 50 l plastic tubs and shielded from artificial light. Synchronous spawning occurred between 21 : 00–22 : 00 h on each night. The released gametes, in the form of buoyant egg-sperm bundles 1–1.5 mm in diameter, were collected from the water surface with gentle suction. Gametes from all colonies were mixed in a single 50 l tub to enable cross-fertilisation, left undisturbed for one hour then transferred to 200 and 380 l plastic tanks for primary rearing. After two hours a 50% water change was made in each tank, then the embryos were left undisturbed with no aeration until the next morning, when gentle aeration was introduced. The larvae were allowed to develop with fresh seawater changes twice per day. Water temperature was logged with an *in situ* recorder in one of the tanks at 15 min intervals and showed a daily range from 28–32 °C (LI, 1996), 22–25 °C (CB, 1997) and 25–30 °C (CB, 1998). The motile, ciliated larvae were maintained in fresh seawater with two 75% water changes per day (LZ, 1996 and CB, 1997) and with semi-continuous water exchange in a floating pond (CB, 1998) moored over the reef.

Embryos and larvae from two natural coral slicks from multi-specific spawnings on March 18, 1998 (SL1) and March 19, 1998 (SL2) were collected from the field by bucket and also maintained in floating, intermittent flow-through ponds at Coral Bay. Direct field observations at night by SCUBA diving confirmed that multiple coral species contributed to the wild slicks used for the experiments. Acroporid species were recorded spawning on both nights especially on the second night (SL2). Subsequent studies on post-settlement mortality (Smith and Heyward, 1999) using larval cultures derived from the same wild slicks confirmed the dominance of acroporids using skeletal characteristics of the well-established primary polyps.

Inducer sample preparation

Live fragments of CRA and coral were collected from the fringing reefs of Lizard Island and Coral Bay. Epiphytic organisms were carefully removed from the single continuous colonies of CRA used in the experiments described. Several species of crustose coralline algae from Lizard Island: *Lithophyllum insispidum* (Adey and Townsend), three colour morphs of *Hydrolithon onkodes* (Heydrich), and Coral Bay: *Neogoniolithon brassica-florida* (Harvey), *Mesophyllum* sp. (after Wommersley 1996), as well as the non-coralline crustose red algae *Peyssonnelia* sp. (after Wommersley 1996) and three species of branching coralline algae *Lithophyllum kotschyanum* (Unger), *Amphiroa anceps* (Lamarck) and *Amphiroa foliacea* (Lamouroux) were used in assays for larval metamorphosis (identifications performed by Cameron Sim, Department of Botany, University of Western Australia).

CRA is composed of a thin outer sheath of live cells that grow in successive layers, leaving behind a calcified skeleton. In each of the experiments, chips consisted of the live surface layer of cells upon a section of calcified skeleton approximately 3 mm thick. In some experiments, CRA6 (*Mesophyllum* sp.) was sectioned by carefully removing the thin live calcified section, resulting in two distinct substrate types, live CRA and the older dead skeleton. Sub-surface pieces of dead reef rubble from approximately 5 cm below the deepest recognisable layer of CRA skeleton were also tested for morphogenic activity.

Live tissue was removed from a common shallow water massive coral, *Goniastrea retiformis*, using high pressure water blasting. Radial slices approximately 6 mm thick were then made through the centre of the spherical colony with a circular diamond saw. Skeletal pieces for use as fragments or in extracts were sampled from the slices in a zone 3–5 cm below the colony surface, i.e. well below the zone of apparent live tissue and active calcification. Both CRA and coral chips were soaked in filtered seawater (0.2 μm) containing antibiotic (oxytetracycline hydrochloride (Pfizer), 2 μg/ml) for 1 h before introduction to individual assay wells.

Metamorphosis assays

Assays for larval metamorphosis were performed in sterile 6-well polystyrene cell culture plates (12 ml, Iwaki, Japan) maintained under the same environmental conditions as the stock larvae cultures. Coral larvae (10–20) were introduced to each well containing 10 ml filtered seawater with antibiotic and a 5 × 5 mm CRA chip. As noted by Harrison and Wallace (1990, see p. 191), settlement and metamorphosis are generally intimately associated, with the result that the two terms are often used interchangeably. Our strict endpoint for scoring metamorphosis differed slightly from that of Morse et al. (1988; 1994), principally because we focused on induction of early metamorphosis rather than subsequent developmental stages of the metamorphosed polyp. However, as in Morse et al. (1994, 1996), our criteria were chosen to reflect the major change from

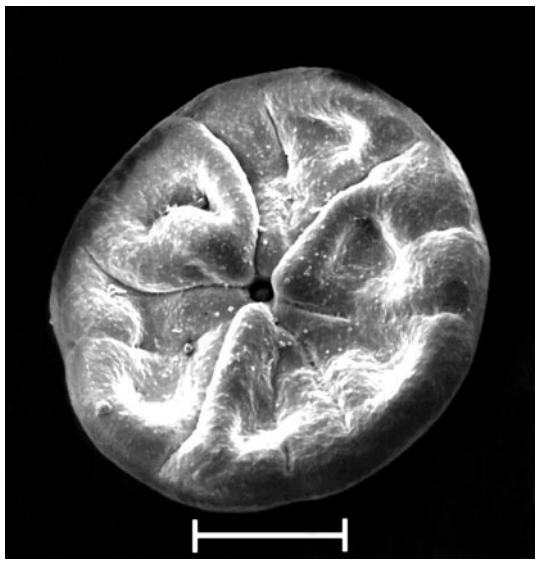

Fig. 1 *Acropora millepora*. Scanning electron micrograph showing early metamorphosis of a typical larvae 12 h after exposure to inducer on the plastic chamber substrate. Scale bar = 200 μm

planula larva to permanently attached and developing primary polyp. For these experiments larvae were defined as metamorphosed when they had changed from either free swimming or casually attached pear-shaped forms to squat, firmly attached disc-shaped structures with pronounced flattening of the oral-aboral axis and typically had obvious septal mesenteries radiating from the central mouth region. e.g. see Fig. 1.

The induction of metamorphosis tended to be relatively rapid. An analysis of variance showed no significant difference in total numbers of metamorphosed versus non-metamorphosed larvae in assays 12 and 24 h after larvae were introduced. Between 12 h and 24 h the continuing development of the metamorphosed primary polyp was apparent. In this study the inducers assays were scored after 24 h. However, some of these metamorphosed polyps were maintained over several days for other experiments and were observed to continue normal development. These observations, together with reports of early metamorphosis as soon as 4 h after treatment for other Pacific acroporids (e.g. Morse et al. 1996) and related methods using 18–24 h assays (Morse et al. 1994), supported the choice of a 24 h end point as a valid and timely indication of early stage metamorphosis. Metamorphosis was scored by direct counting of all larvae in each well using a dissecting microscope.

Screening for active species of CRA and coral was performed using equal sized chips (5 × 5 mm) of the solid biological substrate in individual wells. Equal sized chips of unconditioned terracotta tile and grooved PVC tiles (both commonly used in coral recruitment studies *in vivo*) were introduced to some wells as control substrates in addition to wells containing no substrate. Extracts (see later) were introduced directly to the assay wells via pipette and larval metamorphosis was again scored at 24 h against a control extract (no calcareous substrate). The effect of each treatment was standardised as a percentage of total larvae observed that had attached to the substrate and metamorphosed.

Chemical extract 1: decalcified substrate

The coral skeleton *Goniastrea* and CRA7 were chosen for chemical extraction, which closely followed the procedure developed by Morse et al. (1994). Four g of each substrate were ground and washed in 40 ml filtered seawater with antibiotic. The mixture was then centrifuged 10000 × g, the supernatant discarded and the washing procedure repeated on the pellets twice. The pellets were decalcified using 4 l 50 mM EDTA (Merck), pH 8.2 at 28 °C for 24 h. The decalcified extract was vacuum filtered (Whatman, GFC 47 mm diameter) and adjusted to 2 M NaCl. t-Butyl HIC support (8 g wet weight, BIO-RAD) was introduced to the solution and stirred for 3 h at 4 °C. The resin-adsorbed inducer was collected by vacuum filtration (GFC) and the resin was washed with 2 × 10 ml 2 M NaCl. The resin was blotted dry on paper towel and re-suspended in 1 mM Tris Cl buffer (Sigma Chemicals) at pH 8.2 and transferred to a 0.8 × 12 cm chromatography column. The resin was eluted with Tris buffer and 5mL fractions were collected. The first four fractions assayed were the most potent inducers of larval metamorphosis and these were subsequently combined, then stored frozen as the crude decalcification extract.

Chemical extract 2: methanol

In early trials of the extraction procedure above a bright green residue had collected on the GFC filters used to remove particulates from the decalcified extract. A simple methanol extract of this material displayed highly active morphogenic properties. Subsequently, 4 g each of *Goniastrea* and CRA7 were ground and washed as described and the pellets were stirred in 100 ml HPLC grade methanol (MeOH, Merck) for 3 h at 25 °C. The ground material was filtered (GFC) and the MeOH extraction was repeated twice. All extracts were combined and the MeOH was removed under a stream of nitrogen. The dry extract material was then dissolved in 20 ml 1 mM Tris buffer and stored as the crude MeOH extract.

Statistical analysis

Differences in absolute numbers settling between treatments required that a comparative measure of metamorphosis be obtained using log-transformed data according to Eq. 1 (McCullagh and Nelder 1989). Comparisons were made on all treatment groups within an experiment apart from controls or other treatments where no metamorphosis was observed. Treatments that produced no response were excluded from statistical analysis and degrees of freedom and F statistics were calculated accordingly. An analysis of variance (ANOVA) using The Tukey Honest Significant Difference (for equal or unequal *n*) was performed on each transformed data set using Statistica for Windows, release 5.1 (1997, StatSoft USA).

$$l_{p_i} = \ln((a + 0.5)/(n - a + 0.5)) \quad (1)$$

where l_{p_i} = logit transformed probability of metamorphosis, a = total number of larvae metamorphosed, and n = total number of larvae.

Results

Solid substrates

The metamorphosis of *Acropora millepora* larvae in response to all encrusting coralline algae was consistently high, ranging from 67.4% to 90.8% ($p > 0.05$, $F_{8,51} = 2.30$) (Table 1). The branched coralline algae species, in comparison, displayed a range of activity with BCA2 inducing 69.1% of larvae to metamorphose compared with BCA3 which was inactive. The level of metamorphosis induced by BCA2 was not significantly

Table 1 Mean levels of metamorphosis of 8 day old *Acropora millepora* larvae in the presence of various crustose red algae (CRA), branching coralline algae (BCA) and biologically inert artificial substrates ($\pm$ 1 SE)

Substrate	Abbreviation	Spawning event	Replicates	Metamorphosis (% $\pm$ SE)
Blank, no substrate		All[a]	18	0
Terracotta tile		All[a]	18	0
PVC tile		All[a]	18	0
Lithophyllum insispidum	CRA1	Lizard Is. 1996	6	89.7 $\pm$ 2.9
Hydrolithon onkodes	CRA2	Lizard Is. 1996	6	89.6 $\pm$ 0.7
Hydrolithon onkodes	CRA3	Lizard Is. 1996	6	89.1 $\pm$ 2.9
Hydrolithon onkodes	CRA4	Lizard Is. 1996	6	81.3 $\pm$ 3.5
Neogoniolithon brassica-florida	CRA5	Coral Bay 1997	6	67.4 $\pm$ 4.0
Mesophyllum sp.	CRA6	Coral Bay 1997	6	89.2 $\pm$ 3.0
Peyssonnelia sp.	CRA7	Coral Bay 1998	12	90.8 $\pm$ 1.8
Lithophyllum kotschyanum	BCA1	Coral Bay 1997	6	22.8 $\pm$ 9.2
Amphiroa anceps	BCA2	Coral Bay 1997	6	69.1 $\pm$ 6.3
Amphiroa foliacea	BCA3	Coral Bay 1997	6	0

[a] Control treatments were carried out at each spawning event and in all cases no metamorphosis was observed

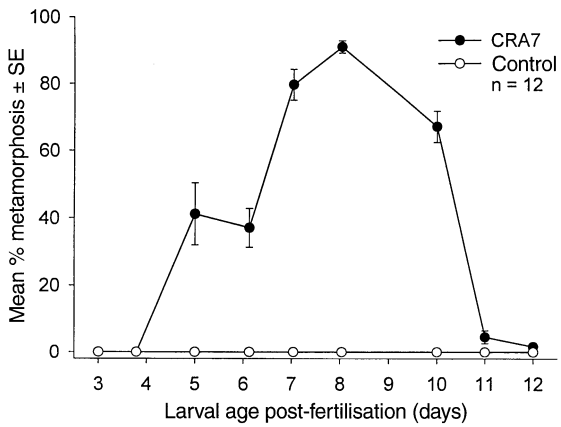

Fig. 2 Percentage metamorphosis of *Acropora millepora* larvae when exposed to crustose red algae (CRA7) versus age of larvae (post-fertilisation). Each point is the mean level of metamorphosis of 12 replicated experiments with error bars representing $\pm$ 1 SE. Control wells contained no CRA

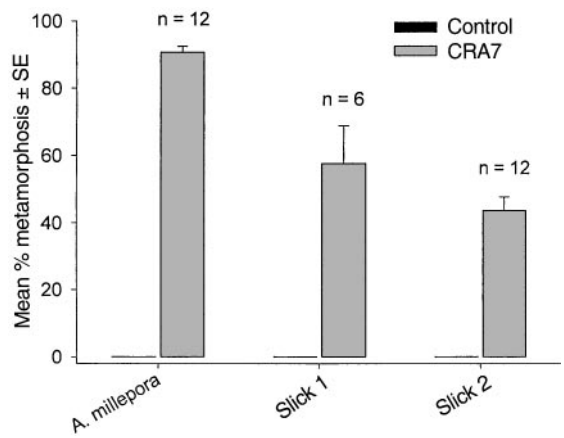

Fig. 3 Percentage metamorphosis of 8 day old *Acropora millepora* larvae and 8 day old larvae cultured from wild coral slicks from successive nights of spawning induced by chips of CRA7. Each bar represents the mean level of metamorphosis of 12 (*A. millepora*, slick 2) or six (slick 1) replicated experiments with error bars representing $\pm$ 1 SE. Control wells contained no CRA

different from the CRA species ($P > 0.05$). The ciliated larvae often attached temporarily on the well surfaces and the CRA substrate, if present, before moving to other sites and re-attaching. This attachment lasted for seconds to hours but the larvae never metamorphosed on the biological substrate, preferring instead the inert well surfaces. Metamorphosis was not observed in control wells, which contained either no substrate, unconditioned PVC plates or unconditioned terracotta tiles (Table 1). Over the course of these experiments larvae were most competent to metamorphose in the presence of CRA7 at an age of 7–9 days post-fertilisation (Fig. 2).

High levels of metamorphosis were also observed for larvae from coral slicks in the presence of CRA (Fig. 3). These levels were lower than for the experiments using only *A. millepora* larvae ($P < 0.01$, $F_{2,27} = 1.10$). Again, all larvae metamorphosed on the base and walls of the wells and no metamorphosis was observed in corresponding control wells that did not contain CRA.

A. millepora larvae metamorphosed in similar numbers in the presence of the live surface of CRA6, the skeleton of CRA6 and chips of CRA6 containing both live and dead material ($P > 0.05$, $F_{4,25} = 0.52$) (Fig. 4). The live section of CRA6 induced behaviour similar to whole chips, with larval metamorphosis observed only on the polystyrene wells. When larvae were exposed to the CRA skeleton a high level ($> 60\%$) metamorphosed directly on the skeleton with the remainder metamorphosing as previously described on the inert surface of the wells. Although the total response was significantly lower ($P < 0.01$, $F_{4,25} = 0.52$) for larvae exposed to reef rubble (27%) compared with CRA, metamorphosis patterns mirrored the CRA6 skeleton and *Goniastrea* with a majority of

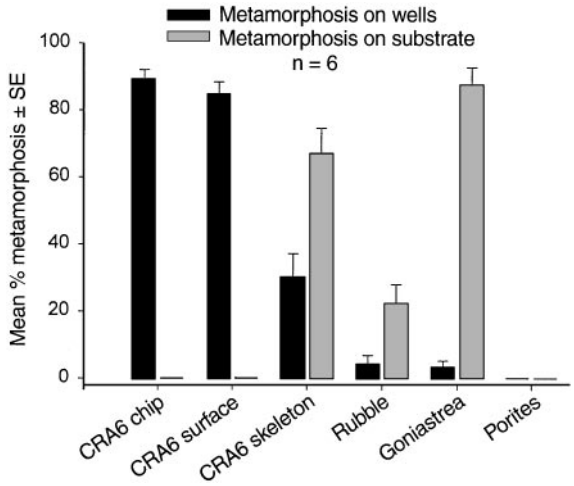

Fig. 4 Percentage metamorphosis of 8 day old *Acropora millepora* larvae induced by a variety of substrates, comparing levels of metamorphosis on the substrate with metamorphosis on the inert well surfaces. Each bar represents the mean level of metamorphosis of six replicated experiments with error bars representing ± 1 SE

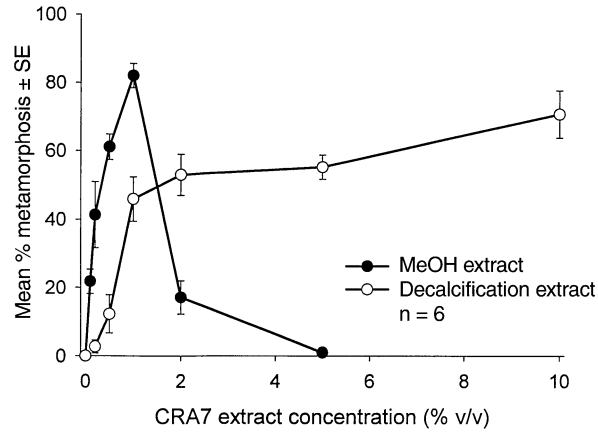

Fig. 5 Dose response curves for metamorphosis of 9 day old *Acropora millepora* larvae induced by chemical extracts of crustose red algae (CRA7). Each point is the mean level of metamorphosis of six replicated experiments with error bars representing ± 1 SE

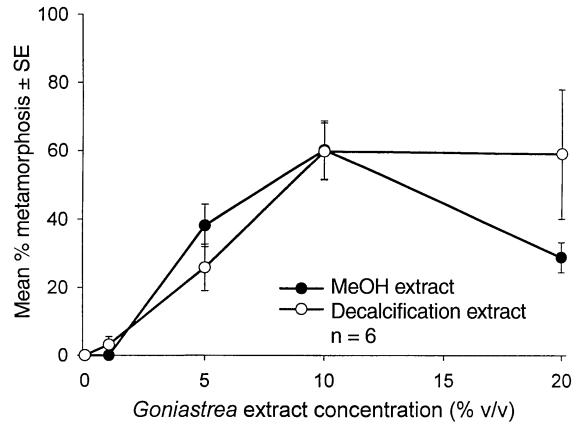

Fig. 6 Dose response curves for metamorphosis of 9 day old *Acropora millepora* larvae induced by chemical extracts of *Goniastrea*. Each point is the mean level of metamorphosis of six replicated experiments with error bars representing ± 1 SE

larvae metamorphosing on the calcareous substrate rather than the well walls (Fig. 4).

When testing settlement plates constructed from skeleton slices of the coral *Goniastrea* for related research, it was observed that a high numbers of larvae were settling on this substrate compared with terracotta tiles conditioned in seawater. When *A. millepora* larvae were exposed to small (5×5 mm) chips of *Goniastrea* skeleton, a similar pattern of metamorphosis was observed as for the CRA skeleton. A majority of metamorphosis occurred on the calcified coral skeleton (Fig. 4), usually in natural pores and often on the underside of the chips. Coral skeleton from the massive coral *Porites* sp. did not induce larval metamorphosis (Fig. 4).

Extracts

Chemical extracts alone of CRA7 and *Goniastrea* each trigged the metamorphosis of *Acropora millepora*, with larvae settling singly or in clumps on the base or corners of the wells. The control extracts where no substrate was extracted were totally inactive, indicating that the extraction procedure itself did not influence metamorphosis. In preliminary experiments, an inducer fraction from a decalcification extract of CRA4 was obtained and 300 µl was added to wells containing larvae and unconditioned terracotta tile chips and grooved PVC plates. Over 90% of larvae underwent metamorphosis but again the majority of larvae metamorphosed on the polystyrene wells with less than 2% settling on the unconditioned artificial substrates.

Dose-response curves were plotted for both extracts of each substrate (Figs. 5 and 6). There was no significant difference in metamorphosis between the decalcified extracts of CRA7 and *Goniastrea* at 10% v/v ($P > 0.05$, $F_{19,97} = 1.56$). The decalcified extracts induced increasing levels of settlement with concentration up to a maximum at 10% v/v for each substrate, although the increase in dose-response for CRA7 was not statistically different ($P > 0.05$, $F_{19,97} = 1.56$) above the 1% concentration. The methanol extracts of both CRA7 and *Goniastrea* on the other hand exhibited peaks in activity at 1% and 10% v/v respectively. The drop in activity at higher MeOH extract concentrations may be due to the presence of a co-extracted toxin in this crude preparation. The maximum level of metamorphosis for the MeOH extract of CRA7 was significantly higher than the metamorphosis

induced by 1% v/v decalcification extract ($P < 0.05$, $F_{19,97} = 1.56$). As an equal mass of substrate was extracted in each case and with final extract volumes also equal, the MeOH extract of CRA7 could be considered more active than our corresponding decalcification extract at this concentration (Fig. 5).

Discussion

Our results contribute to mounting evidence that the larvae of many important reef-building coral species respond to chemical cues present in encrusting and geniculate coralline algae, as well as at least one species of non-calcareous encrusting algae (see Morse et al. 1988, 1996; this study). Additionally, active inducers also appear to be present in calcareous substrates where live crustose algae are not apparent such as non-encrusted coral rubble and coral skeleton of *Goniastrea*.

While there was no significant difference in the ability of encrusting algae to induce metamorphosis of coral larvae, this phenomenon was not a predicable feature of the three branching coralline species (Table 1). The response of coral larvae was not species-specific, since larvae cultured from two wild slicks collected on successive nights were also induced to metamorphose in the presence of CRA. Considering the multi-species composition (see Simpson et al. 1993) of these natural larval cohorts, variance in degree of competency to metamorphose at a single point in time would be expected. Nonetheless, approximately 50% of these wild larvae were induced to metamorphose in response to *Peyssonnelia* sp. This result points towards a common morphogenic pathway in many corals, as proposed by Morse et al. (1996).

Dead CRA layers induce larval metamorphosis, indicating a stable inducer compound is located deep within calcareous layers of CRA and not only at the surface of the substrate. There was no significant difference in the overall induction between the live surface and dead sub-surface layers of CRA. Both these calcareous substrates may contain the same chemical inducer but this has not been confirmed. Coral larvae can come into contact with both surfaces, as the sub-surface CRA layer and older, non-encrusted coral rubble is often exposed following physical disturbance of reef substrates.

The inducer extracted from skeleton of *Goniastrea* by the decalcification method may be of the same biochemical class as that extracted from CRA in this study and by Morse and co-workers (Morse et al. 1988; 1994; 1996; Morse and Morse 1991), since very similar extraction and clean-up methods were used in each of the studies. Disturbance from cyclones or even the minor daily disturbance to reef crests, results in fresh coral skeleton becoming exposed and available for coral larval metamorphosis. Consequently, this study shows that coral larval metamorphosis could occur on coral skeletons even in the absence of CRA.

The trigger for coral larval metamorphosis appears to be primarily chemical, but in the laboratory experiments larvae did not necessarily metamorphose directly on the source of the inducer. Some studies (Van Moorsel 1988; Morse et al. 1996; Smith and Heyward 1999) indicate that when larva are presented with CRA and non-CRA substrates there is a clear preference in favour of metamorphosis on or adjacent to the CRA. For both the coral skeleton and the sub-surface CRA layers, larvae metamorphosed readily on the substrate, but this was not the case for the surface layers of CRA. Further field experiments need to be performed to elucidate the ecological consequences of the larval behaviour patterns reported in these laboratory observations.

We were able to extract a potent inducer fraction from *Peyssonnelia* sp. and *Goniastrea* with MeOH. As the experiments used a crude extract, we are unsure of the chemical nature of this inducer or its similarity to morphogens released by decalcification, nor can we be certain of its ecological role. The ease with which the MeOH soluble inducer was released from CRA and *Goniastrea* does suggest however that, unlike inducers released by decalcification, these are not tightly bound as an integral part or of the calcified cell wall (Morse et al. 1994). Microbial inducer sources have been demonstrated to play a significant role in the metamorphosis of other marine invertebrates (Johnson and Sutton 1994; Kitamura and Hirayama 1987) but their role in the induction of metamorphosis in corals has not been demonstrated.

Coral recruitment is commonly measured by deploying inert substrates such as terra-cotta tiles (e.g. English et al. 1997). Measures of recruit abundance can be significantly influenced by the degree of "conditioning" of the tiles and the nature of the substrate itself (see Harrison and Wallace 1990), being highest on tiles conditioned in seawater for longer periods of time (e.g. Babcock 1992). The surface chemistry of deployed tiles will be subject to the temporal and spatial variation of primary and secondary fouling, which is likely to profoundly influence the attractiveness of the substrate for coral larval metamorphosis. In light of present knowledge of the chemical ecology associated with larval metamorphosis, it seems the degree of tile fouling by encrusting algae will play a significant role in this recruitment process. These factors may introduce additional sources of variance into quantitative recruitment studies that rely on deployment of supposedly standardised inert or conditioned substrates.

Mechanisms for detecting physical signals, such as seawater temperature or lunar and solar light rhythms facilitate spawning synchrony in many coral species and play a major role in fertilisation success. Chemical signals associated with the reef appear to be equally

important in the subsequent life cycle phase of coral recruitment. Planktonic larvae of broadcast spawning corals can respond to chemical signals that the larvae are likely to encounter when they are entrained onto shallow reef environments. While coral larvae can clearly be induced into a precipitous metamorphosis after contact with particular reef organisms and substrates such as CRA or coral skeleton, it is not known whether patterns of recruitment in populations of the major Indo-Pacific reef building species correlate with the distribution of these inducers at fine or coarse spatial scales (e.g. see Hughes et al. 1999). This study adds to recent focus on biochemical morphogenic cues for tropical invertebrates (e.g. Johnson and Sutton 1994; Morse et al. 1996; Hadfield et al. 1997), all of which point to the importance of cellular and sub-cellular components of tropical reefs in the population dynamics of key macrobenthic groups.

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