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Chemically mediated interactions between macroalgae *Dictyota* spp. and multiple life-history stages of the coral *Porites astreoides*

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ABSTRACT: Competition between corals and macroalgae is often assumed to occur on reefs, especially those that have undergone shifts from coral to algal dominance; however, data examining these competitive interactions, especially during the early life-history stages of corals, are scarce. We conducted a series of field and outdoor seawater-table experiments to test the hypothesis that allelopathy (chemical inhibition) mediates interactions between 2 common brown macroalgae, *Dictyota pulchella* and *D. pinnatifida*, and the coral *Porites astreoides* at different life-history stages of the coral. *D. pinnatifida* significantly reduced larval survival and larval recruitment. The extracts of both *D. pinnatifida* and *D. pulchella* significantly reduced larval survival, and the extract of *D. pulchella* also negatively influenced larval recruitment. There was no measurable effect of the crude extracts from *Dictyota* spp. on the photophysiology of adult corals. Our results provide evidence that these *Dictyota* species chemically compete with *P. astreoides* by negatively affecting larval settlement and recruitment as well as the survival of larvae and new recruits. Macroalgae may perpetuate their dominance on degraded reefs by chemically inhibiting the process of coral recruitment.

KEY WORDS: Allelopathy · Coral–algal interactions · *Dictyota* · Chemical defense · Phase shift

INTRODUCTION

Over the past 3 decades, coral reef degradation has occurred on a global scale (Knowlton 2001, Hughes et al. 2003), often resulting in a decrease in live coral cover followed by lasting proliferation of algae, referred to as a phase shift (Done 1992, Hughes 1994, McCook 1999, Hughes et al. 2003, 2007, 2010). Although phase shifts from coral- to algal-dominated communities have been documented on both Atlantic and Pacific reefs, they are most pronounced in the western Atlantic (Gardner et al. 2003). Where corals have been damaged or killed by hurricanes, bleaching and disease, algae have flourished because of depressed herbivory rates due to overfishing and the massive die-off of the sea urchin *Diadema antillarum* (Hughes 1994, Rogers et al. 1997, Mumby & Steneck 2008). Although studies have shown that coral mortality, reduced herbivory and increased nutrient availability can cause or perpetuate phase shifts, few studies have investigated the mechanisms responsible for the maintenance of high algal cover on disturbed reefs (Rogers & Miller 2006, Hughes et al. 2007, 2010, Mumby & Steneck 2008, Mumby 2009).

One consequence of enhanced macroalgal abundance on degraded coral reefs is increased interaction between algae and corals at all life-history stages. Competition for space is an important factor structuring marine benthic communities, particularly on speciose coral reefs (Porter 1974, Jackson & Buss 1975,

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Thacker et al. 1998). Habitat selection, differential growth rates and physical or chemical defenses may be important in mediating competitive interactions among species (Jackson & Buss 1975, Connell et al. 1997, Thacker et al. 1998, Knowlton & Jackson 2001, Ritson-Williams et al. 2009, Rasher & Hay 2010).

Macroalgae are known to negatively influence the settlement and recruitment of coral larvae (Kuffner & Paul 2004, Birrell et al. 2005, Kuffner et al. 2006, Birrell et al. 2008a,b, Diaz-Pulido et al. 2010) and the survival and growth of juvenile corals (Birkeland 1977, Van Moorsel 1985, Box & Mumby 2007, Ritson-Williams et al. 2009). *Dictyota* spp., brown macroalgae common on reefs in the Florida Keys and throughout the Caribbean, caused recruitment inhibition and avoidance behavior in larvae of the coral *Porites astreoides* and increased the mortality rates of recruits (Kuffner et al. 2006). *Dictyota* spp. inhibited larval recruitment through unknown competitive mechanisms that exceeded the effects of space occupation alone. Allelochemical effects could account for this, because *Dictyota* spp. are known to chemically inhibit the settlement of invertebrate larvae (Schmitt et al. 1995, Walters et al. 1996) and can cause bleaching and death of adult corals in direct contact with the algae (Rasher & Hay 2010). *Dictyota* spp. are known to be rich in terpenoid natural products that can function as chemical defenses against generalist herbivores (Hay 1996, Paul et al. 2001, Vallim et al. 2005). To better determine the mechanisms responsible for coral recruitment inhibition, we tested the role of allelopathy (chemical inhibition) in mediating the interactions between 2 chemically rich species of *Dictyota* and 3 life-history stages of *Porites astreoides*. We hypothesized that extracts containing secondary metabolites from *D. pulchella* and *D. pinnatifida* would inhibit recruitment of coral larvae, decrease the survival of new recruits and kill or stress adult corals. These experiments tested which coral life-history stages are most susceptible to competition with *Dictyota* spp. and whether these 2 species of macroalgae had the same competitive effects on corals.

MATERIALS AND METHODS

General experimental design. This study was conducted while based at the Keys Marine Laboratory, Long Key, FL, USA, from 6 to 28 May 2005 and at the Mote Tropical Marine Laboratory, Summerland Key, FL, from 22 May to 10 June 2006. We examined the effects of 2 brown macroalgae, *Dictyota pulchella* Hörnig & Schnetter and *D. pinnatifida* Kützing, on larval settlement and recruit survival of the common reef coral *Porites astreoides* Lamarck. This coral species was chosen because of its abundance on the reefs in Florida and the availability of its brooded larvae. The 2 species of *Dictyota* were selected because they are common throughout the Florida Keys and Caribbean, and they showed different effects on coral recruitment and recruit survival in a previous study that tested the effects of a variety of live algae on larvae of *P. astreoides* (Kuffner et al. 2006). *D. pulchella* had a negative effect on total recruitment and recruit survival, whereas *D. pinnatifida* did not decrease recruitment and only affected the location of recruitment on the settlement tiles.

In May 2005, 30 colonies of the brooding coral *Porites astreoides* were collected on and near the pilings of the Long Key Viaduct, transported to the Keys Marine Lab in coolers and maintained in running seawater. Larvae were collected during the nights of 8 to 14 May 2005 (new moon: May 8), with 29 of the 30 colonies releasing larvae during this time period. After enough larvae were gathered (~20 000) to complete the experiments, the colonies were returned to the sites of collection and reattached to the substratum with Z-Spar® A-788 Splash Zone Compound underwater epoxy.

To obtain larvae, each colony was placed in an individual 3 l Rubbermaid® 'Grip 'N Mix'™ bowl supplied with running seawater. The bowls were tilted such that the positively buoyant larvae spilled over the handles of the bowls into plastic beakers fitted with a 180 µm mesh bottom (larvae were ~1 mm diameter) supported 3 cm off the tank floor by attached silicone stoppers. The water level inside the tank was kept at approximately 15 cm so that the larvae remained in the beaker traps until they were collected each morning shortly after sunrise. Larvae were pooled into a common container prior to being used for the experiments.

The hypothesis that natural products present in *Dictyota* spp. can negatively impact coral larvae and recruits and inhibit coral recruitment was tested by subjecting larvae of *Porites astreoides* to control and treatment conditions (see below for details) inside customized 10.2 cm diameter, 12.7 cm long larval recruitment chambers that were deployed underwater as previously described (Kuffner et al. 2006). The chambers served as a contained environment, while still allowing water circulation via the 180 µm mesh at each end of the chamber that allowed ambient water flow and solar irradiance through the clear, extruded acrylic tubing (Kuffner et al. 2006). Each chamber served as an individual replicate. Chambers were haphazardly deployed at least 1 m apart at shallow, nearshore sites and attached to Dri-Dek® Tiles (rubber mats) with nylon bolts and nuts in order to anchor the chambers upright and parallel to the prevailing currents.

Coral larval survival and recruitment. A test of the effects of the brown macroalgae *Dictyota pulchella* and *D. pinnatifida* and their extracts on the effects of

coral recruitment was conducted from May 10 to 15, 2005. Coral larvae were provided with access to suitable settling habitat (4.5 × 4.5 × 1 cm sections of Sunshine Pavers® wire-cut terracotta tiles that were conditioned at the Tennessee Light CARICOMP site [24° 45.14′ N, 80° 45.70′ W] from 13 March to 9 May 2005). Larvae were subjected to 7 treatments: control (settlement tile with cable tie), macroalgae mimic (seawater-conditioned plastic aquarium plant), live *D. pulchella*, live *D. pinnatifida*, agar control (agar and carrageenan gel strip containing ethanol only), *D. pulchella* lipophilic extract dissolved in ethanol and incorporated in agar/carrageenan at natural wet weight concentration (3.7% wet weight) and *D. pinnatifida* lipophilic extract in agar/carrageenan with ethanol at natural concentration (3.9% wet weight). Clumps of each species of *Dictyota* (roughly 3 cm^3 volume) were attached with a cable tie such that the thalli covered half of the upward-facing space on the tile. The plastic aquarium plant was chosen as a mimic because the size and shape were similar to the macroalgae tested, and the plastic plants would control for effects of shading, abrasion and space occupation.

To prepare organic extracts of the macroalgae, *Dictyota pulchella* and *D. pinnatifida* were collected in the Florida Keys in late May 2004 at Eleven-foot Mound (24° 43.381′ N, 80° 51.696′ W). The algae were immediately frozen and returned to the Smithsonian Marine Station in Fort Pierce, FL, for extraction. The macroalgae were patted dry and weighed to obtain wet weights and then lyophilized to obtain dry weights prior to extraction with a mixture of equal volumes of ethyl acetate and methanol. Three consecutive extractions were dried by rotary evaporator and Speed-vac to yield organic extracts containing relatively nonpolar primary and secondary metabolites (terpenes) from each species. Extract yields were calculated on the basis of both algal wet weight and dry weight as the weight of extract obtained divided by wet weight or dry weight of the algae, multiplied by 100.

Our methods were designed to test the allelopathic effects of the *Dictyota* organic extracts and followed methods previously used to test allelopathic effects of sponge nonpolar compounds (Thacker et al. 1998). We modified the gel to include equal parts of agar and carrageenan because we found that adding the carrageenan created a more rigid gel that better retained the extracts than agar alone. Our goal was to retain the lipophilic compounds in the gel as much as possible and to have the gels mimic the chemical composition of whole algae, so that the larvae would perceive and respond to the compounds on the settlement tiles during the settlement process. A solution of 1.25 g agar, 1.25 g carrageenan and 70 ml tap water was heated to boiling. *D. pulchella* extract (2.8 g) and *D. pinnatifida* extract (3.0 g) were separately dissolved in 5 ml ethanol, and the extracts were stirred into the agar and carrageenan mixture after it began to cool to yield a solution that contained extracts at natural wet mass concentrations. The mixture was then spread into plastic molds backed by fiberglass window screening to form treatment and control strips of agar measuring 2.5 × 35 cm. The strips were allowed to cool and harden onto the window screening before being removed from the molds and cut into 10 pieces, each measuring 2.5 × 3.5 cm with an equal-sized strip of fiberglass window screen attached to each piece.

The control and treatment agar strips were attached (agar side down to better retain the allelochemical effects on the surface of the tiles) to half of the upward-facing space on a settlement tile by a cable tie. Settlement tiles (n = 12 for each of the 7 treatments) were placed individually into larval recruitment chambers with the agar pieces on the top surface of the tiles, and 100 *Porites astreoides* larvae were added to each chamber. Because of the cylindrical shape of the chambers, the settlement tiles did not lie flush with the bottom, allowing larvae easy access to all 6 sides for settlement and recruitment. The chambers were placed in a hard-bottom and sparse seagrass community in Florida Bay, directly behind the Keys Marine Laboratory at 1.5 to 2 m depth. After 5 d, the chambers were brought into the laboratory for analysis. Water inside the chambers was carefully sieved through 180 µm sieves to count larvae still swimming, and the tiles, macroalgae, agar strips and chambers were inspected under dissecting microscopes to record the number and location of recruits. Total survival was defined as all swimming larvae plus live recruits. A recruit was defined as a coral larva that underwent complete metamorphosis, including formation of a calcium carbonate calyx, and was firmly attached to the substratum.

Agar strips were retained after the 5 d experiment to ensure that the composition of the *Dictyota* extracts had not changed over the course of the experiment and that the terpenes were still present in the extracts. Agar was scraped off of the window screen, different treatments were separately extracted with ethyl acetate and methanol, and solvents were dried by rotary evaporator and Speed-vac. Proton nuclear magnetic resonance (NMR) spectra were run on these re-extracted samples and compared with NMR spectra of the original extracts.

Survival data (proportion of 100 larvae that survived and either settled or were still swimming in the chambers) were analyzed by 1-way ANOVA, with treatment as the fixed factor, following arcsine square-root transformation of the data. Two separate ANOVAs were run to compare the data for the aquarium plant mimic control with the whole algae treatments and the agar con-

trol with the 2 *Dictyota* extract treatments. Dunnett's test (2-sided) was used after each ANOVA to compare the treatments with the appropriate control. Recruit data (proportion of 100 larvae that recruited inside the chamber) were analyzed similarly. However, the recruitment data for the agar control and *Dictyota* extracts were subsequently analyzed with a Kruskal-Wallis test followed by all pairwise comparisons, because various transformations of this set of recruitment data did not achieve homogeneity of variance.

Recruit survival and stress. Newly settled coral recruits were tested to determine whether live algae and extracts of *Dictyota* spp. influenced their survival and photophysiology. Settlement tiles with *Porites astreoides* recruits were scraped clean except for one single polyp recruit. Because of limited numbers, the new recruits were from all settlement treatments, but were randomly assigned to the new survival trial treatments. The tiles spent a minimum of 5 d in running seawater before the new trial started to allow time for death to occur in any recruits that were damaged by previous treatments or during the counting process. We only used tiles with intact pigmented recruits on them for the new experiments. The tiles were held in outdoor flow-through seawater tables that were shaded to reduce ambient light to approximate irradiance levels at 2 to 3 m depth as measured with a LiCOR spherical PAR sensor. The mean (±SD) irradiance at noon each day was 982 ± 273 µmol quanta m^{-2} s^{-1} (n = 5). This value was on average 66% of the irradiance that reached the seagrass bed canopy off the point of the lab at 1 m depth, and a 59% reduction of the ambient irradiance striking the surface of the flow-through seawater tables. The new recruits were exposed to treatments on 19 May and the experiment was ended on 23 May 2005.

Individual recruits on tiles were exposed to one of the following treatments: a cable tie control, an aquarium plant as an algal mimic to control for shading, an agar control (with ethanol but no extract added), live *Dictyota pulchella*, live *D. pinnatifida*, the extract of *D. pulchella* incorporated into an agar strip, or *D. pinnatifida* extract in an agar strip. The extracts were prepared in the agar strips as described above and each treatment was attached to the tiles as described above for the larval recruitment experiments. A 10 mm hole was punched out of these agar strips before the strips were placed over the recruits; in this way each recruit was surrounded by the agar strip but not smothered by it. Every tile was an individual replicate and each treatment was replicated with 9 tiles. The treatments were left on the new recruits in the flow-through seawater tables for 5 d before the experiment was scored. The recruits were determined to be alive or dead by looking at them with a dissecting microscope to see whether living tissue remained on the coral skeleton. The number of recruits with live tissue remaining was recorded. Differences in the number of live and dead recruits among treatments were tested with log-linear models. First we tested whether recruit survival differed between the 2 algal species. If the 2 species showed similar responses, data on both species were combined to test for differences between live algae and extracts. If algae and extracts had similar effects, data were combined to test for differences between the controls and treatments. This analytical strategy allowed increased power by increasing sample size after testing different hypotheses and data.

After 4 d of exposure to the treatments, the remaining live recruits were tested for potential stress on coral photophysiology resulting from the various treatments. The treatments were removed, and each coral recruit was dark acclimated for 10 min in an attempt to normalize photosystems and remove effects from the previous irradiance environment. Each recruit was then measured for fluorescence (F), maximum fluorescence (F_m') and quantum yield of photosystem II (Φ_{II}) using a diving pulse amplitude modulated (PAM) fluorometer (WALZ). Measurements were acquired only when momentary fluorescence yield values were between 300 and 500 units to produce accurate Φ_{II} measurements. A flexible fiber optic probe was used for sample illumination and fluorescence collection. The free end of the fiber optic probe was mounted to one side of a magnetic leaf clip. When surveying samples, the leaf clip was gently pressed against each sample, thus darkening it as well as assuring a fixed distance of 5 mm between the probe and the surface of the coral recruit. The data for each measurement (F, F_m', Φ_{II}) were analyzed individually with 1-way ANOVA for differences among treatments as previously described. These data were not transformed as they met the assumptions of normality and homogeneity of variance.

One-year-old recruits were also tested for their susceptibility to the 2 *Dictyota* spp. and their extracts. In May 2005, terracotta tiles with recent recruits (~2 wk old) of *Porites astreoides* were placed on fiberglass rods (so that the tiles were perpendicular to the benthos) and cable tied to the benthos at the Tennessee Reef CARICOMP site at a depth of 12 m. These tiles were left on the reef for 12 mo and were brought back to the laboratory on 20 May 2006. After 12 mo on the reef, most of the tiles had juvenile *P. astreoides* colonies with multiple polyps (12 to 32 polyps). Once in the laboratory, the tiles were maintained in flow-through seawater tables and only individual juvenile corals that had pigmented tissue were exposed to the same treatments as those described above, except the cable tie control was not included because of a limited number of tiles with recruits.

After 5 d of exposure to the treatments, the juvenile corals were scored under a dissecting microscope for the number of coral polyps that retained pigmented tissue, were bleached or dead. There were 5 replicate tiles for each treatment with one small coral colony per tile. The percentage of polyps that retained their pigmentation was calculated for each juvenile coral, and the data were analyzed by 1-way ANOVA. Data were not transformed because they met the assumptions of normality and homogeneity of variance. Two separate ANOVAs were run to compare the data for the aquarium plant mimic (control) with that of the whole algae treatments and the agar control with the 2 *Dictyota* extract treatments.

Adult photophysiology. To determine whether these algae and their extracts negatively stressed adult corals and their ability to photosynthesize, 49 colonies of *Porites astreoides* (all >20 cm in diameter) were maintained in the flow-through seawater tables (shaded with shade cloth as described above) and were exposed to the same 7 treatments as described above for the new recruits. Before the experiment began, 40 colonies were measured for 2 d to determine baseline rates of quantum yield. Nine colonies were added to the experiment on the second day; therefore, only one night of pre-treatment measures were conducted on these colonies. On Day 1 of the experiment, the controls, live algae and extracts in agar were attached to coral colonies that were randomly selected for each treatment. All of the controls and the treatments were attached to the adult colonies using cable ties, and the control colonies were also wrapped with the same number of cable ties. On Day 3 of the experiment, the live algae treatments were replaced with fresh pieces (~2.0 g of tissue). Measurements of quantum yield were made immediately next to treatments or, for the agar treatments, within two 1 cm diameter holes where the agar had been removed by a cork-borer. Φ_{II} from individual adult colonies of *P. astreoides* was measured every day for the 5 d experiment with the diving PAM fluorometer. Fluorescence measurements were acquired on specimens acclimated to dark for a minimum of 2 to 3 h, and 4 to 6 subsamples per colony were used to calculate a mean for each colony each night. A repeated-measures ANOVA was performed on this response variable. The data did not meet the assumptions of the ANOVA model and were therefore rank-transformed prior to analysis.

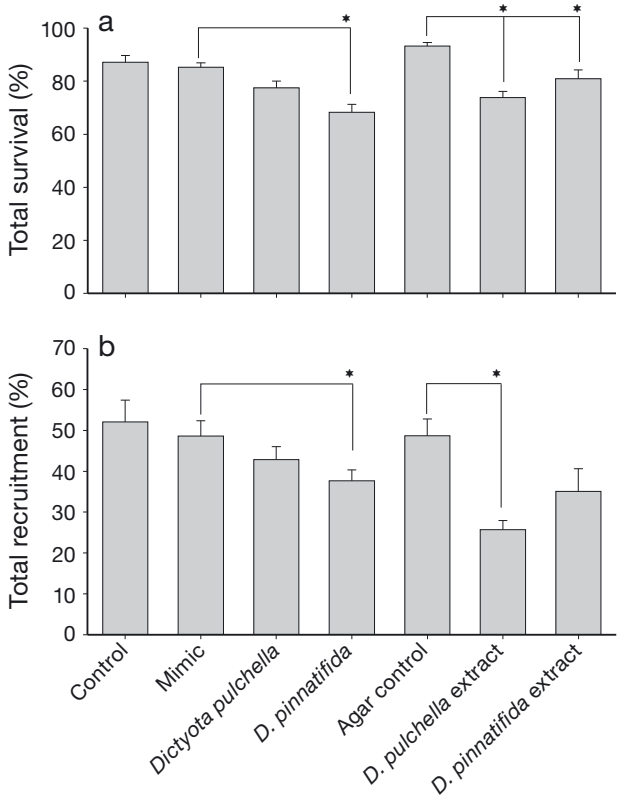

Fig. 1. Larvae of *Porites astreoides*. Survival and metamorphosis in the experimental chambers. (a) Percentage survival of 100 larvae after 5 d of exposure to the treatments. *Significant difference between the treatment and the relevant control (Dunnett's test). (b) Percentage recruitment (total settlement and metamorphosis both on and off the settlement tiles) of 100 coral larvae in response to the treatments for 5 d. *Significantly different from the relevant control by Dunnett's test (for whole algae) or Kruskal-Wallis test followed by pairwise comparisons (for the algal extracts). Data are means + 1 SE (n = 12)

RESULTS

Coral larval survival and recruitment

Total survival rates (swimmers + recruits) differed among treatments (Fig. 1a). One-way ANOVA comparing the algal mimic (plastic aquarium plant) with *Dictyota pulchella* and *D. pinnatifida* (ANOVA, F = 9.88, p = 0.0004) followed by 2-sided Dunnett's test showed that survival of larvae exposed to *D. pinnatifida* was significantly lower than that of control larvae. One-way ANOVA comparing survival of the larvae in the agar control with larvae in treatments with extracts of *D. pulchella* and *D. pinnatifida* (ANOVA, F = 14.21, p < 0.0001) and Dunnett's test indicated that survival of larvae in both extract treatments differed significantly from the agar control. The percentage of larvae that settled and metamorphosed (recruited) in chambers also differed among treatments (Fig. 1b). One-way ANOVA comparing the recruitment of larvae in the algal mimic control with that in the *D. pulchella* and *D. pinnatifida* treatments was not significant (ANOVA, F = 2.86, p = 0.07); however, 2-sided Dunnett's test did show that recruitment of larvae in the treatment with

D. pinnatifida was significantly lower than for control larvae. A significant difference was observed among the recruitment of larvae in the agar control and extract treatments (Kruskal-Wallis test, p = 0.004). Recruitment in the treatment with *D. pulchella* extract differed from that in the agar control but not from that in the *D. pinnatifida* extract treatment. There were no clear patterns in the location of new recruits on or off of the tiles or on the top or the bottom of the tiles among the various treatments (data not shown).

Examination by proton NMR showed that the non-polar extracts of both species contained proton signals indicative of terpenes as well as triglycerides or fatty acids. The *Dictyota pulchella* extract had especially high amounts of acetylated terpenes. The terpenes in these extracts have not been isolated and characterized, and we do not yet know which, if any, of these are the compounds responsible for allelochemical activity. However, proton NMR spectra did determine that the overall composition of the organic extracts of *D. pulchella* and *D. pinnatifida* remained qualitatively the same and that the terpenes and other components did not degrade over the 5 d duration of the recruitment experiments.

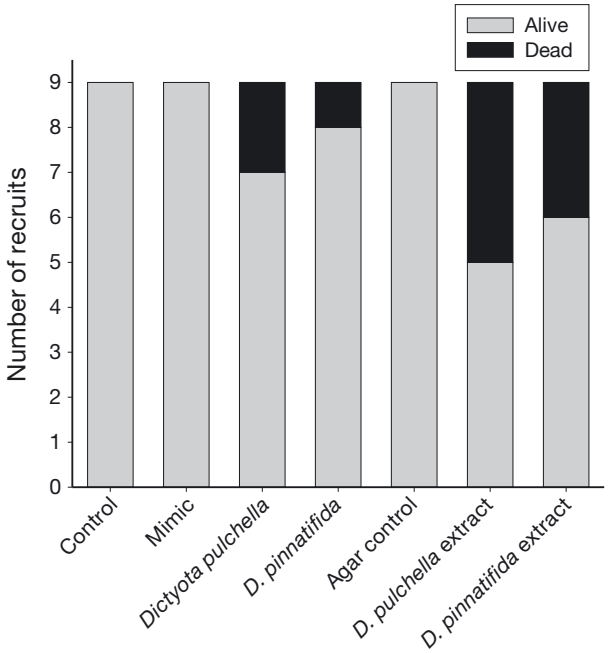

Fig. 2. New recruits of *Porites astreoides*. Survival of 1-wk-old recruits subjected to different algae and algal extracts. Bars represent the number of recruits that were alive or dead after 4 d of exposure to the treatments (n = 9)

Recruit survival and stress

The new recruits had different numbers of survivors in response to the treatments. All 9 of the recruits survived in the 3 control treatments, 1 recruit died in the live *Dictyota pinnatifida* treatment, 2 died in the live *D. pulchella* treatment, 3 died in the *D. pinnatifida* extract treatment, and 4 died in the *D. pulchella* extract treatment (Fig. 2). Log-linear models were used to test whether the 2 algal species or their extracts differed in their effects on recruits. The number of recruits that were dead or alive at the end of the experiment did not differ between either of the 2 live algae (likelihood ratio [LR] = 0.407, df = 1, p = 0.524) or the 2 algal extracts treatment (LR = 0.234, df = 1, p = 0.628), indicating that the 2 algal species had a similar effect on recruit survival. The data from both species were combined to test differences between the effect of the extracts and that of the algae, and none were observed (LR = 2.263, df = 1, p = 0.132). Because the effect of the algal extracts was the same as the effect of the whole algae, all treatment data (live algae and extracts) were pooled and compared with the control data (which were all identical). This test indicated a significant difference overall between the controls and the treatments (LR = 12.592, df = 1, p < 0.001).

For the surviving recruits (Fig. 3), there was no difference in Φ_{II} among treatments (1-way ANOVA, F = 1.49, p = 0.20). In addition, there was no difference in F (ANOVA, F = 1.73, p = 0.13) or F_m' among the treatments (ANOVA, F = 1.78, p = 0.12), although sample sizes for these measurements were small because of low survival of the recruits in some of the treatments (Fig. 2). The overall results were the same if the data were analyzed by two 1-way ANOVA tests comparing the plastic aquarium plant mimic with the live algae and the agar control with the extracts; no significant differences among treatments were observed.

The 1-yr-old recruits were measured for retention of pigmented tissue in response to exposure to the *Dictyota* spp. and their extracts (Fig. 4). No differences were observed among the condition of recruits in the aquarium plant mimic (control) and the live *Dictyota* spp. treatments (ANOVA, F = 0.54, p = 0.60). Corals exposed to the extract treatments, especially the *D. pinnatifida* extract, showed a general trend for fewer pigmented polyps per colony, but variance among replicates was high, and this observation was not statistically significant compared with the agar control (ANOVA, F = 1.80, p = 0.21).

Adult photophysiology

Mean values of Φ_{II} for adult *Porites astreoides* for the 5 d experiment were not statistically different among treatments (repeated-measures ANOVA, p = 0.089; Fig. 5).

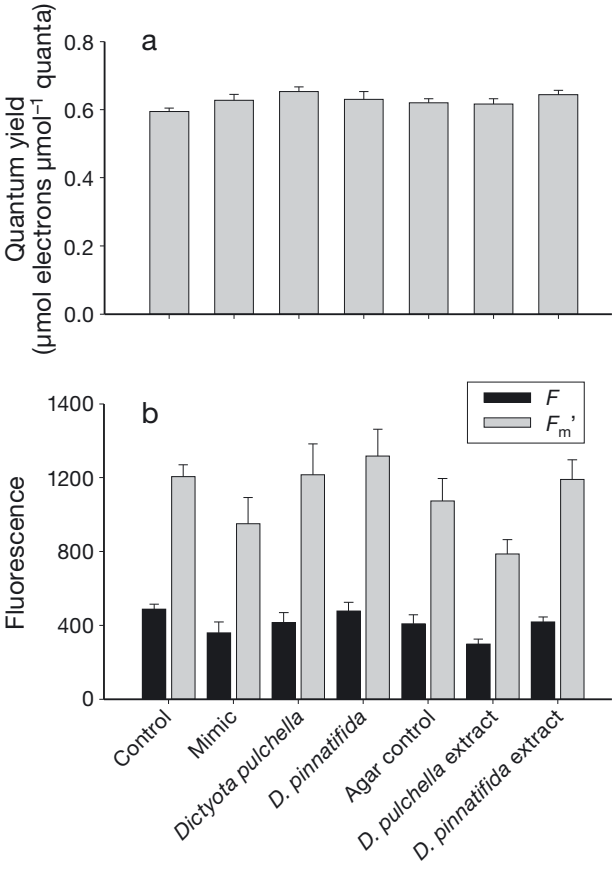

Fig. 3. New recruits of *Porites astreoides*. Photophysiology of 1-wk-old recruits subjected to different algae and algal extracts. (a) Quantum yield of photosystem II of the recruits. (b) Fluorescence (F) and maximum fluorescence (F_m') of the recruits. Data are means + 1 SE; n = 9, 7, 8, 5 and 6 for the controls, *Dictyota pulchella*, *D. pinnatifida*, *D. pulchella* extract and *D. pinnatifida* extract, respectively

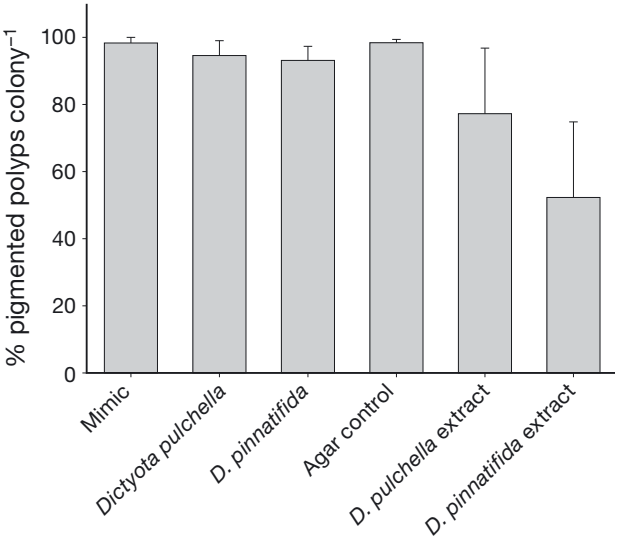

Fig. 4. One-year-old *Porites astreoides*. Percentage of healthy polyps on 1-yr-old recruits after exposure to different algae and algal extracts. Healthy polyps were those that survived and were not bleached after 5 d of exposure to the treatments. Data are means + 1 SE (n = 5)

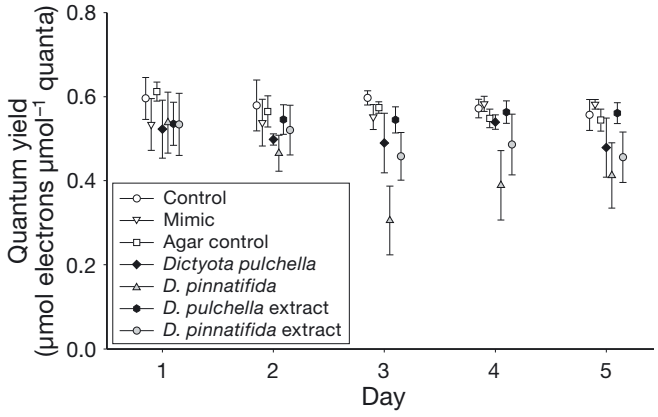

Fig. 5. Adult *Porites astreoides*. Photophysiology of adult colonies after exposure to different algae and algal extracts. Symbols represent the mean quantum yield of photosystem II after exposure to the treatments over time. The same colonies were measured over the duration of the experiment. Error bars are ± 1 SE (n = 7)

DISCUSSION

Coral–algal interactions have become more frequent on coral reefs over the past 3 decades as many reefs have shifted from coral- to algal-dominated communities; however, few studies have tested the mechanisms of competition among macroalgae and other benthic organisms. This study shows that allelopathy may be one mechanism that algae use to successfully compete with multiple life-history stages of coral. In general, early life-history stages of the coral *Porites astreoides* were the most susceptible to the lipophilic extracts of *Dictyota pulchella* and *D. pinnatifida*. *D. pinnatifida* and the extracts of both species of *Dictyota* reduced survival of coral larvae, and *D. pinnatifida* and the extract of *D. pulchella* negatively affected recruitment of *P. astreoides* compared with relevant controls. Survival of new recruits was negatively affected by treatments of live algae and their extracts relative to respective controls.

The larval phase of scleractinian corals is a critical time period when larvae must find habitat favorable for post-settlement survival. Many abiotic and biotic factors can facilitate or inhibit coral larval settlement and survival (Ritson-Williams et al. 2009). Marine algae and cyanobacteria can deter larval settlement and metamorphosis, and allelopathy is one mechanism that may be involved (Baird & Morse 2004, Kuffner & Paul 2004, Maypa & Raymundo 2004, Kuffner et al. 2006, Birrell et al. 2008a,b, Diaz-Pulido et al. 2010).

Waterborne compounds from macroalgae are known to influence settlement and metamorphosis of larvae of corals and other invertebrates, but their effects are variable (Walters et al. 1996, Birrell et al. 2008b, Miller

et al. 2009). Water-soluble compounds from *Padina* sp. inhibited coral settlement, but the seawater conditioned with the brown alga *Lobophora variegata* increased coral settlement compared with controls (Birrell et al. 2008b). An increase in settlement was also found when larvae of the coral *Pocillopora damicornis* were exposed to seawater conditioned with *Sargassum polycystum* and *Laurencia papillosa* (Maypa & Raymundo 2004). Miller et al. (2009) saw variability in the settlement responses of larvae of 3 coral species to exudates from different benthic macroalgal/cyanobacterial assemblages in the Florida Keys, and some of the exudates deterred larval settlement.

The natural products (secondary metabolites) from most macroalgae are lipophilic compounds and are not readily water-soluble; therefore, testing the activity of the organic extracts may provide a clearer test of the allelopathic role of these algae. In one of the few studies that tested lipophilic compounds against invertebrate larvae, surface extracts from *Dictyota menstrualis* inhibited larval settlement of the bryozoan *Bugula neritina* (Schmitt et al. 1995). Our experiments show that organic extracts from both *Dictyota* spp. can inhibit larval survival of *P. astreoides*, and these results are similar to those observed with the whole alga *D. pinnatifida*. The extract from *D. pulchella* inhibited larval settlement and metamorphosis relative to the agar control, although its effect was not significantly different from that of the extract from *D. pinnatifida*. Live *D. pulchella* has been previously shown to affect recruitment of *P. astreoides* larvae and recruit survival (Kuffner et al. 2006), even though its effects were not significant in the present study.

Macroalgae in the genus *Dictyota* are known to produce terpenoid secondary metabolites with multiple ecological functions, including defense against feeding by many generalist marine herbivores (Hay 1996, Paul et al. 2001, Paul & Ritson-Williams 2008). Hundreds of terpenes have been isolated from *Dictyota* spp., including pachydictyol A, dictyol E and acutilols (Hay et al. 1987, Cronin et al. 1997, Vallim et al. 2005). Although we have not yet isolated and characterized their terpenes, proton NMR spectra clearly show that the 2 *Dictyota* extracts used in the present study do not contain the same major compounds. This may explain some of the differences observed in their effects.

Sublethal stress, as measured by PAM fluorometry, was not observed in adult *Porites astreoides* exposed to live *Dictyota pulchella* or *D. pinnatifida* or the algal extracts over a period of 5 d. Other studies have shown that corals have reduced growth rates in the presence of macroalgae (Tanner 1995, McCook et al. 2001, Maypa & Raymundo 2004, Box & Mumby 2007), but the mechanisms of growth inhibition were rarely tested. In the presence of the benthic cyanobacterium *Lyngbya bouilloni*, the coral *Porites lutea* grew less, showed signs of bleaching and had reduced photosynthetic efficiency when compared to the controls (Titlyanov et al. 2007). In another study, *Dictyota* spp. reduced the fecundity of the coral *Montastraea annularis*, and the authors suggested that allelopathy, abrasion and/or overgrowth and shading might explain the negative effects (Foster et al. 2008). Rasher & Hay (2010) showed that several species of coral reef macroalgae, including *D. bartayresiana*, caused bleaching and death of coral tissue in direct contact and could be attributed to allelopathic effects of seaweed lipophilic extracts. We did not observe an effect of nonpolar compounds on the photophysiology of adult *P. astreoides* in the short-term experiments in the present study, but further work with *Dictyota* spp. might show impacts after longer time periods of exposure, and other sublethal effects of these compounds might be observed.

Our study suggests that compounds from species of *Dictyota* common on Caribbean reefs may be involved in allelopathic interactions with coral at multiple life-history stages. These results further support the hypothesis that chemical defenses of macroalgae such as *Dictyota* spp. can facilitate their perpetuation on reefs that have undergone phase shifts by inhibiting coral recruitment (Rogers et al. 1997, Paul et al. 2001, Kuffner et al. 2006). Our research shows that the early life stages, i.e. during larval settlement and early recruit survival, may be especially vulnerable to allelopathic effects. However, allelopathy is not the only mechanism that algae can use to compete with corals (McCook et al. 2001). Algae negatively interact with corals by shading and abrasion (Box & Mumby 2007), releasing dissolved organic carbon that facilitates bacterial growth (Kline et al. 2006, Smith et al. 2006, Morrow et al. in press) and serving as a vector for potentially harmful bacteria (Nugues et al. 2004, Vermeij et al. 2009). There is evidence that the particular mechanisms involved are species-specific, and many macroalgae probably use multiple mechanisms to compete with scleractinian corals.

Coral reefs face a multitude of threats on a global scale (Hoegh-Guldberg et al. 2007). As coral cover on Caribbean reefs declines (Gardner et al. 2003), natural processes of recovery, such as coral recruitment, become increasingly important in restoring coral populations. Macroalgae can reduce coral recruitment by negatively impacting corals at multiple life-history stages, and one management strategy for increasing coral recruitment is to reduce the abundance of harmful macroalgae by increasing local populations of herbivorous fish and invertebrates. Increased levels of herbivory could create more suitable settlement substrata for coral recruitment and reduce competition

between macroalgae and adult corals (Hughes et al. 2007, 2010, Mumby & Steneck 2008, Rasher & Hay 2010). Although the persistence of adult corals and the stability of coral populations may largely depend upon the frequency and intensity of global-scale stressors (e.g. bleaching and disease), improving conditions for coral recruitment is an important method for increasing reef resilience on a local scale.

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