

LETTER

Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality

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Abstract

Declines in coral cover are generally associated with increases in the abundance of fleshy algae. In many cases, it remains unclear whether algae are responsible, directly or indirectly, for coral death or whether they simply settle on dead coral surfaces. Here, we show that algae can indirectly cause coral mortality by enhancing microbial activity via the release of dissolved compounds. When coral and algae were placed in chambers together but separated by a 0.02 µm filter, corals suffered 100% mortality. With the addition of the broad-spectrum antibiotic ampicillin, mortality was completely prevented. Physiological measurements showed complementary patterns of increasing coral stress with proximity to algae. Our results suggest that as human impacts increase and algae become more abundant on reefs a positive feedback loop may be created whereby compounds released by algae enhance microbial activity on live coral surfaces causing mortality of corals and further algal growth.

Keywords

Algae, bacteria, coral disease, coral reef, dissolved organic carbon, macroalgae, microbes, phase-shifts, reef degradation.

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INTRODUCTION

Over the last several decades there has been an alarming decline in coral reefs around the world (McCook 1999; Aronson *et al.* 2003; Bellwood *et al.* 2004; Gardner *et al.* 2005; Pandolfi *et al.* 2005). The causes of reef degradation are often elusive and generally associated with several interacting factors such as global climate change, overfishing, eutrophication and coral disease. The loss of live coral cover is often associated with a phase-shift to a system dominated by fleshy algae (Hughes 1994; McCook 1999;

Fabricius *et al.* 2005). While a number of studies have shown the importance of top-down (grazing pressure) and bottom-up (resources) factors in regulating coral-algal competition (McCook 1999; Miller *et al.* 1999; Smith *et al.* 2001; Jompa & McCook 2002b; McClanahan *et al.* 2002, 2003), much less is known about the specific mechanisms by which algae overgrow coral (McCook *et al.* 2001).

The incidence of coral disease has been increasing and is often positively correlated with increasing algal cover (Goreau *et al.* 1998; Hayes & Goreau 1998; Harvell *et al.* 1999, 2004). These coral diseases are important causes of

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coral reef decline (Bruckner & Bruckner 1997; Goreau *et al.* 1998; Harvell *et al.* 1999; Aronson 2001), but the aetiological agents of these diseases remain largely unknown (Richardson *et al.* 1998; Harvell *et al.* 2004). Recent studies have shown that macroalgae may be important vectors for the transmission of certain coral diseases (Nugues *et al.* 2004), although in most cases it is not clear if a specific pathogen is involved (Richardson *et al.* 1998; Cooney *et al.* 2002; Pantos *et al.* 2003; Casas *et al.* 2004). It is clear, however, that human activity and the incidence of coral disease are strongly correlated (Hayes & Goreau 1998; Green & Bruckner 2000; Lipp *et al.* 2002; Hughes *et al.* 2003). Green & Bruckner (2000) estimate that 97% of coral disease in the Caribbean has been recorded in areas with high human activity. Perhaps, the most poorly understood component of coral decline is how coral diseases, increased algal cover, and human impacts, such as nutrient pollution and over fishing, are related. Understanding these relationships is crucial for the development of conservation, restoration and management plans for coral reefs around the world.

Previous studies have shown that various species or genera of algae can negatively influence corals (McCook *et al.* 2001; Jompa & McCook 2003a,b; Nugues *et al.* 2004). Potential mechanisms include allelopathy, smothering, shading, abrasion, overgrowth (reviewed in McCook *et al.* 2001) and the harbouring of potential microbial pathogens (Nugues *et al.* 2004). However, the indirect effects of algae on coral health have been less considered, in particular the indirect impacts of algal exudates on coral-microbe interactions. It has been shown that most increases in inorganic nutrients (nitrogen and phosphorus) do not directly kill corals, whereas increased organic carbon loading does (Kuntz *et al.* 2005; Kline *et al.* in press). Coral mortality caused by organic carbon loading can be prevented or delayed with the addition of antibiotics (Mitchell & Chet 1975) and is correlated with an increase in microbial activity suggesting that microbes are mediating coral death. Furthermore, several studies in marine, freshwater and terrestrial systems have shown that autotrophs commonly release dissolved organic carbon (DOC) as excess photosynthate into their surrounding media (Cole *et al.* 1982; Aluwihare *et al.* 1997; Collos 1997; Delille *et al.* 1997; Aluwihare & Repeta 1999; Stanley *et al.* 2003; Jones *et al.* 2004). In some studies direct links have been made between release rates of DOC and activity of heterotrophic bacteria (Cole *et al.* 1982; Delille *et al.* 1997). Thus, it would hold that microbial activity may be enhanced on coral reefs with abundant algal populations as a result of high organic carbon exudation from the algae.

The goal of this study was to specifically determine whether the mechanism of algae-induced microbe-mediated coral mortality can occur. Through a combination of both mesocosm experiments and field observations we specific-

ally tested: (a) whether reduced coral health and/or coral mortality could be induced when corals were placed in close proximity, but not direct contact with algae; (b) whether these effects were mediated by microbes; (c) whether these effects are generalizable across various coral-algal species combinations; and (d) whether these patterns could be observed directly in the field.

METHODS

Collection of samples and experimental design

Corals used to develop the protocols for this study (e.g. microprobing, lipopolysaccharide injections, and barrier chambers, see below for details) were provided by the Birch Aquarium (La Jolla, CA, USA). Coral and algal samples used for the experiments were collected using SCUBA at a depth of *c.* 10 m as part of the Scripps Line Islands Expedition (2 August to 5 September 2005). All experiments took place aboard the OR/V White Holly.

Experiment 1

This experiment tested whether a change in coral health could be detected after fragments of corals and algae were placed in close proximity to, but not in direct contact with, one another. Ten replicate samples of the coral *Pocillopora verrucosa* and the green alga *Dictyosphaeria cavernosa* were collected on 19 August 2005 on the southwest side of Palmyra Atoll (5°53.81'N, 162°6.02'W). All samples were stored in Ziploc bags and transported back to the ship in a tub filled with seawater. Once onboard the ship individual fragments of corals (*c.* 3 cm branch length) and algae (*c.* 9 cm² area) were made using a chisel and blade, respectively. Experimental units were made by placing individual fragments of corals and algae into experimental chambers. The chambers were constructed of acrylic with two compartments separated by a 0.02 µm Anodisc filter (Whatman, Maidstone, UK; Fig. 1a). The filter is fine enough to prevent the passage of bacteria and viruses while still allowing passage of dissolved compounds between the compartments.

Five experimental (corals and algae) and five control (corals alone) units were assembled. All units were placed randomly into two 40 L tubs (5 units per tub) and were filled with 20 L of seawater as a water bath. The seawater in both the chambers and the surrounding tubs was changed once daily. After 2.5 days physiological measurements of each coral holobiont (i.e. coral, zooxanthellae and epibiotic microbial community) were made, including oxygen microprobe measurements, estimation of photosynthetic efficiency using pulse amplitude modulation (PAM) fluorometry, and measurements of microbial activity using adenosine triphosphate (ATP) swabs (see below for more detail).

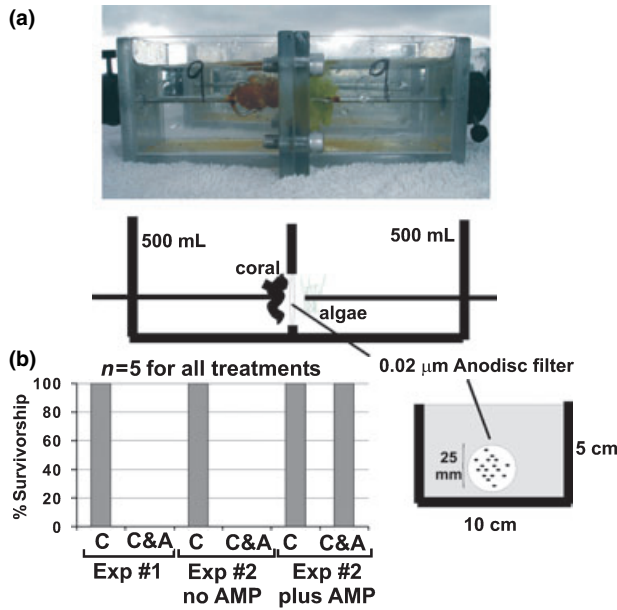


Figure 1 (a) Photograph and schematic of barrier chambers used for coral–algal interaction studies. The 0.02 µm Anodisc filter was chosen because it prevents movement of viruses and bacteria but not dissolved compounds. (b) Coral mortality associated with proximity to algae (first panel, experiment 1) and with and without algae and antibiotics (second panel, experiment 2). C, coral present; C & A, both coral and algae present; AMP, ampicillin at 50 µg mL⁻¹.

Experiment 2

This experiment tested whether a decline in coral health, when in the presence of algae, could be prevented with the

addition of antibiotics, thus suggesting a microbially mediated mortality. Samples for this experiment were collected from reefs on the northwest side of Palmyra Atoll (5°51.91'N, 162°0.70'W) on 23 August 2005. Coral and algal fragments were prepared and experimental units were assembled as described above. Of the 10 chambers, five were filled with seawater alone and five were filled with seawater plus the broad-spectrum antibiotic ampicillin (final concentration 50 µg mL⁻¹). All chambers were placed in 40 L tubs as described above. All water and antibiotic treatments were changed daily. After 2.5 days physiological measurements were made as in experiment 1.

Experiment 3

The final experiment tested the generality of the patterns observed in the first two experiments by monitoring the response of various coral species when placed in close proximity to various algal species. All samples were collected from the outer reef slope at the northeast side of Kingman reef (6°26.72'N; 162°23.80'W), c. 60 km northwest of Palmyra atoll, on 27 August 2005. Divers collected samples of 13 species of coral and 13 species (or functional groups) of algae (Table 1). Upon returning to the ship fragments of corals and algae were prepared as described above (fragments c. 5 cm in length). Coral–algal species combinations were assigned randomly and pairs were connected together and labelled using small cable ties and plastic labels. Four 40 L tubs were filled with 20 L of seawater and the sample units were distributed among the four tubs. Two of the four tubs received ampicillin (final concentration 50 µg mL⁻¹), while the other two contained only seawater. These water baths acted as reef mesocosms where various species of

Table 1 Tabulation of all combinations of corals and algae used in experiment 3 (Fig. 4)

| | <i>Caulerpa</i> | | CCA | | Cyano-bacteria | | <i>Dictyosphaeria</i> | | <i>Halimeda</i> | | <i>Microdictyon</i> | | <i>Peysonnelia</i> | | Turf mixed | | Total | | Sum |
|--------------------|-----------------|---|-----|---|----------------|---|-----------------------|---|-----------------|----|---------------------|---|--------------------|---|------------|----|-------|----|-----|
| | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | |
| <i>Acropora</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 3 | 5 | 8 |
| <i>Favia</i> | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 6 | 7 |
| <i>Fungia</i> | 1 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 5 | 5 | 10 |
| <i>Hydnophora</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 2 | 2 | 4 |
| <i>Montastrea</i> | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 4 | 1 | 5 |
| <i>Montipora</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 2 |
| <i>Pavona</i> | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 1 | 3 | 4 | 8 | 12 |
| <i>Pocillopora</i> | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 3 |
| <i>Porites</i> | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 3 | 6 | 9 |
| <i>Stylophora</i> | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 2 | 3 | 7 | 10 |
| Sum | 4 | 5 | 3 | 6 | 2 | 3 | 0 | 1 | 5 | 10 | 2 | 3 | 1 | 1 | 9 | 15 | 26 | 44 | 70 |

Coral genera are listed along the vertical, while algal genera are listed across the top. + indicates treatments with the antibiotic ampicillin, while - indicates samples grown in seawater alone. The numbers in each cell indicate the number of replicate samples for a given coral–algal pair.

corals and algae were interacting together in the same body of water. While algal exudates were potentially mixing within the water bath in a given tub, the effects of each alga on a given coral are likely to be strongest at the zone of interaction (prior to rapid dilution of exudates away from the alga). All water and antibiotics were changed daily for 4 days. A total of 43 coral–algal pairs were placed in tubs that received ampicillin, while 26 pairs were in tubs with seawater alone. After 4 days coral health was scored as follows: 1, no change; 2, some discoloration; 3, dead next to alga; 4, < 50% dead; 5, 50–99% dead; 6, 100% dead. Physiological measurements were also made on day 4.

For all of the above experiments, tubs containing the water baths and the experimental units were kept on the top deck of the ship in the shade. This light environment (maximum daily irradiance values: 150–300 μM quant $\text{m}^{-2} \text{s}^{-1}$) approximated measurements made in the field at 10 m depth where all samples were collected.

Field observations

Pocillopora verrucosa fragments were collected from Palmyra atoll and Kingman reef where turf algal overgrowth of live coral tissue was evident ($n = 5$ for each atoll). All samples were transported back to the ship as described above. Within 2 h of collection, oxygen measurements were made using microprobes; readings were taken across the coral–algal interfaces as described below to quantify differences in oxygen levels across the interface.

Physiological measurements

Microprobing

Dissolved oxygen concentrations around and on the corals and algae were measured with a Clark-type oxygen microprobe (Unisense; Aarhus C, Denmark). Aerated seawater was used for the 100% dissolved oxygen calibration point. Seawater amended with 0.1 M sodium ascorbate and 0.1 M sodium hydroxide was used as the 0% dissolved oxygen calibration point. All of the microprobing was done under a Leica MZFCIII (Solms, Germany) dissecting microscope. The microprobe was used to measure dissolved oxygen along a transect from the site on the coral surface closest to the Anodisc (in the chambers) or closest to the algal interface (for field samples) to the approximate middle of the coral fragment spanning a total distance of *c.* 3 cm (measurements were taken every 0.5 s). For both experiments 1 and 2, the probe was moved up and down relative to the coral surface to find the range of oxygen maxima (either oxygen release during photosynthesis or the surrounding seawater) and minima (against the coral animal or areas of high microbial activity). Once these regions were found oxygen readings were recorded and averaged over 15 s to obtain

an estimate of oxygen maxima and minima for each sample.

ATP swabs

Measurements of ATP can be used to gauge microbial activity. In the human health and food industry, swabs are used both on food and cooking surfaces to estimate the amount of active microbial activity using the luciferin/luciferase reaction. The swabs are rubbed against a surface, placed into a chamber that activates ATP and initiates the reaction eventually producing light, which is then measured with a luminometer (Hygiena, Watford, UK). For these experiments, the end of an Ultrasnap ATP swab (Hygiena) was placed lengthwise onto the coral surface five times. The swab was then processed according to the manufacturers protocol using a SystemSure II luminometer (Hygiena).

PAM fluorometry

All samples of corals and algae used in the above experiments were assessed physiologically using PAM. PAM technology is commonly used in a number of settings to measure health and physiological status of photosynthetic organisms. Specifically, the diving-PAM (Walz, Germany) has been used to assess the bleaching susceptibility and the response to stressors in a number of coral species *in situ* (Yakovleva & Hidaka 2004; Ralph *et al.* 2005). In our studies, a diving-PAM was used to determine the maximum quantum yield of photosystem II (PS II) measured as the ratio of variable to maximum fluorescence (F_v/F_m) yield. These values were used to assess photosynthetic efficiency of all samples at the end of each experiment (values range from 0 to 1, where healthy higher plant tissue has values near 0.8 regardless of taxa). All samples were dark acclimated for *c.* 2 min prior to providing a saturating pulse. The yield values were compared between experimental and control coral samples for all three experiments. Yield estimates were also made for algal controls in all of the experiments to ensure that the algae were alive and healthy at the end of each incubation. All measurements were made between 3 and 5 p.m.

Statistical analyses

All analyses were done using either R version 2.2.0 or SAS version 9.1. For experiments 1 and 2, observed patterns of mortality were compared against the null expectation of equal probability of mortality among groups using a binomial test. For experiment 3, we tested the effects of antibiotics on the observed patterns of change in coral health (1, no change; 2, some discoloration; 3, dead next to alga; 4, < 50% dead; 5, 50–99% dead; 6, 100% dead) using an ordinal logistic regression.

Where appropriate, physiological data were analysed using non-parametric statistics. For experiment 1, differences in

the ATP data and the PAM data (comparing corals with and without algae in chambers) were assessed using Mann–Whitney *U*-tests. For experiment 2, a Kruskal–Wallis test was used to test for differences among treatments in the PAM data and, if significant, *post-hoc* multiple comparisons were used to identify the experimental treatments that deviated significantly from the control (i.e. corals without algae or antibiotics). Corrections were made for unequal sample sizes and for ties in ranks. For experiment 3, differences in PAM measurements of each coral and alga with and without antibiotics were tested using Mann–Whitney *U*-tests.

RESULTS

Experiment 1

Fragments of *P. verrucosa* placed next to fragments of the green alga *D. cavernosa* in the barrier chambers (Fig. 1a) suffered 100% mortality within 2 days as defined by overall colour and pigmentation of the coral fragments (Fig. 1b) and as evidenced by physiological parameters (see below). Control corals placed in identical chambers without algae present suffered no mortality. The hypothesis that experimental and control corals experienced equal probabilities of mortality was rejected (Binomial test, $P < 0.05$).

All of the physiological measures of coral health also revealed deleterious effects of algae on corals. Oxygen microprobe surveys indicated that the surface of the corals adjacent to the algae was hypoxic (Fig. 2a, $< 1\%$ of the dissolved O_2 in the surrounding seawater). In contrast, corals incubated in chambers without algae displayed $> 100\%$ dissolved oxygen at the coral tissue surface (compared with the surrounding seawater), reflecting the photosynthetic activity of the zooxanthellae (Fig. 2b). There was an increase in the amount of ATP associated with the coral surface next to the algae. The average relative light units (RLU) associated with ATP binding to luciferase was $1371 (\pm 634 \text{ SE})$ on the coral control samples and $2629 (\pm 1580 \text{ SE})$ on the corals placed next to the algae and was significantly higher on corals in the presence of algae ($U = 23$, $n_1 = n_2 = 5$, $P = 0.05$). Photosynthetic efficiency as measured by PAM fluorometry showed that corals in the presence of algae had significantly lower yield values than the coral controls without algae (dark adapted yield = 0.004 ± 0.001 with algae and 0.590 ± 0.033 in controls, Fig. 3a). Photosynthetic efficiency differed between experimental and control corals by over one order of magnitude and these values were statistically significant ($U = 25$, $n_1 = n_2 = 5$, $P = 0.01$) suggesting that the presence of algae had a highly negative impact on the health and physiology of this coral holobiont. Values in this range are indicative of mortality (Yakovleva & Hidaka 2004). In contrast, there was

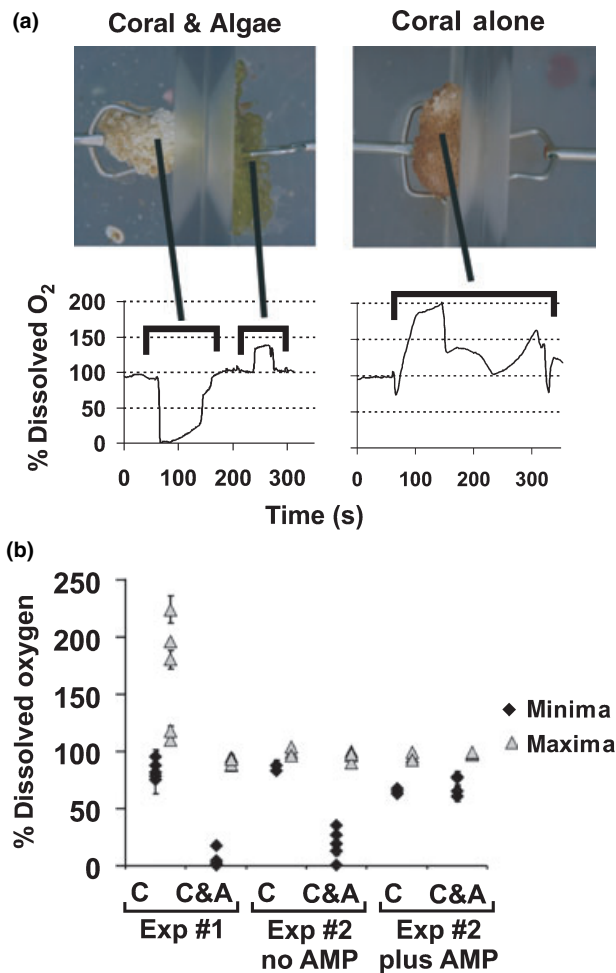


Figure 2 (a) Photographs showing a coral–algal pair in experimental chambers and coral controls from experiment 1. The graphs show oxygen microprobe data taken along transects across the respective organismal surfaces. Readings were taken every 0.5 s. As such, the x-axis of the microprobe data are in units of time and do not correspond to distance along the organism's surface. 100% oxygen saturation represents the background water, any addition above this indicates input via photosynthesis and any decrease indicates oxygen consumption. Note hypoxic conditions on the coral surface near the algae. (b) High and low dissolved oxygen values for coral surfaces with and without algae. The oxygen microprobe was moved up and down relative to the coral surface to find the areas of oxygen maxima (either oxygen release during photosynthesis or the surrounding seawater) and minimum (against the coral animal or areas of high microbial activity). Once maxima and minima were located, data were recorded for 15 s and averaged. These averages ($\pm 1 \text{ SE}$) are graphed above. Data are shown for experiment 1 (left) and experiment 2 (middle and right). C, coral alone; and C & A, coral and algae grown together in experimental chambers (see Fig. 1a).

no difference in the photosynthetic yield for algae grown with or without corals thus indicating that the algae were healthy across both treatments.

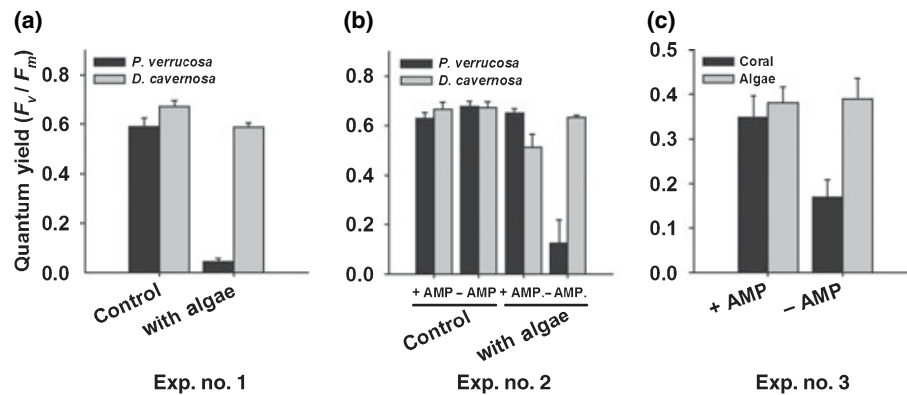


Figure 3 Results of quantum yield of photochemistry for PS II (F_v/F_m) as measured with pulse amplitude modulation fluorometry (PAM). Measurements are of dark-adapted yield (DAY) following saturating pulses for both coral and algae. (a) DAY for *Dictyosphaeria cavernosa* and *Pocillopora verrucosa* following 36 h in experimental chambers in experiment 1 ($n = 5$); (b) DAY for coral and algae grown together with and without antibiotics (Control = coral or algae grown alone, with algae = coral and algae grown together in chambers, +AMP = with ampicillin, -AMP = seawater alone) following 36 h in experimental chambers in experiment 2 ($n = 5$ for experimental units and 3 for controls); (c) DAY for both coral and algae pooled for all species combinations assessed in experiment 3 after 48 h in experimental containers ($n = 20$).

Experiment 2

When *P. verrucosa* and *D. cavernosa* were grown together in chambers separated from each other by a 0.02 μm filter, corals suffered 100% mortality as seen in experiment 1. With the addition of the antibiotic ampicillin there was no mortality or evidence of reduced coral health (Fig. 1b). Again, the hypothesis that experimental and control corals experienced equal probabilities of mortality was rejected (Binomial test, $P < 0.05$). All of the physiological measurements also indicated declines in coral health without antibiotics and no change in health when treated with ampicillin. Dissolved oxygen readings from the oxygen microprobe showed the highest oxygen readings on the control coral surfaces (110–224%, Fig. 2b). These high values reflect the photosynthetic activity of the zooxanthellae. For corals grown with algae and without antibiotics, zones of hypoxia were present on surfaces adjacent to the algae (O_2 range 0.9–36%). These hypoxic zones did not develop on the coral–algal interfaces that were treated with the antibiotic ampicillin (average range 61–78%). Photosynthetic efficiency of corals grown with algae without antibiotics was significantly lower (as measured by dark adapted yield) than the controls (corals alone) and the other two treatments (corals with algae and antibiotics, corals without algae but with antibiotics; $H_c = 10.98$, d.f. = 3, $P < 0.05$, *post-hoc* comparison against control, $P < 0.05$). There was also some variability in the photosynthetic yield of the algae (Fig. 3b). *Dictyosphaeria cavernosa* grown with coral and antibiotics trended towards lower yield values than when grown under the same conditions without coral (0.512 ± 0.052 SE and 0.665 ± 0.029 SE). For algae grown without antibiotics, there was no difference in photosyn-

thetic yield when grown with and without coral. None of these differences in algal photosynthetic yield were statistically significant ($H_c = 5.48$, d.f. = 3, $P > 0.05$).

Experiment 3

Several different combinations of corals and algae were grown together to determine if the patterns observed in experiments 1 and 2 above were taxon-specific (Table 1). Greater than 95% of corals exposed to algae without ampicillin suffered some level of stress from discoloration to 100% mortality within 2 days, and further deterioration on the third day (Fig. 4a). Twenty per cent of the corals in the algae and antibiotic treatment began declining in health but over 80% showed no signs of stress and no further decline was noted between days 2 and 3. The differences between the responses of corals in the antibiotic/no-antibiotic treatments were significant in an ordinal logistic regression for both 2 and 3 day intervals (intercepts = 1.03 ± 0.25 and 1.86 ± 0.36 , respectively, $P < 0.001$ for both tests). *Acropora* showed the greatest susceptibility to the algal treatment (Fig. 4b), with four of six fragments showing 100% mortality by day 2, in both antibiotic and no-antibiotic treatments. Genera such as *Porites*, *Hydnophora* and *Fungia* showed the least amount of mortality or stress following the algal treatment. For most coral genera, treatment with antibiotic appeared to reduce the stress category by one to two steps; however, unequal sample sizes (Table 1) and the diversity of algae and corals requires further experimentation to elucidate any susceptibility hierarchies. Measurements of physiological status indicated similar results. Photosynthetic efficiency as measured by PAM fluorometry was much lower for corals exposed to algae without antibiotics than with (dark adapted

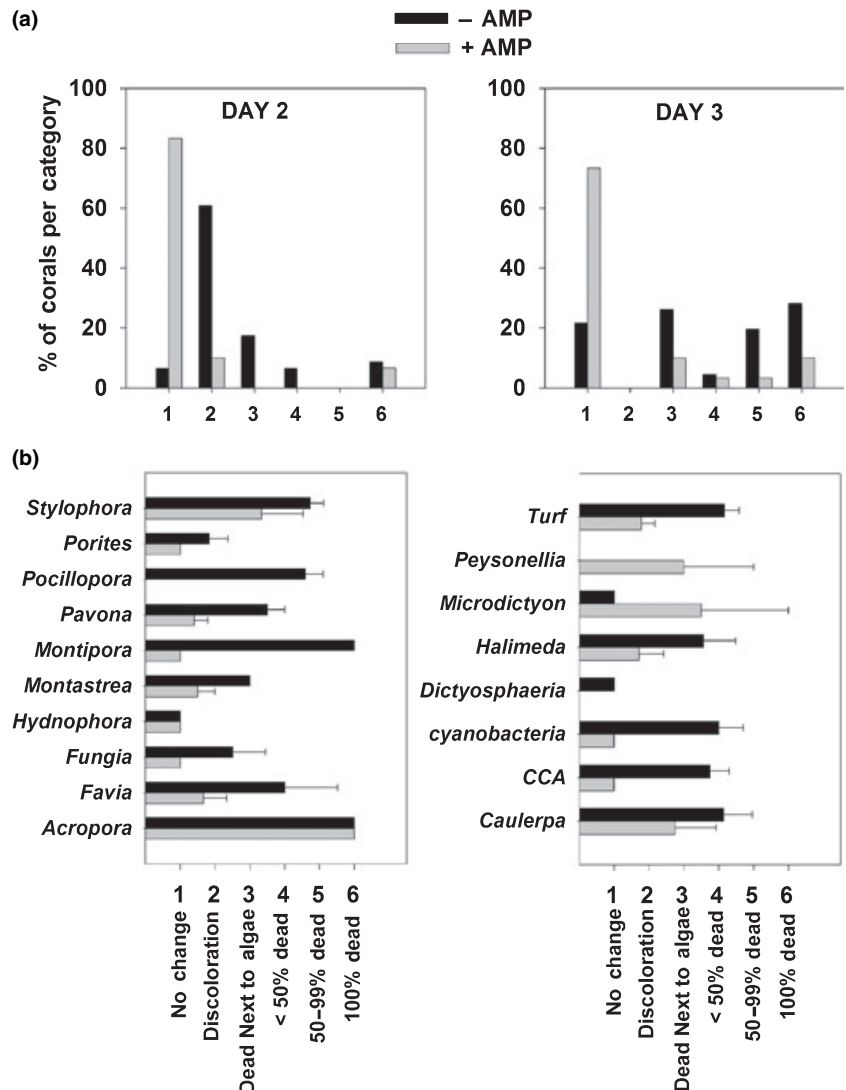


Figure 4 Coral-algal interfaces were created by randomly placing coral and algal fragments together and incubating paired samples in aquaria with and without ampicillin (+AMP or -AMP) amended seawater (experiment 3). The health of the corals was assessed and scored as indicated on the x-axis (1, no change; 2, some discoloration; 3, dead next to algae; 4, < 50% dead; 5, 50–99% dead; 6, 100% dead) after 2 and 3 days. (a) Pooled response of all corals to all algae with and without antibiotics at days 2 and 3, respectively. Data represent the per cent of corals within a treatment that were given each respective score. (b) Detailed response of coral genera to algae and antibiotic treatments (left panel) and variable effects of different algal genera (or functional group) on coral health (right panel) from day 3.

yield: 0.171 ± 0.039 SE and 0.349 ± 0.048 SE, respectively; $U = 292.5$, $n_1 = n_2 = 20$, $P < 0.02$).

Field observations

Coral-algal interfaces were harvested from the field and were assessed using oxygen microprobes. The white light panel in Fig. 5a shows an algae-coral interface where turf algae appeared to be growing over or through the live coral tissue. When viewed under a green fluorescence protein (GFP) filter set (excitation 425/60 nm; emission: 480 nm long pass; LEICA), the GFP within the coral animal was visible, as was the chlorophyll in the zooxanthellae and in the turf algae that were present at the interface. Under a red filter (excitation: 560/40 nm; emission: 610 nm long pass; LEICA), auxiliary fluorochromes in the algae were visible. Additionally, there is a point in the centre of the image (indicated by the arrow in

Fig. 5a) where an endolithic alga is growing up through the coral tissues from below. This site appears bleached in the visible light but is still covered by coral tissue, as evidenced by the GFP signal. The microprobe transect across this interaction zone revealed two areas of hypoxia. First where coral interacts with the surface turf algae (dissolved oxygen < 25%) and second on the edge of the coral and the endolithic algae (dissolved oxygen < 1%). Figure 5b shows the dissolved oxygen readings from the five different coral-algal interfaces. In all of these cases, it was clear that algae were growing on or over live coral tissue. The lowest and highest dissolved oxygen values (averaged over 15 s intervals) on the coral and algal surfaces alone were similar to each other (reflecting active photosynthesis), whereas values recorded from the coral-algal interfaces were consistently low, indicating either reduced photosynthetic activity or active oxygen consumption.

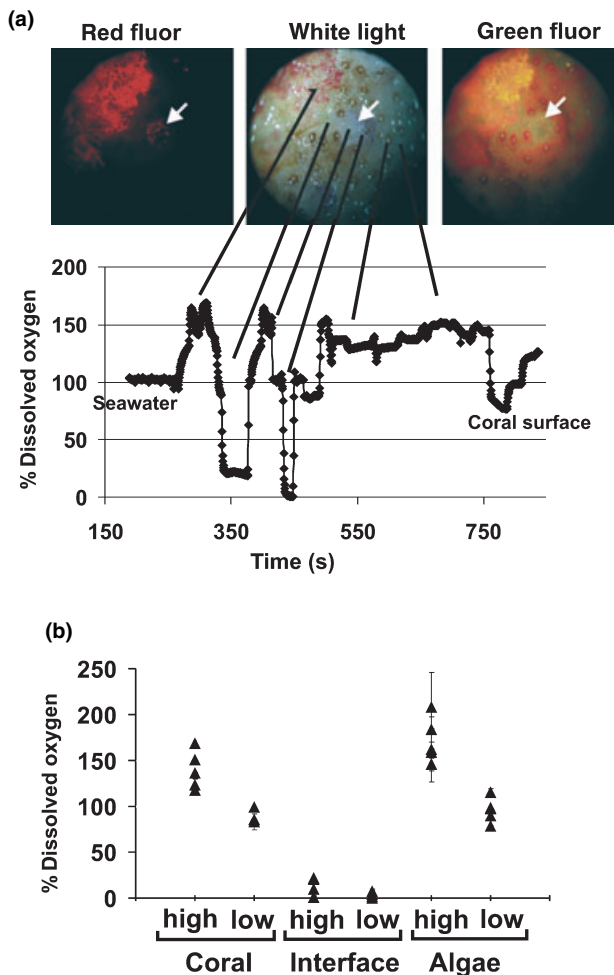


Figure 5 (a) Light and epifluorescent micrographs as well as dissolved oxygen microprobe readings from a natural coral–algal interface. Seawater refers to the surrounding seawater and Coral surface indicates a region where the microprobe was touched to live coral. (b) Maximum and minimum values from dissolved oxygen microprobe readings taken on five different natural coral–algal interfaces. Coral = *Pocillopora verrucosa* and Algae = mixed assemblage of filamentous turf algae.

DISCUSSION

This study has demonstrated that diffusible compounds released by algae mediated coral mortality via microbial activity. While the actual compounds are as of yet unidentified, it is widely known that algae can release excess photosynthate in the form of polysaccharides (Cole *et al.* 1982; Aluwihare *et al.* 1997; Aluwihare & Repeta 1999; Cole 1999; Stanley *et al.* 2003). This DOC can fuel microbial activity and lead to accelerated microbial growth (Cole *et al.* 1982; Aluwihare *et al.* 1997; Aluwihare & Repeta 1999). It would follow that the background microbial community

growing on or near the coral surface may respond to DOC released by algae by exhibiting explosive population growth, eventually creating a zone of hypoxia along the margin or on the surface of the live coral tissue. In our study, all corals exposed to but not in direct contact with algae bleached and were physiologically compromised within 2–4 days. Clearly, some algae may release chemical compounds that may be toxic to other organisms (Walters *et al.* 1996). But here, coral mortality could be completely prevented with the addition of antibiotics, thus supporting the hypothesis that the microbial community was the agent of coral death.

The above results were strikingly consistent for one coral–algal species pair (*P. verrucosa* and *D. cavernosa*). Species-specificity was determined by conducting a larger experiment in which several coral and algal species combinations were randomly assembled and placed together either with or without antibiotics. Most coral species suffered partial or complete mortality when placed with algae, while most coral–algal species combinations survived in the presence of antibiotics. It is possible that corals in the algae treatment were all equally stressed because they were all grown in the same water bath with the potential for mixing of algal exudates. But the effects of a given alga on a given coral are likely to be the strongest at the zone of interaction where the two organisms are in close proximity. As algal exudates diffuse out of the benthic boundary layer they become less available to benthic microbes and therefore will have less of an effect on corals. In this study, we saw differential effects of algae on corals suggesting that not all coral species are equally susceptible to algal mediated mortality and that not all algae will have deleterious effects on corals. Variable responses of corals to different species of algae or different impacts of algae on corals have been previously documented (McCook *et al.* 2001; Jompa & McCook 2002a,b, 2003a,b). Similarly, coral responses to chemical stressors appear variable across coral species and across stressor type (Kuntz *et al.* 2005) and are also concentration dependent (Kline *et al.* in press). Nonetheless, corals generally showed significant declines in health when placed next to algae without antibiotics suggesting that when in close contact, the deleterious effects of algae on corals are common and are mediated by microbes.

Even on healthy reefs algal overgrowth of corals can be observed. Studies have documented or posited various mechanisms by which active overgrowth of live coral tissue can occur and include competition, allelopathy, abrasion and stress (McCook *et al.* 2001). In this study, we were able to collect samples of coral that were being overgrown by turf algae from the nearly pristine reefs of Palmyra atoll and Kingman reef in the northern Line Islands. By physiologically examining the zone of interaction between the coral *P. verrucosa* and turf algae on a number of samples we were able to show an area of hypoxia and accelerated microbial

activity associated with the coral algal border. These field-based observations directly correspond with the patterns observed in our laboratory mesocosm studies suggesting that when in close proximity algae can enhance microbial activity at the coral/algal border and thus facilitate algal overgrowth of 'live' coral tissue. These patterns are likely to be concentration dependent (Kline *et al.* in press) and thus would be strongest in areas that receive a significant input of DOC either from the algae themselves or from anthropogenic sources (e.g. waste water).

An increase in algal abundance on reefs around the world has been attributed to over fishing (or the loss of herbivores), increases in inorganic nutrient concentrations, and/or coral mortality, caused by any number of other factors such as disease, bleaching, sedimentation and destructive fishing practices all of which increase the amount of available space for settlement and growth of algae (Hughes & Connell 1999; Hughes *et al.* 1999; McCook 1999; Knowlton 2001; Aronson *et al.* 2003; Bellwood *et al.* 2004). On healthy reefs, corals are the dominant, habitat-forming organisms and algae are kept at low levels of standing biomass via intense grazing. As reefs become impacted by any number of factors mentioned above and algal production and abundance increases, the amount of DOC released by the algal community as excess photosynthate may increase as well. Once algae become highly abundant and algal tissue begins decomposing even larger amounts of organic carbon can be released into the water column and all of this DOC can potentially be used by the microbial community.

The results of this study suggest that a positive feedback loop may be created during phase-shifts from coral- to algal-dominance on reefs. Increases in algal biomass or productivity (due to natural and/or anthropogenic stressors) can lead to higher levels of DOC in the water column. DOC promotes microbial activity at coral-algal interfaces and the corals then become stressed as a result of hypoxia. Stressed corals suffer localized mortality which subsequently opens more space for settlement and growth of algae. While more research is needed to understand the specific interactions of diverse assemblages of algae, corals and microbes, the above scenario provides a mechanistic basis for how algae can overgrow 'live' coral.

This study sought to identify and describe a mechanism of coral mortality via algal exudates and microbial overgrowth. While there has been an increasing body of literature suggesting that increases in DOC can cause coral mortality via enhanced microbial activity (Kuntz *et al.* 2005; Kline *et al.* in press), the potential sources of excess DOC have not been identified. Several studies in other systems have shown that primary producers release DOC into their surrounding environment and have identified this DOC as an important nutrient source for microbes (Cole *et al.* 1982;

Delille *et al.* 1997; Jones *et al.* 2004). Our study provides the first piece of evidence linking these processes on coral reefs. Furthermore, we have identified that algal exudates not only enhance microbial activity, but can also cause coral mortality. The relative importance of this mechanism across broad spatial and temporal scales and across levels of human impacts is currently unknown, but is of fundamental importance for the conservation and restoration of coral reefs.

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