Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 9-13 July 2012
11C Seaweed-coral competition

Contact with macroalgae causes variable coral mortality in *Montastraea faveolata*

Alexander T. Wolf[1], Christian Wild[1], Maggy M. Nugues[1,2,3]

[1] Coral Reef Ecology Group (CORE), Leibniz Center for Tropical Marine Ecology (ZMT), Bremen, Germany
[2] USR 3278 CNRS-EPHE, Laboratoire d'Excellence "CORAIL", Perpignan, France
[3] Caribbean Research and Management of Biodiversity (CARMABI), Willemstad, Curaçao.
Corresponding author: alexander.wolf@zmt-bremen.de

Abstract
Shifts in benthic reef community structure often involve the replacement of corals by macroalgae. We investigated the response of a scleractinian coral to direct contact with different macroalgae during an *in situ* interaction experiment on Curaçao, southern Caribbean. The macroalgae *Dictyota pinnatifida, Lobophora variegata, Halimeda opuntia* and *Cladophora* spp. were placed onto healthy *Montastraea faveolata* colonies and coral condition was monitored over a period of 15 d. Rapid coral tissue mortality was observed in colonies interacting with *D. pinnatifida* and *Cladophora* spp. In contrast, mortality in the *H. opuntia* and *L. variegata* treatments appeared slowly. At day 3, coral tissue in contact with *D. pinnatifida* and *Cladophora* spp. experienced 55 and 71% mortality, respectively, whereas mortality remained less than 2 % in the *H. opuntia* and *L. variegata* treatments. At day 15, mortality reached 38 % in the *L. variegata* treatment, whereas all other algae caused ≥ 88 % coral mortality. All algae except *L. variegata* caused mortality outside the area overgrown by the transplants, suggesting white plague disease-like processes beyond the area of direct interaction. Such differential coral mortality could be attributed to variable algal-induced exudation of allelochemicals and/or DOC release rates by means of direct toxicity or by fueling microbial activity with ensuing oxygen deficiency.

Key words: Coral-seaweed interaction, Coral mortality, O_2 depletion, Bacterial growth.

Introduction

In the Caribbean, the average cover of hard corals has declined by ~ 80 % in the last 30 years (Gardner et al. 2003). Reef degradation often involves a shift in benthic community structure in which corals are replaced by macroalgae (Hughes 1994). Such changes may be initiated by a combination of several factors such as climate change, over-fishing and human-derived pollution (Hughes et al. 2007), bleaching events (Diaz-Pulido and McCook 2002) and the increase of coral disease outbreaks (Rosenberg and Ben-Haim 2002; Sutherland et al. 2004). However, the extent to which macroalgae drive this shift by outcompeting scleractinian corals is still uncertain (Aronson and Precht 2006; Diaz-Pulido et al. 2009).

Studies investigating the interaction between macroalgae and scleractinian corals provide evidence for: (i) a decrease in fecundity and growth rates of scleractinian corals interacting with algae (Box and Mumby 2007; Birrell et al. 2008; Foster et al. 2008); (ii) negative effects of nutrients on coral-algal competition (Szmant 2002; Vermeij et al. 2010); (iii) negative microbial (Smith et al. 2006); and (iv) allelochemical effects of macroalgae on corals (Rasher and Hay 2010; Paul et al. 2011; Rasher et al. 2011).

In the southern Caribbean, the macroalgae *Halimeda*, *Cladophora*, *Lobophora* and *Dictyota* constitute common and increasingly abundant genera (Nugues and Bak 2008). These algae can impact scleractinian corals via lipid-soluble metabolites transferred via direct contact (Rasher and Hay 2010) or by triggering coral diseases (Nugues et al. 2004). Several recent studies also support that these algae can release dissolved organic carbon (DOC) which in turn affect microbial activity at the coral-algal interface and result in coral death (Wild et al. 2008; Barott et al. 2011).

Here, we describe results from an *in situ* interaction experiment on a coral reef adjacent to Curaçao, southern Caribbean, designed to characterize the species-specific impact of macroalgae on coral health. The observed patterns were discussed in the light of different processes potentially involved in coral-algal interactions. Studies evaluating specific algal characteristics and/or effects help to identify the mechanisms underlying coral-algal interactions and can provide further knowledge to understand the

causation and to improve the management of algal occurrences on Caribbean coral reefs.

Material and Methods

The experiment was carried out in December 2011 at Carmabi Buoy Zero (12°07'N, 69°57'W) on Curaçao, Southern Caribbean. The following macroalgae were used: *Dictyota pinnatifida*, *Lobophora variegata*, *Halimeda opuntia* and *Cladophora* spp. All macroalgae were collected at the experimental site at 5 - 12 m water depth using SCUBA. Back in the laboratory, they were cleaned from epiphytes and invertebrates. Aliquots of algae (70 g wet weight) were placed in small flexible mesh bags (made of nylon fishing nets, 1 cm mesh size) and deployed back at the experimental site onto healthy colonies of the coral *Montastraea faveolata* (n = 5 colonies per algal species) at 6 - 8 m water depth (Fig. 1). Due to the large mesh size and thin structure, nets were assumed not to interfere with flow and fluxes of algal exudates. Nets were attached to rock substrate adjacent to the coral tissue to avoid tissue damage. The health of the coral tissue interacting with the algae was monitored on a daily basis for the first 6 d and thereafter on days 10 and 15 by temporarily removing the nets during the monitoring. Coral condition was differentiated by using healthy, bleached and dead tissue as categories. Tissue which showed a change in coloration only was considered bleached, whereas the dead condition was characterized by decaying and sloughing of tissue. The surface area for each tissue category under the net was measured to the nearest centimeter using a transparent grid (1 cm^2) and expressed as a percentage of the total area overgrown by algae.

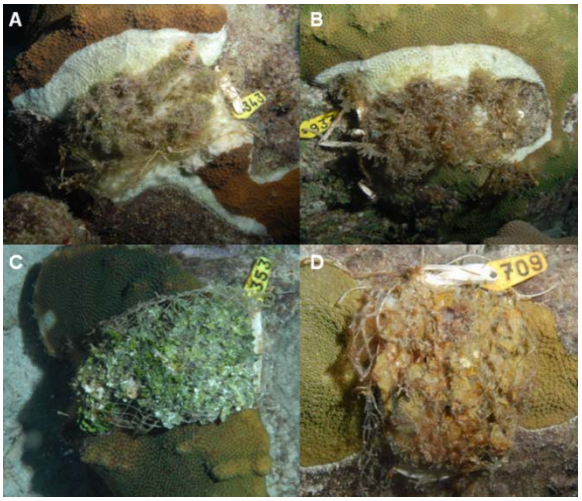


Figure 1: Nets with macroalgae *Cladophora* spp., *Dictyota pinnatifida*, *Halimeda opuntia* and *Lobophora variegata* (A-D) deployed on *Montastraea faveolata* colonies.

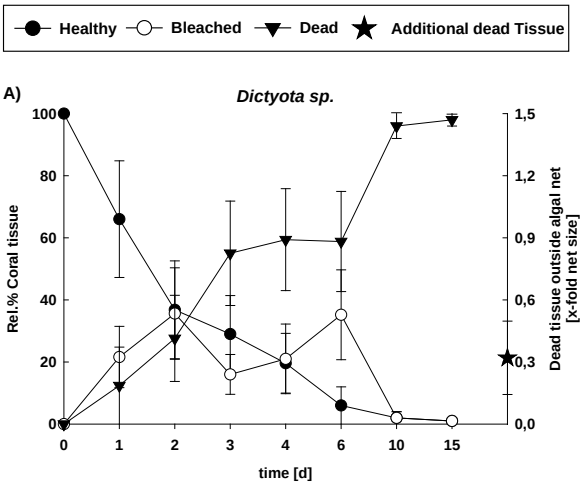
In cases of exceptional tissue degradation beyond the area of direct interaction, the surface areas of dead tissue were also estimated outside the nets using the transparent grid. In addition, the presence of coral derived mesenterial filaments was recorded on a semi-quantitative scale.

Earlier experimental work at the same study sites showed that strict controls (no algae and nets) did not produce any mortality (Nugues et al. 2004). During a previous *in situ* experiment, we deployed 20 identical empty mesh bags as procedural controls on *M. faveolata* corals on the identical site at Buoy Zero. Over 25 days, no or little (< 5 % dead tissue) coral mortality and bleaching on colonies in contact with empty nets only was observed (A. Wolf unpubl. data). Therefore, due to the similarity of the experiments, controls were not used during this experiment.

Repeated measure ANOVAs were used to assess differences among algal species in coral mortality over time. Lack of homogeneity in variances was corrected by *logit*-transformation of the data prior to analysis, as confirmed by Levene's test. All analysis used sphericity correction according to Greenhouse-Geisser and Huynh-Feldt-Lecoutre. To determine differences among specific treatments and time intervals, Tukey's post-hoc test (HSD) was applied.

Results

Rapid tissue deterioration was observed for corals interacting with *D. pinnatifida* and *Cladophora* spp. (Fig. 2A-B). After 3 d of contact, colonies showed 55 ± 17 % (means ± SE) and 71 ± 6 % dead tissue, respectively. In contrast, colonies interacting with *H. opuntia* and *L. variegata* displayed a much slower onset of coral mortality, yet a longer and more pronounced bleaching state, and both treatments caused less than 2 % dead tissue on day 3 (Fig. 2C-D). Over the next 7 d, tissue mortality reached close to



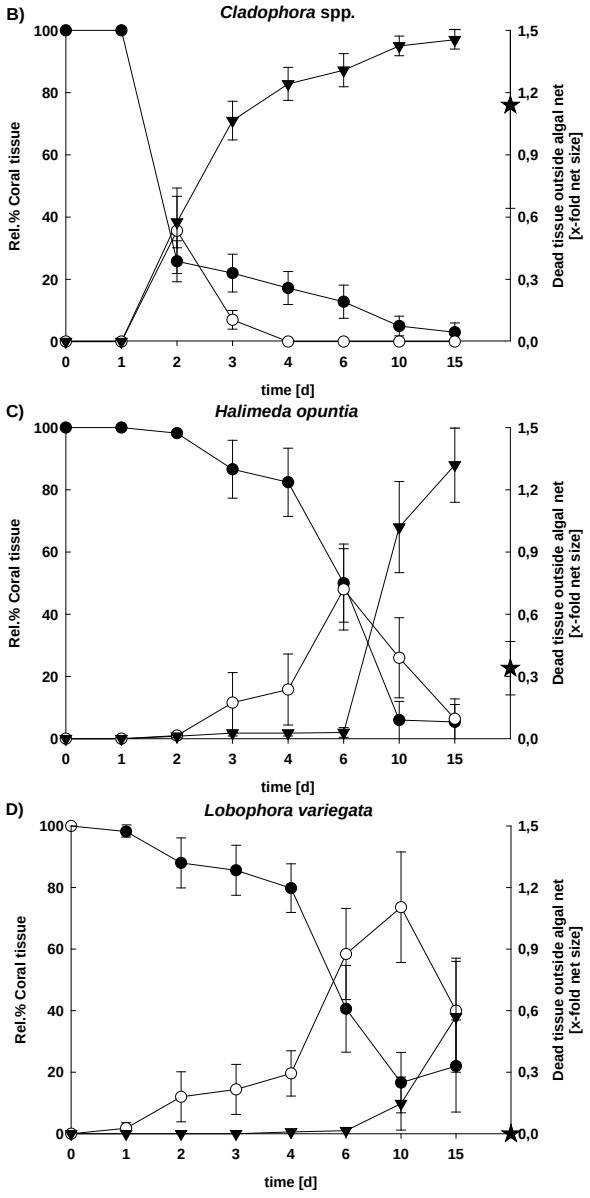


Figure 2: A-D) Changes in coral health categories (healthy, bleached and dead tissue; ± SE; n = 5) in the different algal treatments. Tissue loss outside areas of direct coral-algal interaction (2nd y-axis) is displayed as x-fold algal net size (± SE).

100 % in the *D. pinnatifida* and *Cladophora* spp. treatments and coral bleaching never reached more than 40 % in colonies contacting these algae. *Cladophora* spp. caused the most rapid increase in tissue death and also displayed the shortest period of bleaching (3 d in total) among all algae. In the *H. opuntia* and *L. variegata* treatments, colonies showed 48 ± 13 % and 58 ± 15% bleached tissue on day 6 and both treatments induced bleaching for a period of 13 days in total. After 10 and 15 days, the *H. opuntia* treatment reached coral mortality rates of 68 ± 15 % and 88 ± 12 %, respectively. In contrast, *L. variegata* treatments had the lowest rates of coral mortality, i.e. 10 ± 9 % and 38 ± 18 % on days 10 and 15, respectively. Furthermore, *L. variegata* induced coral bleaching up to 74 ± 18 % on day 10.

Coral mortality outside the area overgrown by the algal transplants was observed for all algae except *L. variegata* (Fig. 1 & 2). The highest value was observed for *Cladophora* spp., which caused a total coral mortality more than twice the size of the algal overgrown area. Mesenterial filaments (MF) were observed from the initiation of the interaction until coral death, with an increase in the number of filaments just prior to coral death (Table 1).

Table 1: Abundance of coral derived mesenterial filaments (MF) over time (day 1-15) for each algal treatment. (-) MF absent, (+) few (1-5) MF, (++) many (>5) MF present per replicate.

***M.faveolata* colony vs.**	**1**	**2**	**3**	**4**	**6**	**10**	**15**
D. pinnatifida	++	-	-	-	-	-	-
***Cladophora* spp.**	++	+	-	-	-	-	-
H. opuntia	+	+	+	+	++	+	-
L. variegata	+	+	+	+	+	+	+

Differences in coral mortality among algal treatments were statistically significant (Table 2). *D. pinnatifida* and *Cladophora* spp. caused significantly higher coral mortality compared to *H. opuntia* and *L. variegata* on days 2 to 6. On days 10 and 15, mortality was less in *L. variegata* compared to all other algal species.

Table 2: GLM Repeated measure ANOVA with algal species as between-factor variable and day as within-factor variable, followed by post-hoc multiple comparisons for each algal treatment.

	Df	**Type I SS**	**MS**	**F**	**P**
Algal species	3	622.3	207.4	28.0	<.0001
Day	6	674.4	112.4	68.1	<.0001[a]
Day*Algal species	18	204.7	11.4	6.9	<.0001[a]

[a] *P < 0.0001 with/without using the sphericity correction factors ε_1 (Greenhouse-Geisser) or ε_2 (Huynh-Feldt-Lecoutre)*

Tukey's post-hoc tests (HSD)

Algal species/Day	**0**	**1**	**2**	**3**	**4**	**6**	**10**	**15**
D. pinnatifida	A	A	A	A	A	A	A	A
***Cladophora* spp.**	A	A	A	A	A	A	A	A
H. opuntia	A	A	B	B	B	B	A	A
L. variegata	A	A	B	B	B	B	B	B

Logit-transformation of the data led to homogeneity of variances expect for day 1 (Levene's test p = 0.023)

Discussion

A number of physical and chemical factors can interact to cause the observed rapid decline of coral health. These include physical abrasion and shading through direct contact between algae and corals (Lirman 2001), mucus production and microbial growth on the coral-algal boundary layer, which in turn may be stimulated by primary or secondary metabolites released by algae (Wild et al. 2008; Rasher and Hay 2010).

An increase in coral mucus will stimulate bacterial growth on the coral surface mucus layer (Segel and Ducklow 1982). A healthy coral holobiont normally actively controls the growth rate of its associated microbes and favors bacteria with a protective role against pathogens by occupying entry niches, or with antibiotic functionality against pathogens (Ritchie 2006). Contact with algae could impair this functionality, resulting in coral pathologies and mortality.

Barrot et al. (2011) carried out direct interaction experiments with *Montastraea annularis*, using *H. opuntia*, *Dictyota bartayresiana* and coral turf assemblages. Coral-associated bacterial density and diversity increased significantly for *D. bartayresiana*, but not for *H. opuntia* interactions. Principal component analysis also showed close clustering for coral- and *H. opuntia* derived bacteria, compared to a distant clustering for *D. bartayresiana* derived bacteria. Comparably, our study showed strong initial differences in coral mortality between the *H. opuntia* and *D. pinnatifida* treatments, the latter one causing very rapid tissue degradation compared to *H. opuntia* treatments. Yet, at the end of the experiment, *H. opuntia* and *D. pinnatifida* produced similar effects on coral mortality. It is plausible that the rapid coral mortality produced by *Dictyota* sp. was caused by the high dissimilarity in bacterial clades between the macroalgae and the corals. At present, the role of algal-derived bacteria in coral-algal interactions is unclear.

Rasher and Hay (2010) found macroalgae-derived secondary metabolites to cause bleaching and mortality in scleractinian corals. In their 20-d assays, lipid-soluble extracts, either excretable or present on the algae's exterior, of *D. bartayresiana*, *H. opuntia* and *L. variegata* also caused a significant, but comparable decrease in photosynthetic activity in *Porites porites*, underlining their potential to poison corals upon direct contact. Interestingly, direct macroalgal contact revealed differences between *D. bartayresiana* and the other two macroalgae in their abilities to suppress photosynthetic activity, lending support for additional, e.g. primary metabolite-induced effects such as DOC release. During our 15-d experiment, *L. variegata* had a lower impact on coral mortality compared with *D. pinnatifida* and *H. opuntia* and *D. pinnatifida* showed a strong initiation of coral mortality. These differences could also be associated with differing release speed of secondary metabolites among algae and/or different allelopathic activity of the same alga in time and space depending on e.g. grazing pressure or nutrients (Paul and Fenical 1986). Under laboratory conditions, Smith et al. (2006) proved that algal-derived, primary metabolites such as dissolved organic carbon can lead to coral mortality. DOC can increase microbial growth by an order of magnitude, suggesting coral-associated microbes to be carbon-limited (Kline et al. 2006). A disrupted coral–microbe relationship due to organic carbon loading (e.g. glucose) could thus directly cause coral mortality by over-stimulating growth of coral mucus-associated microbes. However, these laboratory-based results have yet to be demonstrated *in situ*. The lack of comparative studies on DOC release rates of the investigated macroalgae also precludes comparisons between DOC rates and algal-generated coral mortality. Much of the mucus-associated carbon remains in a refractory form which is not available for microbial growth (Herndl and Velimirov 1986; Wild et al. 2004). *H. opuntia* associated DOC was found to consist of 59 % carbohydrates (Haas and Wild 2010). Haas et al. (2011) showed that *H. opuntia* is releasing high-quality DOC which can be efficiently metabolized by microbes. Therefore even small amounts of DOC could alter microbes in the surface mucus layer (SML) and subsequent coral health. The increase of algal induced DOC in the form of simple sugars may enable microbes to break down more complex and previously unavailable carbon sources via co-metabolism (Dinsdale et al. 2008).

Although anaerobic conditions occur during coral-algal interactions, hypoxia, considered a fluctuating stressor by Wangpraseurt et al. (2012), is unlikely to cause coral mortality by itself. *H. opuntia* was found to rarely and reversibly (after withdrawal) induce hypoxia at the surface boundary layer upon direct contact with *M. annularis*, compared to a more pronounced and partly irreversible hypoxic effect by *D. bartayresiana* (Barott et al. 2009; 2011). Under aerobic conditions, increased DOC availability can fuel bacterial growth by enabling rapid aerobic respiration. A shift towards prolonged anaerobic conditions may facilitate additional fermentative processes by anaerobes. Both metabolic pathways can be fueled by excessive DOC release, thus leading to the observed differences in coral mortality.

Organic carbon treatments can cause pathologies similar to those reported for band diseases, including rapid sloughing of coral tissue (Kuntz et al. 2005). Tissue death occurring outside the area overgrown by our algal transplants was similar to white plague type II like disease signs and lacked any state of bleaching prior to mortality. Effects of algal metabolites have been shown to be restricted to the diffuse boundary layer and to extend only within a few millimeters away from the coral-algal interface in natural conditions (Wangpraseurt et al. 2012). In addition, Pantos et al. (2003) demonstrated a whole-animal response to disease with shifts in the microbial community of even healthy looking tissue. Although

metabolites from macroalgae may have more extensive effects, it is likely that coral mortality beyond the area of algal overgrowth, though initially triggered by algal exudates, was mediated by microbes.

Together our findings suggest that macroalgae cause differential mortality in scleractinian corals. These differences are likely to be associated with a variety of algal characteristics and to depend on the magnitude by which these properties interact to eventually initiate a cascade of microbial processes leading to the observed differences.

Acknowledgements

Authors thank the CARMABI foundation and personnel for continuous technical support. This research has been funded by the Leibniz Center for Tropical Marine Ecology (ZMT) Bremen.

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