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Complex ecological associations: competition and facilitation in a sponge–algal interaction

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ABSTRACT: Over the past few decades, Caribbean coral reefs have undergone a phase shift from coral-dominated to algal-dominated communities due to several factors, including increased input of anthropogenic nutrients. With the decline in coral cover, sponges have also become more dominant members of Caribbean coral reef communities. Increased algal and sponge dominance on Caribbean reefs has led to an increase in the frequency of interaction between these 2 groups. This study used a factorial design to assess the independent and interactive effects of contact and elevated nutrient levels on 2 common members of these communities, the sponge *Aplysina cauliformis*, and the macroalga *Microdictyon marinum*. Algal contact had a significant negative physiological effect on *A. cauliformis*, affecting both the host sponge and its cyanobacterial symbionts. While elevated nutrient levels had some positive effects on the sponge photosymbionts, this only occurred in the absence of algal contact or a shading/abrasion control, and elevated nutrient levels had a negative effect on the sponge holobiont. In contrast, *M. marinum* responded positively to experimentally enhanced nutrient levels and to sponge contact under ambient nutrient regimes, but was not affected by sponge contact under elevated nutrient concentrations. Stable isotope enrichment experiments showed that the alga's positive response to sponge contact was associated with nitrogen transfer from the sponge over the course of the experiment. Thus, while *A. cauliformis* facilitates increased productivity in *M. marinum*, algal contact competitively inhibits sponge condition.

KEY WORDS: *Aplysina cauliformis* · *Microdictyon marinum* · Sponge · Nutrient enrichment · Stable isotope · Macroalga · Coral reef · Cyanobacteria · Symbiont · Caribbean

INTRODUCTION

Predation and pathogenesis are critical ecological processes shaping coral reef communities; however, competition and facilitation are becoming more widely studied as important forces in coral reef structure and function (Bruno & Bertness 2001, Connell et al. 2004). Most macroalgal–coral interactions have been classified as competitive, with the algae negatively affecting the coral through shading, abrasion, overgrowth, or allelopathy (McCook et al. 2001). Even though competition appears to play a significant role in structuring reef communities, there is increasing evidence that facilitation is also important. Facilitation has been documented in many marine habitats (Ellison et al. 1996, Bruno et al. 2003), including coral reefs (Hill 1998, Bruno et al. 2003, Gochfeld 2010). With the continuing decline of coral cover in the Caribbean leading to an increased dominance of algae (Hughes 1994) and sponges (Díaz &

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Rützler 2001, Bell 2008, Loh & Pawlik 2014) on these reefs, it is important to understand the consequences of increasingly frequent interactions between macroalgae and sponges.

Exogenous nutrients are essential to coral reefs, although they can also act as stressors and potential causes of phase shifts in reef communities (Littler & Littler 1984, McCook 1999). For example, nitrogen is often a limiting resource on pristine coral reefs, with typical concentrations below 1 µM (Lapointe 1997). However, in some areas, anthropogenic inputs of nutrients through groundwater seepage and runoff can elevate nitrogen concentrations above normal ranges, resulting in dramatic changes to coral reef community structure (Lapointe 1997, McCook 1999); notably, the increase in algal cover observed throughout the Caribbean (Lapointe et al. 2004). Natural sources of nutrients, such as tidal bores, can bring up nutrient-rich deep water to shallow reefs, providing periodic pulses of high nitrate concentrations to coral reef communities (Leichter et al. 2003). Additionally, nitrogen fixation makes a significant contribution to the amount of 'new' nitrogen available to coral reefs. This process is exclusively prokaryotic, and occurs in both free living and symbiotic microbial communities (Fiore et al. 2010). Marine sponges harbor nitrifying and nitrogen fixing microbial communities that contribute a large part of the available nitrogen on coral reefs (Fiore et al. 2010). While coral reefs are able to deal with periodic increases in exogenous nutrients, with human populations in coastal areas continuing to increase at a rapid rate, understanding anthropogenic influences of elevated nutrients on coral reef communities is increasingly important.

Marine sponges are an important functional group that represents much of the species diversity found in coral reef communities (Díaz & Rützler 2001, Pawlik 2011). These organisms occupy many niches on coral reefs; they provide habitat for other reef organisms, stabilize substrata, and are sources of food (Bell 2008). Sponges harbor diverse microbial communities and are therefore important sources of primary productivity (Erwin & Thacker 2007) and essential contributors to carbon and nitrogen cycling on coral reefs (Taylor et al. 2007, Southwell et al. 2008, Fiore et al. 2010, Maldonado et al. 2012, Thacker & Freeman 2012). These diverse microbial communities are essential for absorption and processing of many inorganic sources of carbon and nitrogen, and transferring them to the host sponge (Thacker & Freeman 2012, Freeman et al. 2013). However, in some cases, sponges and their associated microbial communities can transfer these resources to other organisms on the reef (Ellison et al. 1996, Davy et al. 2002, Pile et al. 2003, de Goeij et al. 2013). Sponges also play an important role in large scale carbon cycling on reefs through benthic–pelagic coupling of these resources (Lesser 2006), supporting the increase of several benthic functional groups, such as macroalgae (Maldonado et al. 2012). This ability of sponges to influence adjacent reef organisms results in the formation of many unique interactions as both competitors (Engel & Pawlik 2005, González-Rivero et al. 2011) and facilitators (Hill 1998, Davy et al. 2002). Understanding these interactions has become increasingly important, as Caribbean coral reefs continue to shift away from coral-dominated communities towards communities dominated by sponges and macroalgae (Nyström et al. 2000, Norström et al. 2009). This study examined interactions between *Aplysina cauliformis,* a common branching sponge species on Caribbean reefs, and *Microdictyon marinum*, a green alga that occupies large areas of substratum on reefs in the Bahamas during the summer months, and how this interaction changes under conditions of elevated anthropogenic nutrient concentrations. This study further used stable isotope enrichment experiments to investigate the carbon and nitrogen dynamics in these organisms and characterize potential mechanisms that contribute to this sponge–algal interaction.

MATERIALS AND METHODS

Study sites

This study was conducted at the Perry Institute for Marine Science on Lee Stocking Island (LSI), Exuma Cays, Bahamas, during May and June 2009. Field experiments and surveys were conducted at Big Point (23° 47.30′ N, 76° 08.12′ W) and Rainbow Gardens (23° 47.78′ N, 76° 08.79′ W), 2 shallow reef sites (3 to 5 m depth) near LSI. All sponge and algal samples were collected from North Norman's reef (23° 47.39′ N, 76° 08.27′ W), 1 km equidistant between Big Point and Rainbow Gardens, from a depth of approximately 5 m.

Field surveys

To measure the frequency of interaction between the sponge *Aplysina cauliformis* and the green alga *Microdictyon marinum*, surveys along 12 band transects (10 × 2 m) were conducted at Big Point

and Rainbow Gardens in May 2009 (n = 6 site^{-1}). Along each transect, all sponges were counted, as well as discrete clumps of *M. marinum* and every organism that was in contact with this alga. The visible outcomes (i.e. abrasion, pigment change, tissue necrosis) of each contact between *A. cauliformis* and *M. marinum* were also recorded. Abrasion was characterized as physical damage to the sponge ectosome, which could also result in altered pigmentation in sponge tissue. However, 'darker pigmentation' was used to describe sponges with altered pigmentation but no visible ectosomal damage. Additionally, percent cover of *M. marinum* was quantified on these transects using a point-intercept method by recording the species under the transect line every 10 cm.

Contact experiment

To test whether contact elicits a measureable physiological effect on the sponge or the alga, a field contact experiment was performed. *A. cauliformis* and *M. marinum* were collected and maintained in the laboratory at LSI in individual containers with flow-through seawater for 2 d prior to the field experiment. The sponges were cut into 10 cm pieces and the algae were separated into clumps of approximately 10 × 10 cm. Initial weights, measurements, and photographs were taken for both sponges and algae, and they were then randomly assigned to 1 of 4 treatment groups (n = 10 treatment^{-1}): (1) sponge alone, (2) algae alone, (3) sponge–algae in contact, and (4) sponge with a shade/abrasion control. The shade/abrasion control was composed of 3 layers of plastic-coated window screen, which resembled the mesh-like morphology and texture of the algae. This screen provided the shading equivalent of an average piece of algae in the sponge–algae contact treatment, as determined by light level measurements with a light meter (LI-COR®) above and underneath algae pieces. In addition to providing shade, contact between the sponge and window screen simulated potential abrasion by the algae.

Each algal or sponge individual, or pair, was attached via cable ties to a 20 × 20 cm plastic rack (Gochfeld et al. 2012a). These racks were attached to the substrate at Big Point, at a depth of approximately 5 m, and left in the field for 4 wk. At the end of the experiment, racks were placed in re-sealable plastic bags filled with seawater and returned to the lab at LSI, where sponges and algae were removed from the racks, weighed, and measured. Small pieces of each sponge (0.5 cm cross-sections) were collected for measurements of chlorophyll *a* (chl *a*) concentration and cyanobacterial symbiont density as measures of symbiont condition, and total protein concentration as a measure of the sponge holobiont (sponge host, its symbiotic cyanobacteria and the sponge-associated microbial community) condition. In addition, algae were collected for chl *a* concentration measurements. All samples were either frozen or preserved (see below) for transport to the University of Mississippi (UM) for further analysis.

Nutrient experiment

To determine the effect of elevated nutrient levels on the interaction between *A. cauliformis* and *M. marinum*, we performed a factorial designed field experiment that investigated the effects of contact and nutrients on algae and sponges in isolation and together. *A. cauliformis* and *M. marinum* were collected and brought back to the lab on LSI, where they were maintained as described above. Initial weights, measurements, and photographs were taken. The next day, organisms were randomly assigned to 12 treatments (n = 10 replicates for each treatment). Each of the 4 treatments used in the aforementioned contact experiment (i.e. sponge alone, algae alone, sponge–algae contact, and the shade/abrasion control) was exposed to 3 nutrient doses. Nutrients were delivered using 10 g of 14-14-14 (N-P-K) Osmocote® slow release fertilizer (Scotts) in packets made of window screen (Thacker et al. 2001, Gochfeld et al. 2012a). Nutrient dose was regulated by varying the distance of samples attached to plastic racks from the nutrient pack. The high dose, at 5 cm from the nutrient pack, was comparable to that used in Gochfeld et al. (2012a); the medium and low doses were dilutions at 25 and 50 cm from the nutrient pack, respectively. Nutrient packs were replaced every 7 d for 4 wk to maintain the approximate nutrient dose over the course of the experiment (Gochfeld et al. 2012a). After 4 wk, the racks were collected and returned to the lab, where cyanobacterial symbiont population condition was assessed via Pulse Amplitude Modulated (PAM) fluorometry (Diving-PAM; Walz) measurements (see 'Fluorescent Yield' section below) on dark-adapted organisms on the night of collection. Sponges and algae were then removed from the racks, weighed, measured, and photographed. As in the contact experiment, sponges and algae were processed for further analysis at UM.

Allelopathy experiment

To determine whether algal allelopathy played a role in the sponge–algal interaction, we performed an assay similar to that described in Thacker et al. (1998). *M. marinum* was collected and the volume to wet weight ratio was calculated using displacement volume. The alga was then lyophilized and extracted in 1:1 dichloromethane:methanol. The extract was added at natural volumetric concentration to 50 ml of a 5% molten agar solution. An equivalent amount of the carrier solvent was added to the control agar. These solutions were poured into plastic molds backed with window screen to form 6 treatment strips and 6 control strips measuring 2 × 6 cm. The agar strips were allowed to harden onto the window screen, and were attached with cable ties to *A. cauliformis* branches on the reef. Each *A. cauliformis* branch (n = 6) had 1 control and 1 treated gel strip spaced at least 10 cm apart. After 1 wk, the *A. cauliformis* branches were collected and brought back to the lab. That evening, strips were removed, PAM readings were taken on the sponge tissue under each gel (Pawlik et al. 2007), and the samples were wrapped in foil and preserved for analysis of chl *a* at UM.

Chl *a* concentration

To assess photosynthetic potential of the sponges' photosymbionts, chl *a* was measured from frozen foil-wrapped samples using methods described in Erwin & Thacker (2007), except that sponge and algae pieces in this study were lyophilized prior to extraction. Briefly, 0.25 g wet weight of *A. cauliformis* or *M. marinum* was placed in a foil-wrapped glass vial with 10 ml of 90% acetone for 18 h at 4°C. Extracts were then transferred to quartz cuvettes and the absorbance of each extract was quantified at 750, 664, 647, and 630 nm on an Agilent 8453 spectrometer. Chl *a* concentrations were calculated using formulas from Parsons et al. (1984) and standardized to the mass of the extracted sponge or algae (µg chl *a* mg^{-1} sponge or algal tissue).

Fluorescent yield

PAM fluorometry was used to measure photosynthetic efficiency of the algae and the sponge's photosymbionts (Gochfeld et al. 2012a). The Diving-PAM provides the organism with an actinic flash of light and measures the maximum fluorescent yield from the organism's photo-system II. This is determined by subtracting the minimum fluorescence (F_0) from the maximum fluorescence (F_m) to calculate variable fluorescence (F_v), and subsequently, by dividing this value by F_m to obtain the maximum quantum yield (F_v/F_m) (Hoegh-Guldberg 1999, Fitt et al. 2001). Three measurements were taken at a standardized distance from different locations on the sample, and the 3 measurements were then averaged (Gochfeld et al. 2012a). The samples were measured in seawater raceways at least 1 h after dark in order to maximize the ability of the photochemical pathways to absorb light energy (Fitt et al. 2001). PAM measurements were collected after samples were retrieved from the field at the end of the nutrient and allelopathy experiments.

Cyanobacterial symbiont density

Symbiont density was quantified using the methods outlined in Freeman & Thacker (2011). Sponges were preserved in 4% paraformaldehyde in 2 ml cryovials at 4°C for 24 h. The paraformaldehyde was then removed and replaced with a 70% ethanol solution. The samples were further dehydrated and embedded in paraffin wax. Cross sections (20 µm) were cut and mounted onto glass slides. Each sample was viewed at 1000× magnification under oil immersion using an epifluorescence microscope. Ten photographs of each sample were taken on haphazardly chosen areas of the sponge sections. The number of cyanobacterial cells was counted using the 'analyze particles' feature in ImageJ software (NIH). For each image, total cyanobacterial cell number was counted, and values from all 10 images were averaged to calculate the mean number of cells in a viewing area of 2886 μm^2.

Protein concentration

Protein content was measured as a proxy for holobiont health in lyophilized sponge samples using the Bradford (1976) assay. Briefly, 5 ml of 1 M NaOH was used to extract 10 mg of lyophilized sponge tissue for 18 h, after which 100 µl of each sample was added to a test tube with 5 ml of Quick Start™ Bradford Dye Reagent (Bio-Rad). The absorbance of each sample was measured at 595 nm using a BioPhotometer V.032 (Eppendorf) and then plotted against a standard curve developed from a bovine serum albumin

sample. Protein concentrations were then standardized to the dry weight of each sponge sample in order to calculate µg protein mg^{-1} sponge tissue.

Sponge and algae growth

Sponge and algal weights were recorded initially and at the end of each experiment. Sponges were briefly blotted with a paper towel to remove excess water before weighing. Algae pieces were not blotted due to their brittle nature, and excess water was removed through gentle shaking for a few seconds. Percent change in weight was calculated using the following formula:

$$[(W_f - W_i) / W_i] \times 100$$

where W_f = final weight and W_i = initial weight of the individual samples.

NO_3^-/HCO_3^- and NH_4^+ experiments

To investigate uptake and transfer of carbon and nitrogen sources, *A. cauliformis* and *M. marinum* were collected and acclimated in the laboratory at LSI in separate containers with flow-through seawater for 1 d. Five individual sponges and 5 pieces of algae were collected and immediately frozen (t_i). An additional 37 sponges were incubated in a solution containing 1 mg l^{-1} 98% $Na^{15}NO_3$ and 1 g l^{-1} 98% $Na^{13}HCO_3$ or 0.1 mg l^{-1} of $^{15}NH_4^+$ tracers for 6 h. After incubation, 5 sponges were collected and frozen (t_0), and 32 sponges were placed into individual containers with flowing seawater for 2 h to rinse out all non-assimilated tracers from the sponge tissue. After this rinse period, sponges were randomly assigned to 2 treatment groups. One group had pieces of algae attached to them with a cable tie, and the other group had pieces of algae placed at 15 and 30 cm from the sponge in its tank. After 12 (t_{12}) and 24 (t_{24}) h, samples from 8 tanks of each treatment group were collected, and sponges and algae were frozen for further analysis.

Analysis of stable isotopes

For each *A. cauliformis* individual, sponge and symbiont cells were separated from the sponge holobiont using methods from Freeman et al. (2013). Briefly, frozen sponges were chopped into small pieces and soaked in an artificial seawater buffer with EDTA at 4°C. Sponges were then homogenized and filtered under low vacuum pressure. The filtrate went through several centrifugation spins that separated out the larger eukaryotic cells from the filtrate at slower speeds (430 to 670 × *g*), and bacterial cells at higher speeds (2100 to 5200 × *g*). Sponge and bacterial cell fraction pellets, and the algal samples, were lyophilized and acidified with 6 M HCl. After acidification, the samples were dried and then weighed into silver capsules for isotopic analysis. Stable isotope analysis was conducted at the Geophysical Laboratory at the Carnegie Institution of Washington (Washington, DC) using a Thermo Delta V Plus isotope ratio mass spectrometer coupled to a Carlo-Erba NC2500 elemental analyzer via a Conflo III open-split interface.

Data analysis

The observed number of *M. marinum* contacts with sponge and coral species was compared to the expected number of *M. marinum* contacts with *A. cauliformis*, based on *A. cauliformis* abundance, using a chi-square analysis. For the contact and nutrient experiments, all response variables were analyzed using a multivariate analysis of variance (MANOVA) in each experiment, followed by univariate analyses of variance (ANOVA) or unpaired *t*-tests as appropriate (see Table 1). Bonferroni corrections were applied to each p-value in the MANOVA to account for the effect of multiple comparisons. Percent change in sponge and alga weights were arcsine transformed before analysis. For the nutrient experiment, sponge cyanobacterial density and both sponge and algal fluorescent yields were omitted from the MANOVA because each metric had a sample size of less than 50% of the other metrics used to estimate sponge condition (i.e. chl *a*, % change in weight, soluble protein concentration); this reduction was due to equipment malfunction. These response variables were evaluated using separate univariate 2-way ANOVAs, and were correlated with the other response variables in the nutrient experiment using 2-tailed Pearson correlations. Within the sponge-alone treatment in the nutrient experiment, chl *a* concentrations were analyzed for the effects of nutrient dose using a univariate 1-way ANOVA. In the allelopathy experiment, chl *a* concentrations and fluorescent yield measurements in *A. cauliformis* were analyzed using paired *t*-tests. For each experiment, Tukey's HSD post-hoc tests were used to detect differences.

Initial values of ^{15}N and ^{13}C for bacterial and sponge cell fractions were compared using a 1-way ANOVA. To test whether sponges became significantly enriched with the ^{15}N and ^{13}C tracers in both experiments, initial isotopic ratios were compared to ratios of samples incubated in the tracer solution using a 2-way ANOVA examining differences in cell fractions, expressed as $\delta^{13}C$ and $\delta^{15}N$, time (t_i vs. t_0) and their interaction. Algal enrichment was analyzed using a 1-way ANOVA for each treatment, comparing experimental ^{15}N and ^{13}C values with initial values in units of $\delta^{13}C$ and $\delta^{15}N$. Tukey's post-hoc tests were used to detect differences in each experiment. All values shown represent mean ±1 SE.

RESULTS

Field surveys

Percent cover of *Microdictyon marinum* was 24.0 ± 7.7% at Big Point. At Rainbow Gardens, *M. marinum* only occurred on 1 transect, where percent cover was 1.2 ± 1.2%. Sponge diversity at the 2 sites combined included at least 22 species. Abundance of these species varied between sites, and *Aplysina cauliformis* comprised 52.0 ± 3.7% (393 individuals) of all sponges at Big Point and 12.0 ± 3.2% (182 individuals) of all sponges at Rainbow Gardens. Both sites combined constituted 506 discrete patches of *M. marinum,* of which 214 were in contact with a total of 20 species of corals and sponges (Fig. 1). Most of these contacts (92%) occurred at Big Point, where abundance and percent cover of *M. marinum* was higher. Of the contacts at Big Point, 37% were with *A. cauliformis*. At Rainbow Gardens, where *M. marinum* abundance was low, 38% of all algal contacts were with *A. cauliformis*. In these surveys, *A. cauliformis* had a significantly higher number of contacts with *M. marinum* compared to all other sponge species surveyed (χ^2, df = 1, $p < 0.0001$) and compared to all coral species (χ^2, df = 1, $p < 0.0001$) except for *Orbicella annularis* (χ^2, df = 1, p = 0.78; Fig. 1). The effects of these *A. cauliformis–M. marinum* contacts varied, with 70% resulting in darker sponge pigmentation at the point of contact, 7% resulting in abrasion damage to the sponge, and 23% showing no visible effects on the sponge.

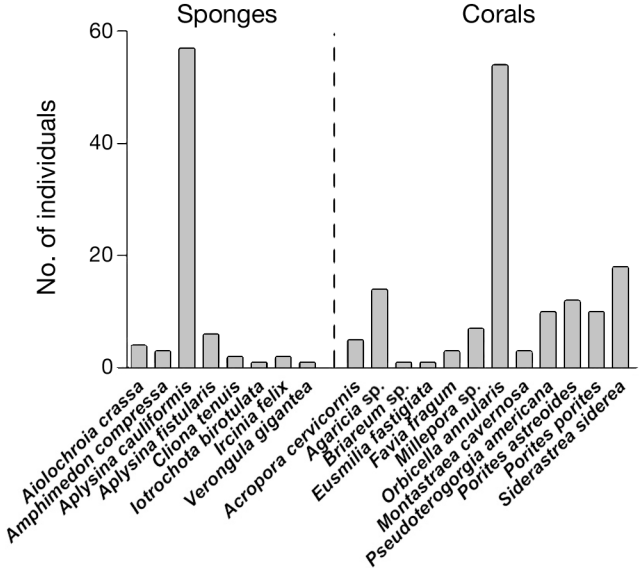

Fig. 1. Number of individuals in contact with *Microdictyon marinum* for each sponge and coral species from surveys at Big Point and Rainbow Gardens

Contact experiment

MANOVA indicated that contact treatment (sponge alone, sponge–algal contact, shade/abrasion control) had a significant effect on overall sponge condition in the contact experiment (Wilks' lambda, df = 8,34, F = 3.934, p = 0.002; Table 1). The MANOVA identified a trend towards a significant effect of sponge contact treatment on algal health (Wilks' lambda, df = 2,11, F = 3.369, p = 0.07; Table 1), and given the limited sample size and its impact on power, we felt this trend warranted further examination. Upon further exploration, a univariate ANOVA indicated a significant impact of contact treatment on algal chl *a* concentration (see 'Chl *a* concentration' section below).

Nutrient experiment

MANOVA results for the nutrient experiment showed a significant effect of algal contact treatment (Wilks' lambda, df = 6,142, F = 4.43, $p < 0.001$; Table 1) and nutrient dose (Wilks' lambda, df = 6,142, F = 6.835, $p < 0.001$; Table 1) on sponge condition, but there were no interactive effects of the 2 variables (Wilks' lambda, df = 12,188, F = 1.489, p = 0.131; Table 1). MANOVA on algal responses indicated a significant effect of nutrient dose (Wilks' lambda, df = 2,48, F = 5.066, p = 0.001; Table 1), but no significant effect of sponge contact (Wilks' lambda, df = 2,48, F = 1.902, p = 0.16; Table 1) or interactive effects between the independent variables (Wilks' lambda, df = 4,96, F = 1.354, p = 0.256; Table 1).

Table 1. MANOVA and ANOVA results for each treatment factor in all experiments. Factors include: algal contact (algal contact, shade control and sponge-alone treatments), nutrient dose (3 distances from the nutrient pack), sponge contact (sponge contact or alga-alone treatments), time (initial, t_i and enriched, t_0 time points), fraction (bacterial and sponge fractions), 15 cm (algae placed 15 cm from sponge in enrichment experiments), and 30 cm (algae placed 30 cm from sponge in enrichment experiments). Positive (facilitation) effects are underlined

Dataset/factors	Statistical test	*F*	df	p	Fig.
CONTACT EXPERIMENT					
Sponge MANOVA	Wilks' Lambda	3.934	8,34	0.002	
Sponge chl *a*	1-way ANOVA	3.605	2	0.04	2A
Cyanobacterial density	1-way ANOVA	10.473	2	0.001	2B
Soluble protein	1-way ANOVA	3.247	2	0.06	
Sponge growth	1-way ANOVA	0.529	2	0.597	
Algal MANOVA					
Sponge contact	Wilks' lambda	3.369	2,11	0.07	
Algal chl *a*	Unpaired *t*-test	7.225	1	0.02	
Algal growth	Unpaired *t*-test	0.222	1	0.48	
NUTRIENT EXPERIMENT					
Sponge MANOVA					
Treatment	Wilks' lambda	4.43	6,142	<0.001	
Nutrient dose	Wilks' lambda	6.835	6,142	<0.001	
Treatment × Nutrient dose	Wilks' lambda	1.489	12,188	0.131	
Sponge chl *a*	2-way ANOVA				
Algal contact		7.456	2	0.001	3A
Nutrient dose		0.229	2	0.796	
Algal contact × Nutrient dose		2.447	4	0.05	
Sponge fluorescent yield	2-way ANOVA				
Algal contact		10.9	2	0.0001	3B
Nutrient dose		0.46	2	0.63	
Algal contact × Nutrient dose		0.49	4	0.74	
Cyanobacterial density	2-way ANOVA				
Algal contact		4.99	2	0.01	3C
Nutrient dose		0.63	2	0.54	
Algal contact × Nutrient dose		0.26	4	0.9	
Soluble protein	2-way ANOVA				
Algal contact		8.263	2	0.001	3D
Nutrient dose		21.473	2	<0.001	
Algal contact × Nutrient dose		0.915	4	0.46	
Sponge growth	2-way ANOVA				
Algal contact		0.1	2	0.905	
Nutrient dose		0.005	2	0.54	
Algal contact × Nutrient dose		0.592	4	0.67	
Algal MANOVA					
Treatment	Wilks' lambda	1.902	2,48	0.16	
Nutrient dose	Wilks' lambda	5.066	4,96	0.001	
Treatment × Nutrient dose	Wilks' lambda	1.354	4,96	0.256	

Dataset/factors	Statistical test	*F*	df	p	Fig.
Algal chl *a*	2-way ANOVA				
Sponge contact		0.297	1	0.59	4
Nutrient dose		10.09	2	<0.001	
Sponge contact × Nutrient dose		0.057	2	0.945	
Algal fluorescent yield	2-way ANOVA				
Sponge contact		0.63	1	0.44	
Nutrient dose		0.054	2	0.95	
Sponge contact × Nutrient dose		0.79	2	0.47	
Algal growth	2-way ANOVA				
Sponge contact		3.318	1	0.07	
Nutrient dose		0.646	2	0.528	
Sponge contact × Nutrient dose		2.688	2	0.078	
NO_3^-/HCO_3^- EXPERIMENT					
Sponge enrichment: nitrogen	2-way ANOVA				
Time		8.89	1	0.009	5A
Fraction		1.15	1	0.3	
Time × Fraction		2.96	2	0.1	
Sponge enrichment: carbon	2-way ANOVA				
Time		209.5	1	<0.0001	5A
Fraction		25.2	1	0.0001	
Time × Fraction		28.2	2	<0.0001	
Algal enrichment: nitrogen					
Sponge contact	1-way ANOVA	0.65	1	0.65	
15 cm	1-way ANOVA	1.88	1	0.18	
30 cm	1-way ANOVA	0.29	1	0.75	
Algal enrichment: carbon					
Sponge contact	1-way ANOVA	2.14	1	0.14	
15 cm	1-way ANOVA	3.99	1	0.04	
30 cm	1-way ANOVA	3.95	1	0.04	
NH_4^+ EXPERIMENT					
Sponge enrichment: nitrogen	2-way ANOVA				
Time		7.52	1	0.01	5B
Fraction		0.14	1	0.71	
Time × Fraction		0.61	2	0.45	
Alg al enrichment					
Sponge contact	1-way ANOVA	6.14	1	0.01	6
15 cm	1-way ANOVA	0.45	1	0.64	
30 cm	1-way ANOVA	35.11	1	0.0001	

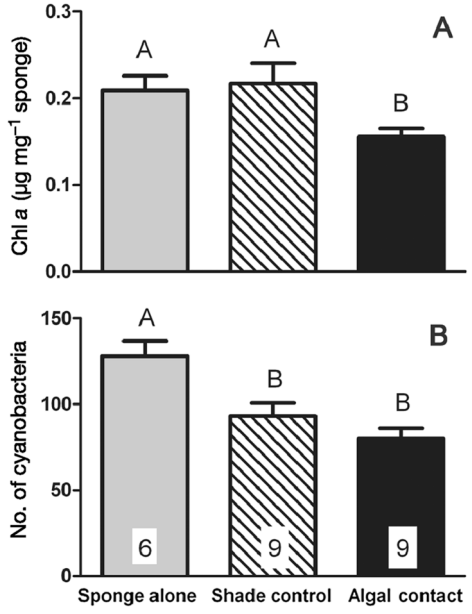

Fig. 2. *Aplysina cauliformis*. Mean (± SE) (A) chl *a* concentration and (B) cyanobacterial symbiont density after 28 d in contact with algae or a shade/abrasion control in the contact experiment. Histograms with different letter groups are significantly different by ANOVA. Numbers within the histograms represent adjusted sample sizes

Chl *a* concentration

In the contact experiment, chl *a* concentrations in *A. cauliformis* were significantly affected by algal contact (Table 1, Fig. 2A). Contact with *M. marinum* resulted in significantly lower chl *a* concentrations in *A. cauliformis* compared to the other 2 treatments (Tukey's HSD test, $p < 0.05$). Chl *a* concentrations in *M. marinum* in the contact experiment were significantly affected by sponge contact, resulting in a higher chl *a* concentration (0.026 ± 0.002 µg chl *a* mg^{-1} algal tissue) than in the algae alone (0.021 ± 0.0009 µg chl *a* mg^{-1} algal tissue; unpaired *t*-test, df = 1, $F = 7.22$, $p = 0.02$).

In the nutrient experiment, chl *a* concentrations in *A. cauliformis* were also significantly affected by algal contact (Table 1, Fig. 3A). Sponges in the algal contact and shading/abrasion control treatments had significantly lower chl *a* concentrations than did the sponge alone (Tukey's HSD test, $p < 0.05$). Within the sponge-alone treatment, there was a significant effect of nutrient dose (1-way ANOVA, df = 2, $F = 4.16$, $p = 0.027$; Fig. 3A), and chl *a* content increased significantly as the nutrient dose increased (Tukey's HSD test, $p < 0.05$). Algal chl *a* concentrations in the nutrient experiment also increased significantly with increasing nutrient dose (Table 1, Fig. 4).

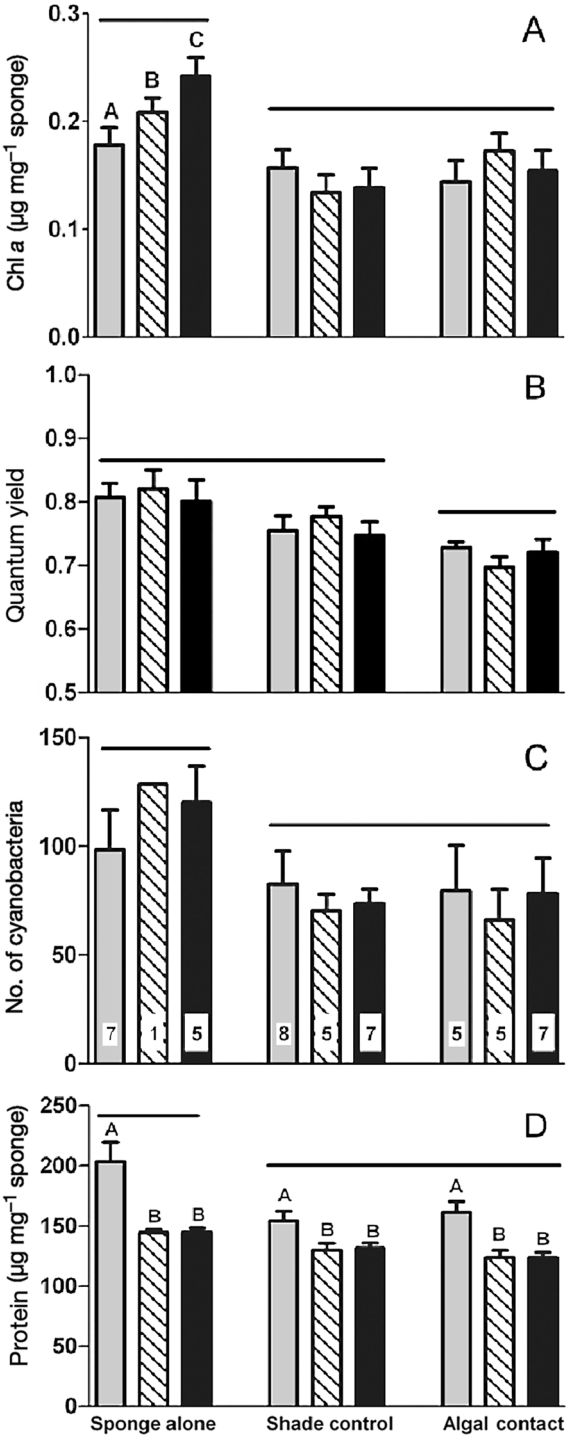

Fig. 3. *Aplysina cauliformis*. Mean (± SE) (A) chl *a* concentration, (B) quantum yield, (C) cyanobacterial abundance, and (D) protein concentration under manipulations of contact and nutrient dose in the 28 d nutrient experiment. Gray bars, hatched bars, and black bars represent the low, medium and high nutrient doses, respectively. Numbers within the histograms represent adjusted sample sizes. Histograms with different letters are significantly different by ANOVA. Separate solid lines over the histograms represent significant differences between treatment groups

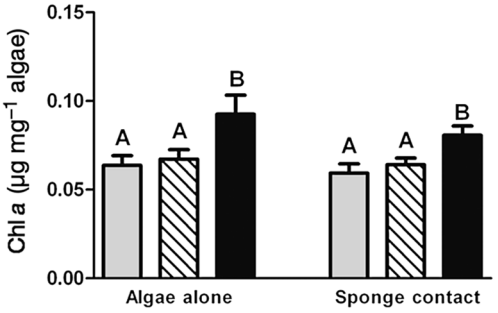

Fig. 4. *Microdictyon marinum*. Mean (± SE) chl *a* concentration under different treatment conditions after the 28 d nutrient experiment. Gray bars, hatched bars and black bars represent the low, medium and high nutrient doses, respectively. Histograms with different letters are significantly different by ANOVA

In the allelopathy experiment, chl *a* concentrations in *A. cauliformis* showed no differences between the solvent control and the *M. marinum* extract treatment (0.24 ± 0.15 and 0.22 ± 0.002 µg chl *a* mg^{-1} sponge tissue, respectively; paired *t*-test, df = 5, p = 0.19).

Fluorescent yield

Fluorescent yield measured in *A. cauliformis* was significantly affected by contact treatment in the nutrient experiment (Table 1). Fluorescent yield of *A. cauliformis* in the algal contact treatment was significantly lower than in either the shade/abrasion or sponge alone treatments (Tukey's HSD test, $p < 0.05$; Fig. 3B). This result was significantly correlated with chl *a* concentration (Pearson correlation, p = 0.02), but was not significantly correlated with change in sponge weight or soluble protein concentration (Pearson correlation, p = 0.27 and p = 0.15, respectively). Fluorescent yield in *M. marinum* was not significantly affected by contact treatment, nutrient dose, or their interaction (Table 1). In the allelopathy experiment, fluorescent yield of *A. cauliformis* was not affected by contact with *M. marinum* extract (mean ± SE = 0.415 ± 0.022 for controls and 0.409 ± 0.021 for extract treatments; unpaired *t*-test, df = 5, p = 0.8232).

Cyanobacterial symbiont density

Symbiont density in *A. cauliformis* was significantly reduced by shading and by algal contact in the contact experiment (Tukey's HSD test, $p < 0.05$; Table 1, Fig. 2B) and in the nutrient experiment (Tukey's HSD test, $p < 0.05$; Table 1, Fig. 3C). Cyanobacterial density was not significantly affected by nutrient dose, nor were there significant interactions across the treatments. Cyanobacterial density was significantly correlated with chl *a* and soluble protein concentrations (Pearson correlation, p = 0.0003 and p = 0.02, respectively).

Protein concentration

In the contact experiment, protein concentrations in *A. cauliformis* were not significantly different among treatments (413.5 ± 25.5 µg protein mg^{-1} sponge for the sponge-alone treatment, 436.7 ± 39.9 µg protein mg^{-1} sponge for the shade control treatment, and 377.9 ± 15.6 µg protein mg^{-1} sponge for the algal contact treatment; Table 1). Total protein concentration in *A. cauliformis* in the nutrient experiment was significantly affected by contact treatment and nutrient dose, but there were no interactive effects (Table 1). In the nutrient experiment, the sponge-alone treatment had significantly higher protein concentrations compared to the other 2 treatments, and sponges at the low nutrient dose had significantly higher total protein concentrations than at the high and medium doses (Tukey's HSD test, $p < 0.05$; Fig. 3D).

Sponge and algae growth

Percent change in sponge weight was not significantly affected by algal contact (0.001 ± 0.01% for sponge-alone treatment, 0.01 ± 0.02% for shade control treatment, and 0.005 ± 0.01% for algal contact treatment; Table 1). Percent change in algal weight in the contact experiment also was not affected by sponge contact (0.47 ± 0.08% and 0.39 ± 0.06% for the algae-alone and sponge contact treatments, respectively; Table 1).

In the nutrient experiment, percent change in sponge weight was not affected by algal contact, nutrient dose, or their interaction (Table 1). Percent growth of sponges in the sponge-alone treatment was 0.08 ± 0.14%, –0.06 ± 0.09%, and 0.02 ± 0.03% for the low, medium and high nutrient doses, respectively. Percent growth of the sponge in the shade control was 0.01 ± 0.01%, 0.02 ± 0.02%, and 0.04 ± 0.04% for the low, medium and high nutrient doses, respectively. Sponges in the algal contact treatment had a percent change in weight of 0.02 ± 0.04%, 0.09

± 0.06%, and 0.02 ± 0.02% for the low, medium and high nutrient doses, respectively. Algal growth in the nutrient experiment was also not significantly affected by sponge contact, nutrient dose, or the interaction of the 2 factors (Table 1). Percent growth in algae from the algae-alone treatment was 0.28 ± 0.15%, 0.22 ± 0.06%, and 0.29 ± 0.04% for low, medium and high nutrient doses, respectively, while percent growth of algae in the sponge contact treatment was 0.14 ± 0.07%, 0.15 ± 0.06%, and 0.15 ± 0.06% for the low, medium and high nutrient doses, respectively.

NO_3^-/HCO_3^- experiment

Initial sponge samples showed significant differences in $\delta^{15}N$ between cell fractions (0.8 ± 0.2‰ and 3.1 ± 0.1‰ for bacterial and sponge cell fractions, respectively; 1-way ANOVA, F = 162.5, p < 0.0001). Bacterial and sponge cell fractions showed no differences in $\delta^{13}C$ (−19.5 ± 0.2‰ and −19.1 ± 0.2‰ for bacterial and sponge cell fractions, respectively; 1-way ANOVA, F = 2.71, p = 0.13). There was a significant ^{15}N enrichment of samples at t_0 (i.e. enriched samples) compared to t_i (initial) samples (1.9 ± 0.4‰ and 12.4 ± 3.7‰ for initial and enriched samples, respectively; Fig. 5A), but there was no effect of cell fraction or interaction of the 2 variables (Table 1). For the $NaH^{13}CO_3$ tracer, there was significant enrichment of samples at t_0 (−19.3 ± 0.1‰ and 1.51 ± 2.8‰ for t_i and t_0, respectively), with the bacterial fraction, on average, higher than the sponge fraction, and a significant interaction between the 2 terms (Table 1). Tukey's HSD test revealed that both post-enrichment fractions were significantly enriched with ^{13}C over the initial samples, but also that the post-enrichment bacterial fraction was more enriched than the sponge cell fraction from the same time (Fig. 5A). Sponges maintained significant enrichment over initial concentrations for both the carbon and nitrogen tracers (data not shown).

Concentrations of ^{15}N and ^{13}C in algal samples in this experiment were not significantly different than initial values. $\delta^{15}N$ values for *M. marinum* samples indicated no significant enrichment at either 12 or 24 h compared to initial samples (Table 1), although results were highly variable, suggesting that some individuals may have been enriched with ^{15}N. Algae also did not absorb ^{15}N at either 12 or 24 h when placed either 15 cm (−0.3 ± 0.2 and −0.2 ± 0.2 for 12 and 24 h, respectively), or 30 cm from an enriched sponge (−0.09 ± 0.2 and 0.28 ± 0.6 for 12 and 24 h, respectively; Table 1). There were no differences in $\delta^{13}C$ in algal samples collected at 12 and 24 h, suggesting no transfer of carbon resources from the sponge. Additionally, there was no evidence for uptake of sponge-derived carbon in algae at 15 cm from the enriched sponge (−14.7 ± 0.4 and −13.9 ± 0.2 for 12 and 24 h samples), as the $\delta^{13}C$ in fact decreased significantly from initial samples at 12 h (Tukey's HSD test, p < 0.05). A significant decrease in $\delta^{13}C$ was also observed at 12 h for algae at 30 cm from an enriched sponge (Tukey's HSD test, p < 0.05), but algal samples at 24 h showed no significant differences from initial samples (−13.3 ± 0.3, −14.4 ± 0.3 and −14.3 ± 0.3 for t_i, t_{12} and t_{24} algal samples, respectively; Table 1).

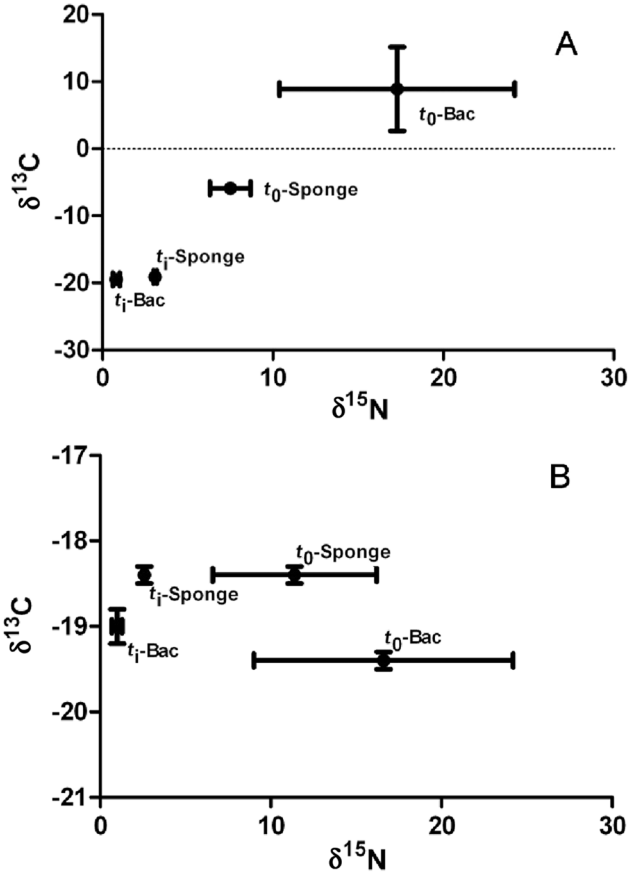

Fig. 5. (A) Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ in initial (t_i) and enriched (t_0) sponges. Sponges collected from the incubation chamber were significantly enriched with $Na^{15}NO_3$ and the $Na^{13}HCO_3$ tracer (p = 0.009 and p < 0.0001 for $\delta^{15}N$ and $\delta^{13}C$, respectively). (B) Mean (± SE) $\delta^{15}N$ in t_i samples and t_0 sponges. Sponges collected from the incubation chamber were significantly enriched with the $^{15}NH_4^+$ tracer (p = 0.01) after 6 h. Bac: bacterial cell fraction; Sponge: sponge cell fraction

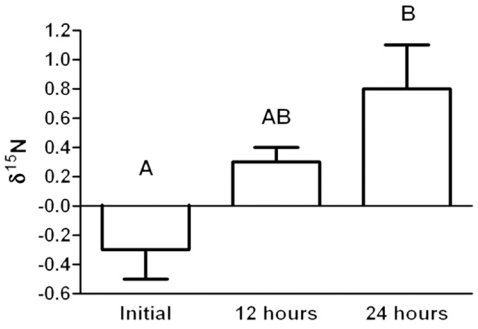

Fig. 6. Mean (± SE) $\delta^{15}N$ enrichment of *Microdictyon marinum* of initial alga samples and after 12 and 24 h in contact with an *Aplysina cauliformis* individual that was enriched with $^{15}NH_4^+$. Significant algal enrichment with sponge-derived $\delta^{15}N$ was shown after 24 h ($p = 0.01$). Histograms with different letter groups are significantly different by ANOVA

NH_4^+ experiment

In the NH_4^+ experiment, significant differences in $\delta^{15}N$ were shown between fractions in t_i sponges (0.8 ± 0.3 ‰ and 2.5 ± 0.1‰ for bacterial and sponge fractions, respectively; 1-way ANOVA, $F = 33.5$, $p = 0.0004$). After 6 h of incubation (t_0) in 0.1 mg l^{-1} of $^{15}NH_4$, sponges became significantly enriched compared to initial samples (1.7 ± 0.3 and 14.0 ± 4.3 for t_i and t_0 samples, respectively), but there was no effect of cell fraction type or interaction of the 2 terms (Table 1, Fig. 5B). Sponges in this experiment maintained significant nitrogen tracer enrichment over initial concentrations (data not shown).

Algae in the NH_4^+ experiment showed significant enrichment with the ^{15}N tracer relative to initial samples after 24 h in contact with the sponge (Table 1, Fig. 6). Algae at 15 cm from an enriched sponge showed no significant differences from initial $\delta^{15}N$ values (−0.3 ± 0.2, −0.2 ± 0.3, and −0.5 ± 0.2 for t_i, t_{12} and t_{24} samples, respectively), suggesting that they obtained no sponge-derived nitrogen resources. Algae at 30 cm showed a significant decrease in $\delta^{15}N$ values at 24 h compared to initial values, but no differences from initial values were observed at 12 h (−0.6 ± 0.1 and −1.7 ± 0.1 for 12 h and 24 h samples, respectively; Table 1).

DISCUSSION

This study identified a complex interaction between *Aplysina cauliformis* and *Microdictyon marinum,* in which both competition and facilitation appear to play a role. While the alga had a negative competitive effect on sponge physiology, the sponge facilitated the production of chl *a* in the alga, likely through a transfer of nitrogen. Elevated nutrient concentrations appear to benefit the alga, and have a positive effect on the sponge symbionts in the absence of other stressors. However, exogenous nutrient addition above concentrations that would likely be supplied by the sponge had a greater effect on algal heath, further supporting the contention that nutrient transfer from the sponge is beneficial to *M. marinum*.

Studies of algal interactions with other reef species have largely focused on corals, and have mainly been labeled as competitive interactions (reviewed in McCook et al. 2001), but sponge interactions with other coral reef organisms have been gaining attention (González-Rivero et al. 2011, Pawlik 2011, Slattery et al. 2013). Despite their high abundance on Caribbean coral reefs, few studies have investigated the effects of sponge–algal contact *in situ* (López-Victoria et al. 2006, González-Rivero et al. 2012). In one such study, González-Rivero et al. (2012) showed that contact with the brown alga *Lobophora variegata* had a negative effect on the sponge *Cliona tenuis* through reduction of its lateral growth rate. The success of algal interactions with other reef organisms appear to rely on 3 mechanisms: shading, abrasion, and allelopathy (McCook et al. 2001, River & Edmunds 2001), although more recently the effects of dissolved organic matter and microbial interactions have been recognized (Smith et al. 2006). Some algae possess allelopathic compounds that can damage competitors (de Nys et al. 1991, Rasher et al. 2011, Slattery & Lesser 2014), and while *M. marinum* can have allelopathic effects on the hard coral *Orbicella annularis* (C. G. Easson & D. J. Gochfeld unpubl. data), an allelopathic effect was not observed against *A. cauliformis*. This difference could be due to a temporal factor, as the allelopathy experiment in the current study lasted just 1 wk, whereas the contact and nutrient experiments ran for a longer time period, as have some other algal allelopathy studies employing similar methods (Rasher & Hay 2010, Rasher et al. 2011, Slattery & Lesser 2014). Alternatively, *A. cauliformis* may have some resistance to allelopathic compounds from *M. marinum*, the allelopathic compounds that would be active against the sponge may be hydrophilic (Harder et al. 2004) and therefore not represented in the organic extraction used, or the seasonality of the alga may provide periodic relief from any stress associated with algal contact (Lirman 2001), since the alga is not present on the reef during the winter months (C. G. Easson & D. J. Gochfeld pers. obs.).

M. marinum can grow as a canopy over the substrate during the summer months, enabling it to shade other reef organisms (Kramer et al. 2003). In this way, *M. marinum* may be able to overgrow small and repent growth forms of *A. cauliformis*, as well as shading and possibly weakening the bases of large upright *A. cauliformis*. Because *A. cauliformis* has been documented to receive up to 75% of its energy budget from its photosymbionts (Freeman & Thacker 2011), a reduction in irradiance would likely decrease the energy resources available to the sponge. Even under shaded conditions, *A. cauliformis* maintains its relationship with its symbionts, receiving the majority of its carbon and nitrogen from them (Freeman & Thacker 2011). The maintenance of this relationship, coupled with the reduction in symbiont abundance observed in response to algal contact, could lead to a reduced energy budget in the host. The results of this study suggest that shading may be the main stressor resulting from algal contact, although the sponges were able to maintain high chl *a* concentrations in the shade/abrasion control treatment in the contact experiment despite lower cyanobacterial abundance. Sponges were not able to compensate for this shading in the algal contact treatment, suggesting a more complex interaction between the alga and the sponge than just a shading effect. While *A. cauliformis* growth was not affected in the current study, similar studies with this sponge have exhibited mixed results regarding growth rates as a metric for sponge health (e.g. Freeman & Thacker 2011, Gochfeld et al. 2012a). This discrepancy may be due to a combination of factors, including seasonality and high variability in growth rates over short temporal scales (Duckworth & Battershill 2001, McMurray et al. 2008, Pawlik et al. 2008); however, the biochemical endpoints used in this study represent responsive proxies for sponge health (Erpenbeck & van Soest 2007).

Whereas *M. marinum* caused several negative effects on the sponge, this interaction appeared to benefit the alga. *A. cauliformis* possesses a diversity of chemical defenses (Puyana et al. 2003, Gochfeld et al. 2012b), yet there was no evidence that *A. cauliformis* released allelopathic compounds that damaged *M. marinum*. In contrast, the nutrient transfer experiments indicated that this increased algal productivity was due to nitrogen transfer from the sponge to the alga. Transfer of nitrogen from sponges to plants and macroalgae has been demonstrated in previous studies (e.g. Ellison et al. 1996, Davy et al. 2002, respectively). Davy et al. (2002) showed that sponge-derived nitrogen was transferred to the red alga *Ceratodictyon spongiosum* when the 2 species were in contact. However, while the net-like morphology of *M. marinum* maximizes its surface area to volume ratio and would be expected to favor efficient uptake of nutrients, the current study only demonstrated significant uptake of the nitrogen substrate ammonium. There may be several reasons for differential uptake of nitrate and ammonium. From the standpoint of algal uptake, ammonium is a more biologically available source of nitrogen than nitrate, especially to photosynthetic eukaryotes in the marine environment (Zehr & Ward 2002). Differential uptake by the alga could also be due to sponge processing of nitrogen resources. Many sponges that host dense microbial communities are able to quickly uptake nitrogen from both ammonium and nitrate, and incorporate it into their biomass (Thacker & Freeman 2012, Freeman et al. 2013). However, proteins essential for assimilation of both of these resources have only been found in the cyanobacteria and sponge-associated microbial communities, and not in the eukaryotic sponge cells (Zehr & Ward 2002, Taylor et al. 2007), indicating that both nitrogen substrates are assimilated by sponge microbial communities and then translocated to the sponge host in a form that can be incorporated into the sponge biomass (Taylor et al. 2007, Freeman et al. 2013). The limiting factor might be the processing rate of these 2 substrates, as ammonium can be directly assimilated into microbial and sponge biomass, whereas nitrate requires additional conversion steps for assimilation (Taylor et al. 2007). In the current study, these properties may have played a role in the availability of the 2 nitrogen substrates to the alga, even though *A. cauliformis* can excrete both NH_4^+ and NO_x^- into the water column (Maldonado et al. 2012). The current study showed no carbon transfer from sponge to alga, which could be attributed to a variety of causes, including minimal heterotrophic feeding by *A. cauliformis*, sufficient abundance of carbon in the environment, or net production of dissolved carbon by *M. marinum* during periods of adequate irradiance (Taylor et al. 2007, Maldonado et al. 2012, Thacker & Freeman 2012).

This study demonstrated that increased exogenous nutrient levels enhanced the condition of the algae, as measured by chl *a* concentration, supporting studies that implicate nutrients as a cause of increased algal abundance on reefs (Lapointe 1997). In contrast, Szmant (2002) has argued that evidence is lacking for nutrient enrichment directly causing increased algal abundance and decreased coral abundance on reefs. Furthermore, published results showing nutrient effects often use concentrations that are orders of magnitude higher than found on

reefs (reviewed in Szmant 2002). The present study used nutrient concentrations documented in previous field experiments with *A. cauliformis* (Gochfeld et al. 2012a), and showed that while nutrients benefited the sponges' photosymbionts, as exhibited by increased chl *a* content in the sponges, they also led to reduced sponge holobiont health, as demonstrated by the reduction in total protein content. This dichotomy could be due to reduced sponge metabolic activity, or it may be a symptom of nutrient stress changing the sponge–symbiont relationship (sensu Fiore et al. 2010). While this was not observed in *A. cauliformis* under shading stress (Freeman & Thacker 2011), the addition of nutrients may release the symbionts' dependence on host-derived sources of nitrogen (reviewed in Fiore et al. 2010).

While it is important to understand the consequences of sponge–algal interactions and of elevated nutrients, it is also important to understand how these potential stressors function together. In a coral–algal interaction, Jompa & McCook (2002) found that increased nutrient loads increased growth of the alga, which subsequently caused greater coral tissue mortality, although a high level of herbivory was able to mask the effects of elevated nutrients. Slattery et al. (2013) observed a similar response in a natural experiment that assessed the impact of cave sponge nitrate efflux onto nearby patch reefs. Additionally, elevated nutrients can interact with other stressors that cause coral reef decline, such as disease progression (Voss & Richardson 2006, but see Gochfeld et al. 2012a). In our study, presenting these stressors both separately and in combination enabled us to determine a potential mechanism for facilitation of the alga in this interaction. While the addition of nutrients did not appear to alter the alga's impact on sponge condition, elevated nutrients reduced the sponge's ability to compensate for reduced irradiance in the shade control, causing these sponges to group with the algal contact treatment rather than the sponge-alone treatment in terms of chl *a* concentrations. The protein data from the nutrient experiment also suggests that nutrient addition and algal contact, both separately and in combination, elicited negative effects on the sponge. Whereas cyanobacterial abundance was unaffected by nutrient addition, protein concentration in the sponge holobiont was inversely proportional to nutrient dose. These data suggest that the observed protein reduction was likely related to sponge host condition rather than symbiont condition.

The interaction between *A. cauliformis* and *M. marinum* exhibits characteristics of both competition and facilitation, sometimes referred to as ecological antagonism. While *M. marinum* has a detrimental physiological effect on *A. cauliformis*, the alga appears to benefit from contact with the sponge, through the transfer of nitrogen resources. However, our survey data suggest that sponge contact is not required for this alga to be prolific on the reef. Several studies have indicated that many sponges are net sources of nitrogen on coral reefs (Southwell et al. 2008), and their ability to efflux nitrogen could increase local concentrations of these limited resources (Maldonado et al. 2012). Assimilation of sponge-derived nitrogen by neighboring reef organisms is significant (de Goeij et al. 2013), perhaps especially in closely associated organisms such as the algae and sponge in the current study. Indeed, although the nitrogen budgets of only 22 Caribbean sponge species have been reported, 20 of these species were shown to have an overall net efflux of dissolved nitrogen in the form of NH_4^+ and/or NO_3^- (Maldonado et al. 2012). Because *M. marinum* interacts with several of these species, interactions like those reported in the current study may be prevalent on these shallow reefs. If sponge-derived nitrogen boosts algal productivity, this might directly impact algal abundance on these reefs, potentially leading to circumstances supporting algal dominance on reefs where overfishing, anthropogenic nutrients, and disease have already tipped the scale in favor of the algae.

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