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ALLELOPATHY IN THE TROPICAL ALGA *LOBOPHORA VARIEGATA* (PHAEOPHYCEAE): MECHANISTIC BASIS FOR A PHASE SHIFT ON MESOPHOTIC CORAL REEFS?[1]

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Macroalgal phase shifts on Caribbean reefs have been reported with increasing frequency, and recent reports of these changes on mesophotic coral reefs have raised questions regarding the mechanistic processes behind algal population expansions to deeper depths. The brown alga *Lobophora variegata* is a dominant species on many shallow and deep coral reefs of the Caribbean and Pacific, and it increased in percent cover (>50%) up to 61 m on Bahamian reefs following the invasion of the lionfish *Pterois volitans*. We examined the physiological and ecological constraints contributing to the spread of *Lobophora* on Bahamian reefs across a mesophotic depth gradient from 30 to 61 m, pre- and post-lionfish invasion. Results indicate that there were no physiological limitations to the depth distribution of *Lobophora* within this range prior to the lionfish invasion. Herbivory by acanthurids and scarids in algal recruitment plots at mesophotic depths was higher prior to the lionfish invasion, and *Lobophora* chemical defenses were ineffective against an omnivorous fish species. In contrast, *Lobophora* exhibited significant allelopathic activity against the coral *Montastraea cavernosa* and the sponge *Agelas clathrodes* in laboratory assays. These data indicate that when lionfish predation on herbivorous fish released *Lobophora* from grazing pressure at depth, *Lobophora* expanded its benthic cover to a depth of 61 m, where it replaced the dominant coral and sponge species. Our results suggest that this chemically defended alga may out-compete these species in situ, and that mesophotic reefs may be further impacted in the near future as *Lobophora* continues to expand to its compensation point.

Key index words: **allelopathy; herbivory; lionfish; *Lobophora*; mesophotic reefs**

The loss of coral cover worldwide (Gardner et al. 2003, Bruno and Selig 2007), to anthropogenic and/or natural disturbances (Hughes and Connell 1999, Roff and Mumby 2012), is often associated with an increase in algal biomass and a "phase shift" in coral reef community structure (Hughes 1994, Norstrom et al. 2009). However, the proximate causes of these phase shifts are still subject to considerable debate (McManus and Polsenberg 2004, Mumby and Steneck 2008, Dudgeon et al. 2010, Mumby et al. 2013). In particular, significant research effort has focused on perceived competing theories of either nutrients (= bottom-up processes: LaPointe 1997) or grazers (= top-down processes: Hughes et al. 1999) as the causal factor controlling phase shifts, and while there is increasing awareness that these forces do not function in isolation, the preponderance of experimental data show that top down processes explain most of the observed effects (e.g., Smith et al. 2001, Diaz-Pulido and McCook 2003, Burkepile and Hay 2006). Recently, questions have refocused on the magnitude of these phase shifts (Bruno et al. 2009) and the stability of alternate states (Mumby 2009, Dudgeon et al. 2010, Mumby et al. 2013). For example, Rasher and Hay (2010) demonstrated that 40%–70% of Caribbean and Pacific algae suppressed coral growth by contact-mediated allelopathic interactions. They noted that, in addition to the direct effect of these allelochemicals on the competitive dominance of algae over corals, the negative feedback loops that increase algal abundance would further enhance the stability of an alternate algal state by increasing algal-coral contact rates.

The brown alga *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira (hereafter *Lobophora*) has a circumtropical distribution (e.g., Ruyter van Steveninck and Breeman 1987, Jompa and McCook 2002), as well as a broad depth range that encompasses the upper mesophotic reef zone in the Bahamas (i.e., 30–60 m; Aponte and Ballantine 2001, Lesser and Slattery 2011). *Lobophora* often dominates the benthic cover and biomass of coral reefs undergoing phase transitions (Renken et al. 2010). *Lobophora* does exhibit differential susceptibility to herbivores within and between sites (reviewed in Coen and Tanner 1989, Bolser and Hay 1996, Downie et al. 2013) that may be a function of

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variability in putative chemical defenses (Steinberg and Paul 1990). However, greater concentrations do not always predict protection against herbivory (Targett et al. 1995, Targett and Arnold 1998), and these compounds may only be functional against a subset of the herbivore guild at mesophotic depths. In addition, on shallow reefs *Lobophora* competes with at least four species of coral, *Agaricia* sp., *Montastraea annularis* (= *Orbicella annularis* complex: Budd et al. 2012), *Porites cylindrica* (Jompa and McCook 2002, Box and Mumby 2007, Foster et al. 2008), and *P. porites* (Rasher and Hay 2010) as well as the sponge *Cliona tenuis* (Gonzalez-Rivero et al. 2012), via shading, abrasion, and allelopathic mechanisms. Conversely, and surprisingly, the presence of this alga has also been shown to facilitate the settlement of *Acropora millepora* larvae by 40% over control substrata (Birrell et al. 2008).

Mesophotic coral reefs, at depths of 30–150 m, have been the subject of increasing research efforts, since they are physically and biologically coupled to their shallow-water counterparts (Lesser et al. 2009, Slattery et al. 2011), have been hypothesized to act as potential refugia for shallow-reef species (Lesser et al. 2009, Bongaerts et al. 2010), and might therefore contribute to the potential resilience of degraded shallow coral reefs (Hughes et al. 2010). While strong gradients of down-welling irradiance significantly influence the structure of the deep reef community (Lesser et al. 2009), photo-acclimatization and heterotrophy provide mechanisms by which corals and algae can survive at these depths (Runcie et al. 2008, Lesser et al. 2010). Recent evidence indicates that deep reef communities are more genetically isolated than once thought (Lesser et al. 2010, Slattery et al. 2011), so the generality of the deep reef refugia hypothesis is constrained by site-specific variability (van Oppen et al. 2011, Brazeau et al. 2013). The stability of deep reef coral communities (Bak et al. 2005) has recently been challenged using a comparative analysis which quantified significant increases in algal cover over time (Stokes et al. 2010), although the specific reasons for increasing algal abundance at depth were not identified. In contrast, Lesser and Slattery (2011) quantified a significant phase shift to algal dominance over time that began with the arrival of the invasive lionfish (*Pterois volitans*) on Bahamian deep reefs. Their results showed that the loss of herbivorous fishes at mesophotic depths to this novel predator (Albins and Hixon 2008) resulted in an increase in algal biomass and cover without any increase in nutrients (e.g., Sotka and Hay 2009) or evidence of community altering stressors (i.e., bleaching, disease, hurricanes, and/or overfishing: e.g., Hughes 1994). In fact, prior to the lionfish invasion coral and sponge cover represented 50% and 82% of the benthos, while abiotic substrate accounted for 19% and 10%, at 46 and 61 m respectively (Lesser and Slattery 2011). However, that study did not provide a mechanistic basis to explain how the increase in algal cover resulted in the decline of corals and sponges that formerly dominated these depths. Since *Lobophora* represented the dominant space occupying species of macrophyte observed during this phase shift, we asked three questions relative to its biology and ecology to describe its potential to compete with corals and sponges at depths it did not previously occupy. First, is there any proximate physiological reason why the expansion of *Lobophora* did not occur prior to the lionfish invasion? Second, what impact do grazers have on mesophotic populations of *Lobophora*? Finally, does *Lobophora* have allelopathic effects on corals and sponges of the mesophotic reef?

MATERIALS AND METHODS

Study site. *Lobophora* surveys and collections were conducted on two mesophotic coral reef systems near Lee Stocking Island, Exuma Cays, Bahamas. Bock Wall (BW; 23°49.915′ N, 76°09.179′ W) is the site of our long-term deep reef studies that include community changes following the lionfish invasion (Lesser and Slattery 2011). The BA transect (BAT; 23°46.818′ N, 76°05.007′ W) represents a site where frequent deep reef submersible surveys date back 20 years (e.g., Aponte and Ballantine 2001). *Lobophora* cover at these sites averaged 80% at 30 m, 30% at 46 m, and 5% at 61 m (Lesser and Slattery 2011) prior to the lionfish invasion in 2005 (Albins and Hixon 2008). The distribution and abundance of *Lobophora* on these deep reefs, as well as interactions with corals and sponges, were recorded between 2003 and 2011 using techniques described in Lesser and Slattery (2011). The physical environment of these mesophotic reefs has been described previously (Lesser et al. 2010, Lesser and Slattery 2011) and those results are contemporaneous with the research described here. Replicate algal samples composed of multiple blades of the decumbent form of *Lobophora* ($n = 5$ from each site) were collected from mesophotic depths (30 and 61 m), at least 5 m apart, and segregated into individual resealable plastic bags for processing and subsequent analyses. In addition, interactions involving direct contact between *Lobophora* and the coral *Montastraea cavernosa* or the sponge *Agelas clathrodes*, as well as the condition of each, were recorded by divers.

Lobophora *physiology.* To assess the depth-specific physiological limitations of *Lobophora* related to their expansion into mesophotic depths, active fluorescence was measured on dark acclimated (30 min) individual blades ($n = 3$) from independent *Lobophora* samples, collected as described above, using a pulse amplitude modulated (PAM) fluorometer (Walz Inc., Effeltrich, Germany). All measurements were taken at the same distance and probe angle, and measurements of minimum (F_o) and maximum (F_m) fluorescence were used to calculate variable ($F_v = F_m - F_o$) fluorescence and subsequently the maximum quantum yield of photosystem II (PSII) fluorescence (F_v/F_m) or the number of functional photosystem II reaction centers (Warner et al. 2010).

Samples of individual *Lobophora* blades ($n = 3$) from each sample were frozen, lyophilized, ground to a powder with a mortar and pestle, acid treated with 1 N HCl to remove any calcium carbonate from calcareous epiphytes, rinsed with distilled water and allowed to dry. Samples were then sent to the Marine Biological Laboratory (Woods Hole, MA, USA)

for the analysis of particulate carbon (C) and nitrogen (N), as well as the natural abundance of the stable isotopes $\delta^{15}N$ and $\delta^{13}C$. Samples were analyzed using a Europa ANCA-SL elemental analyzer-gas chromatograph attached to a continuous-flow Europa 20-20 gas source stable isotope ration mass spectrometer. The analytical precision of the instrument is $\pm 0.1‰$, and the mean precision of sample replicates for $\delta^{13}C$ and $\delta^{15}N$ was $\pm 0.4‰$ and $\pm 0.2‰$ respectively. The carbon isotope results are reported relative to Pee Dee Belemnite and the nitrogen isotope results are reported relative to air; both are expressed using the delta (δ) or the permil notation (‰).

Discrete seawater samples ($n = 3$ at each depth) were collected in May 2005 ($n = 3$ separate days) and Aug 2005 ($n = 2$ separate days) at 30, 45, and 61 m, and in May 2006 ($n = 4$ separate days) and May 2009 ($n = 4$ separate days) at 30, 45, 61, 76, and 91 m. The samples were analyzed using an Oakton PT 35 pH meter, which has an accuracy of ± 0.01 pH units, calibrated to a 3-point standard curve (pH buffers: 4.01, 7.00, and 10.01). These results were further standardized against seawater samples collected in 2010 at 10, 30, 45, 61, 76 and 91 m. These samples were analyzed for total alkalinity (TA), dissolved inorganic carbon (DIC) and pCO_2 by the University of New Hampshire Ocean Process Analysis Laboratory (OPAL). TA was analyzed using an Apollo Sci-Tech AS-A2 automated analyzer (Bogart, GA, USA), which employs the Gran titration procedure. This analysis has a precision of 0.1% and the initial pH for the titration was measured on the same sample using a Thermo Orion combination electrode (precision ± 0.027 pH units). DIC was analyzed from acidified 1–3 mL samples using an Apollo Sci-Tech DIC analyzer with a precision of 0.1%. Certified reference materials were used to ensure the precision of the TA and pH determinations (Dickson et al. 2007). pH and HCO_3 were then calculated with CO2calc software (Robbins et al. 2010) using the in situ temperature and salinities at the time of sample collection from the same depths of collection and the inorganic carbon dissociation constants of Dickson and Millero (1987).

For all analyses described above, tests for normal distribution and homogeneity of variances were conducted and data were transformed where needed, or transformed *a priori* (e.g., ratios). A one-way ANOVA with a fixed effect of depth was then used to test for significant differences between treatment means, followed by post-hoc testing (Scheffe's) if required. The Oakton-analyzed and OPAL-analyzed pH data were regressed against depth, and the error between these procedures was identified.

Lobophora *recruitment.* To assess algal recruitment patterns and rates within mesophotic reef depths, replicate 1 m^2 plots ($n = 4$) were cleared at each of two depths (30 and 61 m) at BW in May 2005. A 1 m^2 quadrat was affixed to the reef via stainless steel spikes, and the area within, as well as a 15 cm "buffer" strip surrounding the quadrat, was scoured to hardpan using paint scrapers and wire brushes. The quadrat was removed between sampling periods, but the position was identified subsequently using the marker spikes. At each sampling period the buffer strip was scraped to ensure that recruitment into the plots represented spores/zygotes settlement, and not lateral expansion of foliose algae. The percent cover and identity of algae within these quadrats were recorded in August 2005 and May 2006 (prior to the lionfish population explosion), and again in May 2009 (~2 years following significant lionfish recruitment to local reefs: Albins and Hixon 2008). The algal recruits to these plots were broadly characterized as crustose coralline reds (CCR), turf (unidentified filamentous greens and reds), *Lobophora variegata* (decumbent morphotype), and *Peyssonnelia inamoena*. The percent cover of hardpan and/or deposited sand at any given point in time was recorded as abiotic substrate. These plots were also assessed for grazing rates by herbivorous fishes, by a diver observing from a distance of ~1.5 m over a 10 min period ($n = 3$–8 replicate observations for each plot), during each of the time points. Specifically, the fish species and number of bites in the recruitment plots were recorded. During the observation period, the specific type of algae consumed was identified and recorded. Recruitment to these plots was analyzed using a repeated-measures ANOVA, with depth as the fixed factor. Herbivory was analyzed using a two-way ANOVA, with depth and time as fixed factors.

Lobophora *feeding assays.* To determine the effect of algal compounds on controlling herbivory of *Lobophora* on mesophotic coral reefs, algae were extracted in methanol:dichloromethane (MeOH:DCM), evaporated under pressure, and the percent extract per gram dry weight of algal tissue was determined on a microbalance. The MeOH:DCM crude extract was further separated using high pressure liquid chromatography, and a single peak was isolated and identified by comparing ^1H and ^{13}C nuclear magnetic resonance chromatography and high resolution mass spectrometry to published data (Cantillo-Ciau et al. 2010). Phlorotannin concentration of the extract was assessed using the Folin-Denis assay, as described in Boettcher and Targett (1993). The arcsin-transformed data were analyzed using a two-way ANOVA with depth and site as fixed factors.

Preweighed clumps of *Lobophora* were offered to ecologically relevant herbivores (*Acanthurus coeruleus* and *Sparisoma atomarium*) and an omnivore (*Canthigaster rostrata*), maintained in replicate 19L aquaria ($n = 5$ per species) for 24 h, and change in mass was used to estimate algal palatability. While these preliminary assays indicated that each species was similarly capable of feeding on *Lobophora*, they demonstrated that the herbivores were less tolerant of laboratory conditions. Therefore, we used the pufferfish *C. rostrata* as a model grazer for laboratory feeding assays since it is commonly found grazing at mesophotic depths (Lesser and Slattery 2011), as well as on shallow reefs, and it incorporates a variety of algae into its diet (Randall 1967, Slattery pers. obs.). The laboratory assay has been described (Gochfeld et al. 2012); briefly, natural concentrations of the crude extract or the purified compound from *Lobophora* were embedded in a mixture of 2% sodium alginate and pureed squid mantle at a protein concentration equivalent to that of *Lobophora* (= 4%; Bolser and Hay 1996, Slattery unpubl. data), while control pellets were prepared similarly but without extracts or compounds. Prior studies have used nondeterrent algae as an ecologically relevant protein alternative to squid mantle (e.g., *Ulva* sp.; Bolser and Hay 1996), however, when our herbivory experiments were conducted these palatable species were locally grazed to minimal biomass; thus, the well-described assay using squid as a protein source was utilized. These were offered to pufferfish, who either consumed or rejected the pellets based on individual food preferences (Gochfeld et al. 2012). Feeding assay data were analyzed using Fisher's Exact test.

Lobophora *competition assays.* To determine the consequences of direct interactions between *Lobophora* and the coral, *Montastraea cavernosa*, or the sponge, *A. clathrodes*, a series of contact experiments were conducted in flowing seawater tanks. These species were chosen since they represent the dominant coral (Lesser et al. 2010) and one of the most dominant sponges (Slattery and Lesser, pers. obs.) across the mesophotic reef gradient, and both species interact regularly with *Lobophora*. For these experiments, replicate individuals ($n = 10$ genets) of the coral and sponge species were "cloned" into three ramets that were used in one of three treatments: (i) interspecific contact between the alga and the coral or sponge, (ii) a contact control (a sheet of industrial grade plastic cut to the size of a *Lobophora* blade; 10 cm^2), and (iii) a noncontact control. After "cloning," the ramets were maintained in a raceway for 10 d to heal, and then pre-exposure

measurements of active fluorescence were recorded, as described above, for *Lobophora* and *M. cavernosa*. Since *A. clathrodes* lacks photosymbionts, we measured metabolic oxygen demand with a microrespirometer (Unisense Inc., Aarhus, Denmark). Briefly, four Clark-type microelectrodes (detection limit = 0.050 μM) simultaneously recorded metabolic oxygen demand at the surface of each sponge ramet in a treatment block, as well as a calibrated water sample, for 1 h of real-time measurements; the average consumption was recorded as μmol O_2 $h^{-1} \cdot g^{-1}$ dry weight of sponge. The experiment was initiated when each ramet was placed into its own 8 L Plexiglas® (Evonik Industries, Parsippany, NJ, USA) aquarium under the previously described treatment conditions. These aquaria received a constant flow of ambient seawater, and consistent temperature was maintained by "floating" the aquaria in a large raceway. The tanks were further covered with neutral density shade-cloth, that lowered light levels to an equivalent depth of 30 m (i.e., depth of collection, or ~180 μmol quanta · $m^{-2} \cdot s^{-1}$ of photosynthetically active radiation; Lesser et al. 2010), and the experiment was maintained for 96 h before the samples were recovered for postexposure PAM or microrespirometry. Both PAM and microrespirometry provide an assessment of acute (= short-term responses) nonlethal effects in these controlled experiments as opposed to field assays where chronic (= longer term responses) bleaching of corals was observed. For each species, differences between contact treatments were assessed using a two-way ANOVA, blocked by genets, with treatment and time as fixed factors.

To assess the potential impact of *Lobophora* allelochemicals on coral and sponge competitors for mesophotic coral reef space two cytotoxicity assays were conducted; these were run contemporaneously with the aforementioned laboratory contact assays described above. The effect of the *Lobophora* extracts and pure compound on corals was assessed in situ at BW and BAT using a modification of the technique described in Slattery et al. (2008). Briefly, natural volumetric concentrations of the crude extract or purified compound from *Lobophora* were embedded into phytagel discs, and paired treatment and control discs were tied to the surface of the coral *M. cavernosa* ($n = 10$ replicate colonies). These discs were removed after 1 week and the area beneath was photographed. The digital images from each coral colony were analyzed using ImagePro® Plus Version 4.5 (Media Cybernetics, Rockville, MD, USA), and the percent of bleached tissue under paired control and treated discs were assessed by subtracting discolored area from total covered area (Gochfeld et al. 2006). Since the PAM fluorometer has a maximum operational depth of 46 m we utilized the more chronic endpoint of bleaching in our field assays.

A laboratory cell lysis assay was used to examine the effect of the *Lobophora* extracts from BW and BAT and the pure compound, on the sponge *A. clathrodes*. Replicate sponges ($n = 10$) were disassociated into cell suspensions by soaking in calcium-/magnesium-free filtered seawater (CMFSW) for 10 min and forcing the suspension through sterile gauze. These slurries were further cleaned of lysed material and aggregates via filtration, centrifugation, and layering with a Percol/CMFSW mixture (Willoughby and Pomponi 2000). Individual cells were added to replicate ($n = 10$ each) Sedgwick Rafter counting chambers in the presence or absence of the *Lobophora* extract or pure compound, as well as 3-([3-cholamidopropyl]-dimethylammonio) propanesulfonate (CHAPS) as a positive lysis control, or CMFWS as a negative control, and these were maintained in an incubation chamber at ambient temperature (25°C) and a 12:12 light: dark cycle. After 48 h, the first one hundred cells "encountered" under a light microscope were scored as intact or lysed. Differences in coral bleaching or sponge cell lysis between paired controls and treatments were analyzed between sites, and the pooled data were examined using a two-way ANOVA with depth and treatment as fixed factors, followed by a Scheffe's *post hoc* test as needed.

RESULTS

Dark-adapted F_v/F_m on blades of *Lobophora* from 30 to 61 m were not significantly different from one another (Table 1). The mean F_v/F_m values ±1SE were 0.81 ± 0.053 for algae collected from 30 m and 0.82 ± 0.023 for algae collected from 61 m. Additionally, the $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures were not significantly different between depths. The mean $\delta^{13}C$ values ±1SE were −18.7 ± 1.39 at 30 m and −18.63 ± 0.07 at 61 m. The mean $\delta^{15}N$ values ±1SE were 0.767 ± 0.05 at 30 m and 0.933 ± 0.07 at 61 m. Finally, C:N ratios were not significantly different among depths. The mean C:N ratios ±1SE were 53.6 ± 5.92 at 30 m and 62.7 ± 9.51 at 61 m.

Discrete seawater samples collected between May 2005 and May 2009 at various depths shows variability between sampling periods, as well as a significant reduction in pH at the lower limits of the upper mesophotic zone (i.e., 61 m; Fig. 1A). Specifically, pH dropped from an average of about 8.1 at 30–45 m to about 8.0 at 61 m, however, there was no significant effect of time and there was no depth x time interaction (Table 1). In 2010, depth-dependent pH values showed a trend of decreasing values with depth that was not significant (Fig. 1B) and the cross-calibration of laboratory pH values in 2010, using the field determinations, revealed an error of 2.2% (Fig. 1C). The associated depth-related increases in pCO_2 (μatm) and HCO_3 (μmol kg per seawater) were also not significant.

Lobophora recruitment to bare plots at 30 and 61 m occurred rapidly; within 3 months of clearing the benthos, this alga represented 3%–10% of the benthic cover (Fig. 2), although percent cover had not increased significantly by the following year. However, by May 2009 *Lobophora* represented 60%–75% of the benthic cover in these plots, and these levels persisted through 2010 and 2011 (Slattery pers. obs.). There was a significant increase in *Lobophora*, relative to the other benthic constituents (i.e., algae and abiotic substrata) through May 2009 (Table 1). Turf algae were only found within select quadrats at 30 m, and only during August 2005. By May 2006, CCR was established at both depths and at about 10% of the benthic cover. *Peyssonnelia inamoena* was recorded in May 2009 in quadrats at 61 m. In contrast, *Halimeda copiosa*, another common alga on mesophotic reefs, was never observed within these plots. There was also a significant effect of depth on benthic cover within the plots, but there was no interaction effect (Table 1).

Contact between *Lobophora* and *M. cavernosa* were observed a total of 13 times in the field, and in 61.5% of these interactions the underlying coral

Table 1. Statistics summary table. Datasets refer to specific metrics collected during this project, and correspond to a particular figure in the paper. Statistic provides the specific test, as well as the F-statistic, degrees of freedom, and P value for each treatment factor.

Dataset/factors	Statistic	*F*	df	*P*	Figure
F_v/F_m					
Depth	One-way ANOVA	0.028	1,5	0.875	
$\delta^{13}C$					
Depth	One-way ANOVA	0.005	1,5	0.945	
$\delta^{15}N$					
Depth	One-way ANOVA	0.386	1,5	0.577	
C:N ratio					
Depth		1.999	1,5	0.230	
pH [sensor]					
Depth	Two-way ANOVA	13.736	2,3	≤0.0001	Fig. 1A
Time		0.803	2,3	0.495	
Interaction		1.860	2,6	0.095	
pH [calculated]					
Depth	One-way ANOVA	2.051	5,12	0.143	Fig. 1B
pCO_2					
Depth	One-way ANOVA	1.616	5,12	0.230	
HCO_3					
Depth	One-way ANOVA	2.133	5,12	0.131	
Recruitment					
Depth	Repeated Measures ANOVA	221.562	1,4	≤0.0001	Fig. 2
Time		33.434	4,12	≤0.0001	
Interaction		0.608	4,12	0.831	
Grazing- acanthurids					
Depth	Two-way ANOVA	144.345	1,2	≤0.0001	Fig. 3
Time		216.548	1,2	≤0.0001	
Interaction		30.365	1,2	≤0.0001	
Grazing- scarids					
Depth	Two-way ANOVA	27.091	1,2	≤0.0001	Fig. 3
Time		80.005	1,2	≤0.0001	
Interaction		6.940	1,2	0.0018	
Phlorotannin					
Depth	Two-way ANOVA	0.125	1,1	0.728	
Site		1.724	1,1	0.208	
Interaction		0.001	1,1	0.974	
Feeding assay					
Extract- 30 m	Fisher's Exact			≥0.9999	
Extract- 61 m				≥0.9999	
Extract- BW				≥0.9999	
Extract- BAT				≥0.9999	
Compound- 30 m				≥0.9999	
Compound- 61 m				≥0.9999	
Compound- BW				≥0.9999	
Compound- BAT				≥0.9999	
Contact- coral					
Treatment	Two-way ANOVA	0.105	1,2	0.900	
Time		0.896	1,2	0.355	
Interaction		0.044	1,2	0.957	
Contact- sponge					
Treatment	Two-way ANOVA	0.017	1,2	0.983	
Time		0.139	1,2	0.711	
Interaction		0.085	1,2	0.919	
Reciprocal effects					
Treatment	Two-way ANOVA	0.169	1,2	0.845	
Time		0.011	1,2	0.981	
Interaction		0.019	1,2	0.916	
Discs- coral					
Treatment	Two-way ANOVA	578.286	1,2	≤0.0001	Fig. 4
Depth		1.251	1,2	0.268	
Interaction		0.151	1,2	0.861	
Discs- sponge					
Treatment	Two-way ANOVA	906.244	1,3	≤0.0001	Fig. 5
Time		0.113	1,3	0.738	
Interaction		2.392	1,3	0.101	

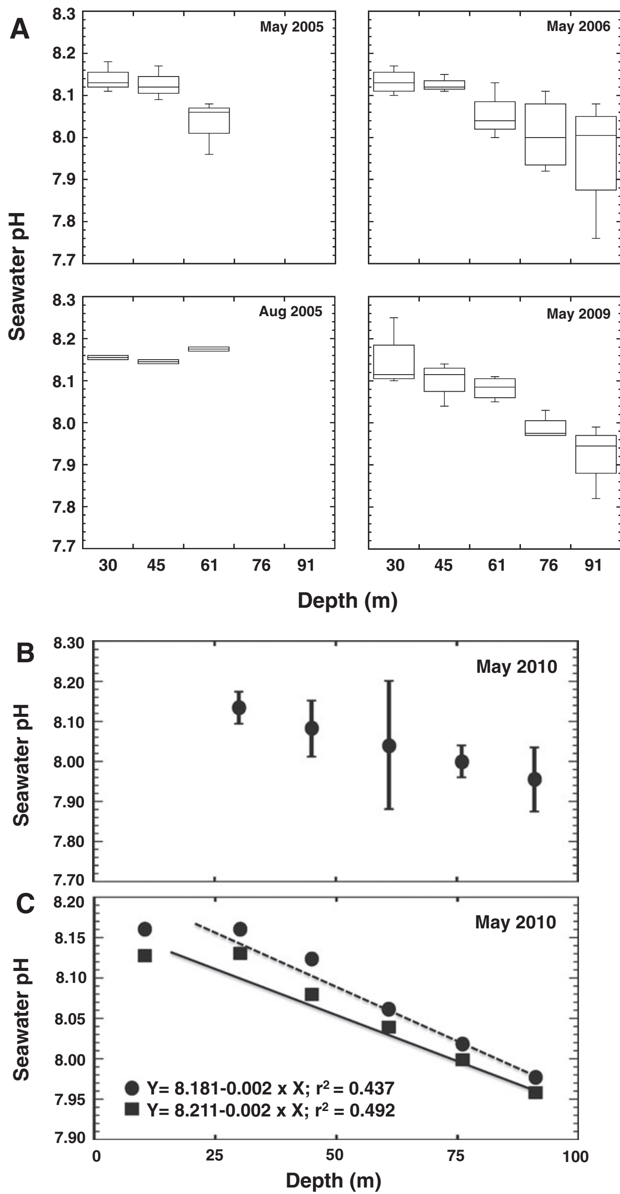

Fig. 1. Seawater pH across depth. (A) Change in pH across depth between May 2005 and 2009 determined by field sensor. Replicate water samples ($n = 3$) were collected at each depth, and on multiple days in any given time period (box and whisker plots represent the median surrounded by the upper and lower quartiles, as well as the maximum and minimum data points). (B) Change in pH across depth in May 2010 determined by titration. (C) Calibration of the pH field sensor to samples processed via titration in May 2010. Data indicates an error of 2.2% between the techniques (Regression: $Y = 0.434 + 0.95 \times X$, $r^2 = 0.978$).

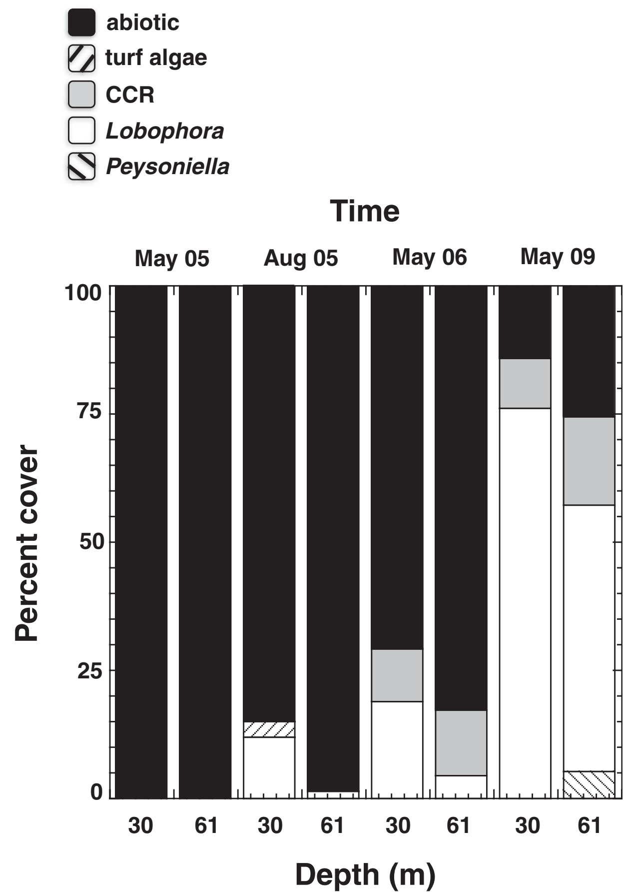

Fig. 2. Algal recruitment plots. Histograms represent the mean algal constituents within replicate ($n = 4$) m^2 recruitment plots, at two depths, through time. Error bars have been removed for visual purposes.

tissue exhibited evidence of bleaching and/or tissue necrosis. Contact between *Lobophora* and *A. clathrodes* was only observed seven times in the field, and only 28.6% of the interactions resulted in sponge tissue necrosis.

For both scarids and acanthurids there was a significant depth x time treatment effect (Table 1) confounding any significant effects of either depth or time independently. As a result *post hoc* multiple comparison tests were conducted only on the significant effect of time x depth. For scarids grazing rates (Fig. 3; primarily *Sparisoma atomarium*, but including *Scarus coelestinus* and *Cryptotomus roseus*) there were significant differences between depths in both 2005 and 2006 while in 2009 grazing rates were significantly lower than previous years and at both depths (i.e., 30 and 61 m). For acanthurids (Fig. 3; primarily *Acanthurus coeruleus*, but including *Acanthurus chirurgu*) grazing rates were significantly different between depths in 2005 and 2006, but unlike scarids the rate of grazing was also significantly greater at 30 m in 2006 compared to 2005. In 2009, and as was the case for scarids, grazing rates in 2009 were significantly lower than 2005 and 2006 at both depths (Fig. 3).

Phlorotannin concentrations did not vary between depths (30 m = 14.45 ± 0.82% vs. 61 m = 16.01 ± 0.77% [mean ± 1SE]) or between sites (BW = 15.02 ± 0.87% vs. BAT = 15.44 ± 0.80%), nor were there significant interactions (Table 1). The crude extract from *Lobophora* did not deter feeding by the pufferfish *C. rostrata* in laboratory

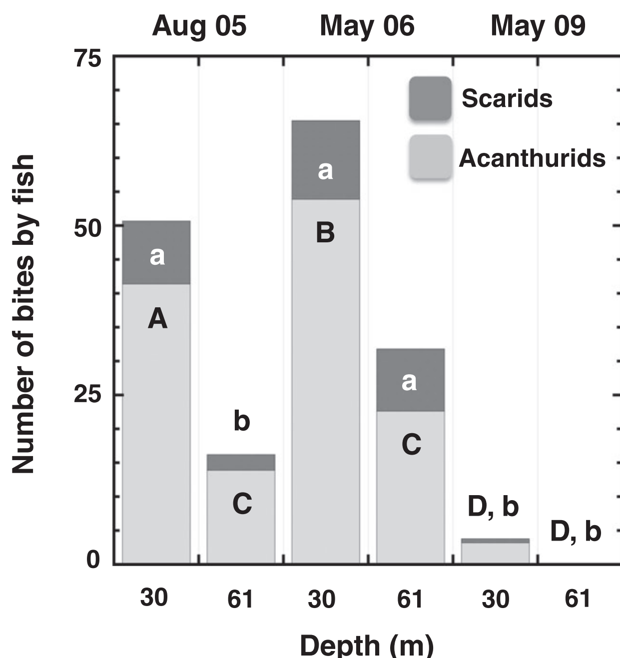

Fig. 3. Herbivore grazing in recruitment plots. Stacked bar plots represent the mean number of bites by scarids (dark portion) and acanthurids (light portion) within recruitment plots, at two depths, through time. Error bars have been removed for visual purposes. Treatment groups with similar letter groups (lower case for scarids, and upper case for acanthurids), are not statitistically different from each other (Tukey's $P > 0.05$).

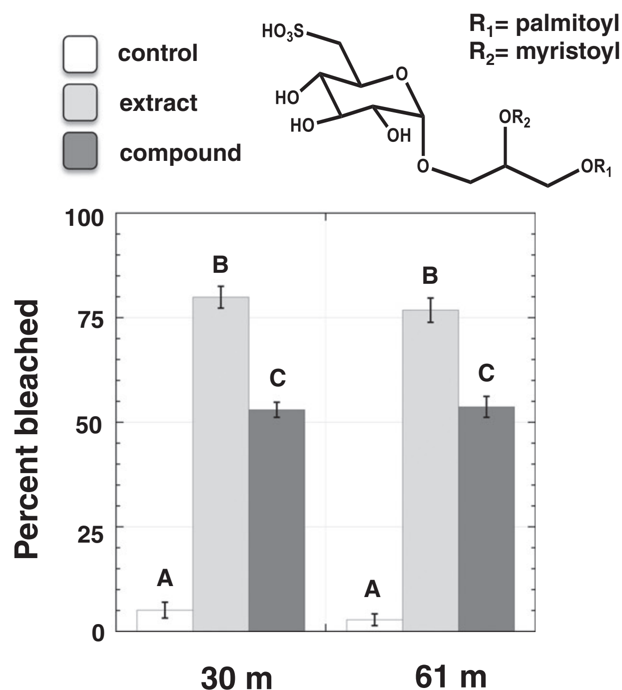

Fig. 4. Coral allelopathic response. Histograms represent the mean ± 1SE percent area of corals bleached in response to either control or *Lobophora* treated allelopathy discs. Bars with different letter groups are significantly different by ANOVA. Structure represents a pure compound isolated from the *Lobophora* extract and tested at natural concentrations.

assays as a function of depth (100% of treated pellets consumed), or site (93% of treated pellets consumed; Fisher's Exact Tests: $P \geq 0.9999$, Table 1). A purified compound representing 0.034 ± 0.007% (mean ± 1SE) of the algal dry mass, identified as 1-*O*-palmitoyl-2-*O*-myristoyl-3-*O*-(6‴-sulfo-α-D-quinovopyranosyl)-glycerol (SQDG: Fig. 4), was similarly not a feeding deterrent at natural concentrations at either depth (93% of treated pellets consumed) or site (100% of treated pellets consumed; Fisher's Exact Tests: $P \geq 0.9999$, Table 1).

In the competition experiment, there was no significant effect of *Lobophora* contact on *M. cavernosa* maximum quantum yields in either of the controls or the treatment, or between initial and final time-points, nor were there significant interactions (Table 1). The mean F_v/F_m values in the contact treatment ±1SE were 0.77 ± 0.019 at the beginning of the experiment and 0.78 ± 0.028 at the end, while the contact controls were 0.78 ± 0.027 at the beginning and 0.77 ± 0.034 at the end, and the noncontact controls were 0.79 ± 0.018 at the beginning and 0.76 ± 0.032 at the end. However, there was an observable difference in the coral color (= bleaching) under algae relative to controls. There was also no significant effect of *Lobophora* contact on *A. clathrodes* metabolic oxygen demand in either of the controls or the treatment, or between initial and final time-points, nor were there significant interactions (Table 1). The mean ± 1SE for metabolic oxygen demand of sponges in the contact treatment were 24.54 ± 2.05 and 24.98 ± 1.65 μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ dry wt of sponge at the beginning and end of the experiment, respectively. The contact control and noncontact controls, respectively, were 24.34 ± 2.16 and 24.49 ± 2.55 μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ dry wt of sponge at the beginning of the experiment, and 25.19 ± 2.70 and 24.52 ± 1.86 μmol $O_2 \cdot h^{-1} \cdot g^{-1}$ dry wt of sponge at the end of the experiment. In both experiments, reciprocal effects of coral or sponge contact on *Lobophora* F_v/F_m were not significant for either of the controls or the treatment, or through time, nor were there significant interactions, and the values were similar to those reported above from our depth surveys.

Discs containing the crude extract and pure compound from *Lobophora* caused significant visible bleaching in the coral *M. cavernosa*, relative to control discs (Fig. 4). The extract was ~50% more bioactive than the pure compound (Scheffe's test: $P < 0.0001$). However, there was no significant effect of depth for the response to the discs, nor were there significant interactions (Table 1). The crude extract and pure compound from *Lobophora* also caused significant lysis of cells from the sponge *A. clathrodes*, relative to control cells incubated in CMFWS (Fig. 5), and the extract exhibited greater

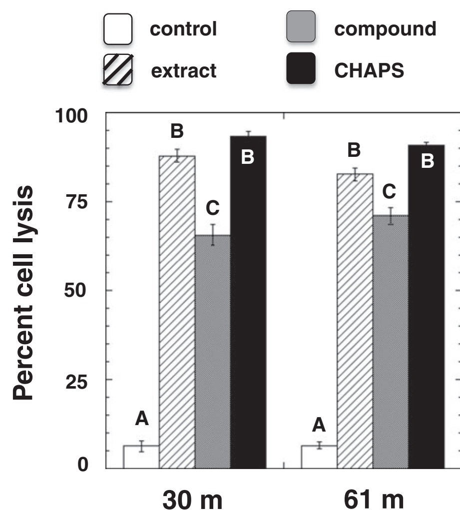

Fig. 5. Sponge allelopathic response. Histograms represent the mean ± 1SE percent sponge cells lysed in response to either controls or *Lobophora* extract or compound. Bars with different letter groups are significantly different by ANOVA. See Figure 4 for structure of pure compound.

bioactivity than the pure compound (Scheffe's test: $P < 0.0001$). In fact, *Lobophora* extract lytic activity was identical to the negative control, CHAPS (Scheffe's test: $P = 0.203$). However, there were no significant differences between algal extracts collected at 30 and 61 m, nor were there significant interactions (Table 1).

DISCUSSION

The depth distributions of marine algae are often set by physiological adaptations to abiotic factors (i.e., light quality and quantity, temperature, and nutrients: e.g. Runcie et al. 2008), and *a priori* it was predicted that one or more of these factors had previously limited the distribution of *Lobophora variegata* into mesophotic depths. However, *Lobophora* is a well-described low-light adapted macrophyte (Peckol and Ramus 1992, Runcie et al. 2008), and has been reported to grow to depths of 81 m with >50% cover (Littler et al. 1986). At our study sites, overall algal cover at 30 m was 83% and at 61 m was 92% by 2009; and the dominant alga was *Lobophora* (Lesser and Slattery 2011). Prior to the lionfish invasion, cover of *Lobophora* had been reported to be 23% at 45 m, 15% at 60 m, and 0.5% at 75 m at our study sites (Aponte and Ballantine 2001, Lesser and Slattery 2011). Light and nutrient levels at these sites have been previously reported (Lesser et al. 2009, Lesser and Slattery 2011), and they show that neither resource would be limiting for *Lobophora* to a depth of ~80 m. Additionally, despite evidence for the upwelling of deep water, and its associated lower pH, no measurements of carbonate chemistry at this site varied significantly with depth or remotely approached concentrations associated with carbon limitation and its concomitant effects on photosynthesis or growth (Holbrook et al. 1988, Israel and Hophy 2002).

The $\delta^{13}C$ stable isotope signatures of samples from 30 and 61 m were not significantly different and were indicative of macrophytes using a carbon concentrating mechanism (Giordano et al. 2005). Additionally, the pCO_2 and HCO_3 values along the depth gradient were not significantly different and should not have affected the availability of inorganic carbon for photosynthesis. The active fluorescence data show that algae from 30 to 61 m had similar light harvesting efficiency, as indicated by the similar proportion of functioning photosynthetic reaction centers. The $\delta^{15}N$ isotope signature in *Lobophora* is consistent with upwelled nitrate, with a depleted $\delta^{15}N$ isotope signature from the mineralization of organic material originating from a source that consumed nitrogen fixers (see discussion in Slattery et al. 2011). Upwelling, caused by internal waves, is a common feature of the Bahamas (Leichter et al. 2006) and is likely responsible for both the depleted $\delta^{15}N$ isotope signature in *Lobophora* and the variability in pH observed at mesophotic depths. The carbonate chemistry reported here shows that DIC is variable, but when pH is either invariant with depth or lower pHs are observed at mesophotic depths, DIC (both TCO_2 and HCO_3^-) never reaches concentrations required to cause carbon limitation or photoinhibition of photosynthesis, as observed experimentally in *Lobophora* (Holbrook et al. 1988). Taken together, there is no indication that any of the major physiological controllers of algal productivity were limiting for *Lobophora* at our mesophotic sites in the Bahamas, to a depth of at least 61 m suggesting a biotic control on its lower depth distribution (e.g., Spalding et al. 2003).

Lobophora from the upper mesophotic reef zone at BW and BAT lack feeding deterrent properties, both in its crude extract (that includes bioactive phlorotannins; e.g., Targett and Arnold 1998), as well as a dominant purified compound from the extract with cytotoxic properties (SQDG; Cantillo-Ciau et al. 2010). Phlorotannin concentrations in these populations of *Lobophora* can exceed levels that have caused feeding deterrence to grazing invertebrates and fish in other sites (e.g., Boettcher and Targett 1993). However, our data support the hypothesis that *Lobophora* lacks effective chemical defenses against the dominant herbivorous fishes on shallow and deep reefs in the Caribbean (Lewis 1985) and in the Pacific (Steinberg and Paul 1990). Our findings also suggest that phlorotanins in general do not function as effective feeding deterrents in tropical systems, except where chemically tolerant herbivores are locally absent. In fact, in situ observations of herbivorous fishes (i.e., Acanthurids and Scarids) grazing on *Lobophora* within our recruitment plots suggest that these algal populations lack appropriate chemical defenses to deter these

ecologically relevant consumers. Moreover, deep reefs often contain fewer herbivores than shallow reefs (Brokovich et al. 2010, Lesser and Slattery 2011), so the selective pressure for grazer deterrent compounds is likely to be reduced since physical parameters that influence growth are relatively constant across depth (this study; but see Hay 1981). Thus, our data suggest that herbivory acted as a limit to the depth distribution of *Lobophora* at BW and BAT prior to the lionfish invasion and the subsequent decrease of this functional guild of fish (Lesser and Slattery 2011). In fact, significantly fewer herbivores were observed at 30 m, and none at 61 m, in May 2009, 2–3 years after the lionfish population increase and expansion into the mesophotic zone. At that point, *Lobophora* cover was as high, or higher, than on many local shallow reefs (Slattery and Lesser pers. obs.). The fact that there was available substrate for *Lobophora* recruitment, as well as excess nutrients, and a lack of other stressors, throughout the study period, supports our hypothesis that deep reef algae were subject to top-down control (e.g., Hughes et al. 1999) prior to the lionfish invasion.

Observations of field interactions between *Lobophora* and the coral, *M. cavernosa*, or the sponge, *A. clathrodes*, provided initial support for contact-mediated competition between these species. However, contact experiments in the laboratory were inconsistent with these results. The difference in time scales between the field observations (weeks to months) and the laboratory assays (96 h) likely account for this discrepancy, and a recent field-based study by Rasher and Hay (2010) indicates that significant effects are manifested over 20 d. The crude extract and a purified compound, SQDG, from *Lobophora* caused beaching in *M. cavernosa* and cytotoxic responses in *A. clathrodes* further supporting contact-mediated and water-borne allelopathy respectively. Moreover, these effects were similar to those observed in field interactions. Given the time-frame and nature of our laboratory assays, which likely released allelopathic compounds from inside algal cells (e.g., Dworjanyn et al. 1999), it is possible that these compounds are exuded by *Lobophora* more slowly in the field (Jennings and Steinberg 1994), although they may be diluted by flow under specific deep reef conditions (i.e., tidal bores; Leichter et al. 2006). *Lobophora* is increasingly recognized as a competitive dominant of corals and sponges (Jompa and McCook 2002, Foster et al. 2008, Gonzalez-Rivero et al. 2012), although previous evidence for allelopathy has been inconsistent (Box and Mumby 2007, but see Rasher and Hay 2010). Despite differences in assay procedures, *Lobophora* extracts were significantly more active against corals and sponges than was SQDG in isolation (Figs. 3 and 4). These data indicate that multiple compounds are likely acting in an additive manner within the crude extract (e.g., Slattery et al. 2008, Rasher et al. 2011). Phlorotannins serve a variety of structural and defensive roles within brown algae (Targett and Arnold 1998), and as dominant constituents of the *Lobophora* crude extract (Arnold et al. 1995, Targett et al. 1995), it is possible these compounds account for the observed differences in bioactivity between the crude extract and SQDG (Lau and Qian 2000). However, brown algae often contain a diverse suite of secondary metabolites, and minor constituents can serve important ecological roles against potential herbivores and competitors (Deal et al. 2003, Rasher et al. 2011). For example, SQDG is a minor component of the *Lobophora* extract (= 0.034% of the algal dry mass) but it accounts for over 75% of the observed cytotoxicity. While our assays did not assess specific mechanism (s) of action, SQDG is known to inhibit DNA polymerase, enhance immunosuppression, and kill tumor cells and microbes (Cantillo-Ciau et al. 2010), so it is possible that similar mechanistic pathways are responsible for the coral bleaching and sponge necrosis responses observed. Our results add to recent discoveries on the importance of bioactive natural products in competitive interactions between corals and algae (e.g., Rasher and Hay 2010, Rasher et al. 2011).

Decreased herbivory appears to have provided an opportunity for *Lobophora* to advance from a depth of 30–61 m (Lesser and Slattery 2011), where it replaced corals and sponges, likely via contact and/or water-borne mediated interactions, and ultimately has become the dominant space occupying species in the upper mesophotic zone. These results are apparently contradictory to those from the deep reefs (30–40 m) of Curacao where recent increases in *Lobophora* benthic cover followed the loss of corals to bleaching, disease and storm damage (Nugues and Bak 2008), which suggested that corals maintained some control over the distribution of *Lobophora*. In fact, Ruyter van Steveninck et al. (1988) documented a 35% reduction in *Lobophora* growth rates when in close proximity to five species of coral; coral overgrowth, however, was possible when herbivory was relaxed due to the high turnover rates of algal blades. Similarly, Diaz-Pulido et al. (2009) reported a rapid recovery in coral cover following a *Lobophora* population explosion on the Great Barrier Reef, however, they noted specific differences between Caribbean and Pacific coral reefs, including the temporal and spatial scale of the disturbance that likely resulted in the coral resilience they observed. In contrast, *Lobophora* out-competed corals when herbivory was relaxed (Jompa and McCook 2002), and there is compelling evidence that reduced herbivore diversity and abundance can lead to *Lobophora*-mediated phase shifts on the Great Barrier Reef (Cheal et al. 2010). These data indicate that interactions between *Lobophora* and corals/sponges are more complex than phase shift paradigms suggest (Dudgeon et al. 2010), and that

competitive dominance by *Lobophora* is manifested in the presence of a significant and continuous disturbance such as overfishing or the lionfish invasion.

Macroalgae have also been indirectly implicated in coral mortality by increasing dissolved organic carbon (DOC) release that changes the microbial community and causes microbe-induced necrosis (Smith et al. 2006, Vega-Thurber et al. 2012). One essential component of this indirect effect is that the increase in microbial activity results in zones of hypoxia adjacent to coral tissues that facilitates the activity of bacteria that are opportunistic pathogens (Barott et al. 2012). However, recent studies have shown that these zones of hypoxia or anoxia associated with algal interactions are not significantly different from those normally experienced by corals at night (Wangpraseurt et al. 2012) and that microbial respiration contributes little to these zones (Brown and Carpenter 2013), which is consistent with previous tissue measurements of oxygen concentration around and in coral tissues (Dykens and Shick 1982, Kuhl et al. 1995). Additionally, allelochemicals released by *Lobophora* have been shown to be effective inhibitors of fungal and bacterial growth (Kubanek et al. 2003, Morrow et al. 2011 respectively). Therefore, the change in the mesophotic coral/sponge community is likely due to *Lobophora* allelopathy, and not to any indirect effects (i.e., hypoxia mediated by *Lobophora*-released DOC) of this increase in algal percent cover.

The physiological constraints of conducting research at mesophotic reef depths preclude traditional manipulative ecology experiments. However, a combination of water column sampling for the abiotic conditions experienced by *Lobophora* at depth, as well as field surveys, and laboratory assays using ecologically relevant predators and competitors, have provided significant insights into the mechanistic processes that control deep reef communities. Specifically, there is no *a priori* abiotic rationale for the limited depth distribution of *Lobophora* pre-lionfish. Our laboratory assays and field observations also indicate that *Lobophora*, and its propagules, is actively consumed by herbivorous and omnivorous fishes that were present on these reefs prior to the lionfish invasion, but significantly reduced post-lionfish. In addition, compounds within *Lobophora* have the potential to cause physiological damage to corals and sponges from the deep reef. Thus, the most likely mechanistic explanation for the replacement of corals and sponges on these mesophotic reefs is allelopathic competition.

The BW and BAT mesophotic reefs have undergone a major phase shift from coral/sponge dominance to *Lobophora* dominance to depths of 61 m (Lesser and Slattery 2011). While deep reefs have been viewed as potential refugia for stressed shallow water species (Lesser et al. 2009, Bongaerts et al. 2010), and as more stable communities than shallow reefs (Bak et al. 2005), our data indicate that this is not always the case. The BW and BAT deep reef structure had been stable for at least a decade (Aponte and Ballantine 2001, Slattery and Lesser pers. obs.), but this phase shift, in a relatively short time span (= 2–3 years; Lesser and Slattery 2011) underscores the ephemeral quality of these communities (Stokes et al. 2010). *Lobophora* colonization from 30 to 61 m occurred despite limited propagule dispersal potential, likely due to reduced grazer pressure (Ruyter van Steveninck and Breeman 1987). Cover and biomass of *Lobophora* in the upper mesophotic zone (= 30–61 m) now equals or exceeds levels recorded on regional shallow coral reefs (Ruyter van Steveninck and Breeman 1987, Renken et al. 2010). This phase shift is almost certainly due to a lionfish-mediated trophic cascade. Specifically, lionfish predation on herbivorous fish guilds and mesograzers has removed a constraint on algal growth, although this selective pressure was arguably never great (Brokovich et al. 2010, Lesser and Slattery 2011). However, it is possible that previous levels of herbivory on the deep reefs were more important in removing propagules instead of biomass (e.g., Carpenter 1981, Ruyter van Steveninck and Breeman 1987, Morrison 1988), and observations of grazing foci within our recruitment plots seem to support this hypothesis. While *Lobophora* blades show evidence of Acanthurid grazing, the substrate representing CCR, turf, and potentially *Lobophora* propagules was heavily impacted by Scarids (Slattery pers. obs.). More germane to the community dynamics is the fact that *Lobophora* has the capacity to compete successfully with a locally important coral, *M. cavernosa*, and sponge, *A. clathrodes*, of the deep reefs (Lesser et al. 2010, Garcia-Sais 2010, respectively), in contrast with reports from other regions (Ruyter van Steveninck et al. 1988, Diaz-Pulido et al. 2009). The traditional mechanism of algal competition (i.e., shading; McCook et al. 2001) is probably less important on deep reefs that are characterized by reduced irradiances and low-light adapted species (Lesser et al. 2009). Instead, allelopathic interactions, whether contact-mediated or water-borne, could be more important at these depths (e.g., Gross 1999), and *Lobophora* is apparently a strong competitor (Jompa and McCook 2002, Foster et al. 2008, Gonzalez-Rivero et al. 2012). Rasher and Hay (2010) demonstrated that allelopathic algae, including shallow-water *Lobophora* from Panama, do inhibit coral health with implications for community resilience. It is likely that these same effects will drive the deep reef community structure of the Caribbean from a stable coral/sponge dominated system, to one characterized by fleshy macro-algae (Lesser and Slattery 2011). Although these algae ultimately will be constrained by the photosynthetic compensation depth (about 80 m on BW and BAT; Lesser et al. 2009), the loss of grazers to the invasive lionfish removes one bar-

rier to algal dispersal to these depths, and likely will lead to continued loss of deep reef community diversity.

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