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Productivity links morphology, symbiont specificity and bleaching in the evolution of Caribbean octocoral symbioses

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Many cnidarians host endosymbiotic dinoflagellates from the genus *Symbiodinium*. It is generally assumed that the symbiosis is mutualistic, where the host benefits from symbiont photosynthesis while providing protection and photosynthetic substrates. Diverse assemblages of symbiotic gorgonian octocorals can be found in hard bottom communities throughout the Caribbean. While current research has focused on the phylo- and population genetics of gorgonian symbiont types and their photo-physiology, relatively less work has focused on biogeochemical benefits conferred to the host and how these benefits vary across host species. Here we examine this symbiosis among 11 gorgonian species collected in Bocas del Toro, Panama. By coupling light and dark bottle incubations (P/R) with ^{13}C-bicarbonate tracers, we quantified the link between holobiont oxygen metabolism with carbon assimilation and translocation from symbiont to host. Our data show that P/R varied among species, and was correlated with colony morphology and polyp size. Sea fans and sea plumes were net autotrophs (P/R > 1.5), while nine species of sea rods were net heterotrophs with most below compensation (P/R < 1.0). ^{13}C assimilation corroborated the P/R results, and maximum $\delta^{13}C_{host}$ values were strongly correlated with polyp size, indicating higher productivity by colonies with high polyp SA:V. A survey of gorgonian-*Symbiodinium* associations revealed that productive species maintain specialized, obligate symbioses and are more resistant to coral bleaching, whereas generalist and facultative associations are common among sea rods that have higher bleaching sensitivities. Overall, productivity and polyp size had strong phylogenetic signals with carbon fixation and polyp size showing evidence of trait covariance.
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Introduction

Since the discovery of coral-hosted *Symbiodinium*, nutrition has been a major focus of coral symbiosis. Hand and Muscatine (1958) were among the first to demonstrate that *Symbiodinium* provide photosynthates to their hosts, thus settling debate over the functional significance of the intracellular algae. Since then, numerous studies have quantified the contribution of autotrophic and heterotrophic nutrition to the coral holobiont for a number of scleractinian species, even to sub-cellular scales (Yellowlees *et al.*, 2008; Houlbreque and Ferrier-Pages, 2009; Pernice *et al.*, 2014). We now know that *Symbiodinium* translocate carbon-based photosynthates in excess of host needs and nitrogen in the form of amino acids derived from host wastes and dissolved inorganic nitrogen from the water column. By coupling the coral polyp's opportunistic heterotrophy with *Symbiodinium* photosynthesis, the coral holobiont can thrive despite the severe nutrient limitation typical of tropical seas (Yellowlees *et al.*, 2008; Houlbreque and Ferrier-Pages, 2009). However, holobiont performance and host benefits are poorly understood across different host–symbiont interactions and changing environmental conditions.

Our understanding of how coral–*Symbiodinium* interactions vary across diverse associations remains limited. Most studies have focused on several model coral species such as *Stylophora pistillata*, *Pocillopora damicornis* and members of the genus *Acropora* (Muller-Parker *et al.*, 1994; Hoegh-Guldberg and

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Williamson, 1999; Grover et al., 2008). These species of photophilic corals all possess branching colony morphologies and small polyps and are thus too similar in their morphology and nutritional physiology to reveal the full breadth of coral–*Symbiodinium* functional diversity (Tremblay et al., 2012; Baker et al., 2013). Fewer nutritional studies have compared species within and between genera and even fewer in non-scleractinian anthozoans (Lasker, 1981; Lasker et al., 1983; Wilkerson and Muscatine, 1984; Ribes et al., 1998; Swanson and Hoegh-Guldberg, 1998). Such critical gaps limit our understanding of the functional, ecological and evolutionary significance of these symbioses.

The evolution of feeding structures occurs when species compete for limited nutritional resources (Grant and Grant, 2006). For corals, niche partitioning and competition likely drive the diversity of colony and polyp morphologies. Divergence towards the utilization of novel resources at the extremes of the heterotrophic – autotrophic continuum (that is, from plankton to dissolved inorganic nutrients) can reduce competitive interactions and drive speciation. Porter (1976) argued that variation in coral colony morphology, specifically the ratio of surface area to volume (SA:V), is central to understanding the nutritional strategies of scleractinian corals. Indeed, Porter predicted that autotrophy should be maximized in corals with high SA:V, with the specific idea that branching corals would exhibit higher photosynthesis/respiration (P/R) relative to massive (non-branching) species. Moreover, such a relationship would promote the evolution of 'layered' colony morphologies, which Porter identified as a convergent character in terrestrial plants. At a smaller scale, variation in polyp size is often posited as an indicator of heterotrophic capacity as larger polyps possess larger tentacles and gastrovascular cavities for prey capture and digestion. Conversely, small polyps have higher SA:V, which maximizes the light received by *Symbiodinium*, inhabiting the gastroderm at densities up to 10^6 cells cm^{-2}, and increases the surface area for nutrient uptake. Taken together, increasing or decreasing SA:V can occur at the colony and polyp scale. Here we test Porter's hypothesis by quantifying productivity (P/R and ^{13}C assimilation) and utilizing morphological, phylogenetic and ecological data on a diverse collection of Caribbean octocorals (gorgonians).

In the Caribbean Sea, gorgonians hosting *Symbiodinium* are especially diverse with at least 51 described species (Sánchez and Wirshing, 2005). Moreover, there is great variation in gorgonian morphology, with many reefs dominated by species with fan, plume or rod morphologies. Perhaps due to the morphological similarity to aposymbiotic deep water species, and the paucity of symbiotic species in the Indo-Pacific, there is a perception that symbiotic gorgonians gain most of their nutrition via passive suspension feeding (Leal et al., 2014). To date, the evidence to support this contention is limited. Indeed, symbiotic gorgonians differ in their ability to capture particles from the water column (Lasker, 1981). Although some species obtain sufficient carbon from particulate organic matter (Murdock, 1978; Coffroth, 1984), they still require nitrogen supplemented via *Symbiodinium* photosynthesis (Wainwright, 1967). Unlike Indo-Pacific soft corals (Fabricius and Klumpp, 1995) previous studies of Caribbean gorgonian productivity (as determined by oxygen metabolism) support a predominantly autotrophic nutrition in many species.

Here we expand on previous gorgonian productivity studies by using light and dark bottle incubations to estimate P/R in combination with inorganic stable carbon isotope tracer additions to assess autotrophic productivity among 11 species of symbiotic gorgonians. We test the following null hypotheses: (1) all gorgonian symbioses are mutualistic where the host benefits from symbiont photosynthesis, (2) the mutualisms are invariant with respect to the amount of carbon translocated to the host and (3) the host-derived benefits of mutualism are unrelated to morphology, (that is, polyp size) of a species. Further, we examined the functional significance of varied productivity through a posteriori correlations between productivity measures and eco-physiology, including symbiont specificity and thermal bleaching resistance.

Materials and methods

Field collections

Five whole colonies of 11 gorgonian species were collected using SCUBA from reefs nearby the Smithsonian Tropical Research Institute's (STRI) field station in Bocas del Toro, Panama in September 2010. A total of three sites (depths of 3–8 m) were visited to obtain a diverse collection. The sea plume, *Antillogorgia acerosa* (AACE), and sea rods *Pterogorgia anceps* (PANC) and *Eunicea succinea* (ESUC) were collected from Boca del Drago (9°25'21.52"N; 82°19'35.78"W). The sea rods *Plexaura homomalla* (PHOM), *Plexaurella fusifera* (PFUS), *Plexaurella nutans* (PNUT), *Eunicea calyculata* (ECAL), *Eunicea flexuosa* (EFLE), *Eunicea mammosa* (EMAM), sea plume *Antillogorgia acerosa* and sea fan *Gorgonia ventalina* (GVEN) were sampled from Crawl Cay Channel (9°15'36.88"N; 82°6'32.78"W). The sea rod *Briareum asbestinum* (BASB) was collected near South Carenaro Island (9°20'36.19"N; 82°14'17.15"W). A subsample from each collected specimen was treated with dilute bleach to isolate sclerites, which were used to establish species identification following Bayer (1961). Calyx depth, the distance from the surface edge of the aperture (where the polyp emerges from the colony) to the base where the polyp is anchored within the colony, was used as a proxy for polyp size (Thibaudeau, 1983). The calyx is the anatomical space occupied by a single polyp, from which the polyp can extend into the environment.

P/R and ^{13}C Incubation Experiment
Detailed experimental procedures are provided as Supplementary Online Material. In short, five colonies of each gorgonian species were fragmented into three clonal replicates, with one replicate preserved as an initial control. The remaining replicates were incubated in light and dark bottles for an assessment of net primary production and respiration, respectively, measured as change in dissolved oxygen concentration following Freeman *et al.* (2013). Seawater in each bottle was amended with 98% $NaH^{13}CO_3$, such that the resulting carbon stable isotope ratios ($\delta^{13}C$) could be used to trace photosynthetic assimilation by *Symbiodinium* and subsequent transfer to host cells (Baker *et al.*, 2013; Freeman *et al.*, 2013).

Stable isotope analysis
For details of sample preparation please refer to the Supplementary Online Methods. In brief, each dried gorgonian fragment was physically homogenized, and the host and symbiont fractions were separated using centrifugation of a suspended homogenate. The supernatant (host) and pellet (symbiont) were dispensed into silver capsules, acidified and dried before stable isotope analysis.

All samples were analyzed using a Thermo Delta V isotope ratio mass spectrometer coupled to a Carlo-Erba NC2500 elemental analyzer via a Conflo III open-split interface at the Geophysical Laboratory. Analytical precision was determined by repeated analysis of an internal acetanilide standard ('acet 6'; 70% C). Standard precision during runs of initials and dark incubated enriched samples was +/− 0.2‰, and +/− 0.4‰ for the light incubated enriched samples.

Gorgonian phylogenetic signal and trait correlation
Sequences of two mitochondrial protein-coding genes, *ND2* and *msh1*, were obtained from GenBank to construct a phylogeny of the species sampled in this study (McFadden *et al.*, 2006; for details of tree construction see Supplementary Online Methods; Supplementary Table S2). Phylogenetic signal describes the degree to which more closely related organisms share more similar traits. We used the 'phylosignal' function of the R package Picante to test whether P/R ratios, $\delta^{13}C_{host}$, $\delta^{13}C_{symbiont}$, C:N$_{host}$, C:N$_{symbiont}$ and calyx depth displayed significant phylogenetic signal given the host phylogeny (that is, whether more similar values were associated with more closely related gorgonians more often than expected by chance; refer to Supplementary Online Methods for further detail and references).

A posteriori tests
Following our experimental work, we obtained data from (1) GeoSymbio, a quality controlled database containing phylogenetic data on *Symbiodinium* isolated from diverse invertebrate hosts including gorgonians (Franklin *et al.*, 2012), and (2) Prada *et al.* (2010), the most comprehensive report on gorgonian thermal bleaching (loss of *Symbiodinium*) from the 2005 mass coral bleaching event in Puerto Rico. These data were used to test the hypotheses that symbiont specificity and bleaching resistance are correlated with our productivity measurements (P/R, $\delta^{13}C_{host}$ and $\delta^{13}C_{symbiont}$).

Results

Gorgonian productivity
P/R values varied significantly among the 11 species of Caribbean gorgonians measured (ANOVA; $F = 5.26$; $P < 0.0001$; Figure 1). All sea rods had average P/R values <1.5, which is below a conservative threshold for autotrophy (Wilkinson, 1983). Five out of nine sea rod species had mean values below compensation (P/R = 1.0). In contrast, a sea fan (*G. ventalina*) and sea plume (*A. acerosa*) were both net autotrophs with P/R ~ 1.8 with some replicate fragments exceeding 2.5. P/R was strongly correlated with autotrophy (NPP$_{light}$; $R^2 = 0.84$, $P < 0.0001$, $n = 11$), not respiration (NPP$_{dark}$; $R^2 = 0.16$, $P = 0.22$, $n = 11$; Supplementary Table S1). However, the highest dark bottle respiration was observed in *A. acerosa* (mean NPP = − 2.03 mg O_2 l^{-1} g^{-1} h^{+}) and generally declined with increasing colony volumes with *P. nutans* and *P. fusifera* having the lowest observed respiration (Supplementary Table S1).

$\delta^{13}C$ - natural abundance and tracer experiment
Natural abundance $\delta^{13}C$ ranged from − 20.4 to − 14.9‰ with a mean of − 17.3‰. There was a strong correlation between the mean natural abundance $\delta^{13}C_{host}$ and $\delta^{13}C_{symbiont}$ inclusive of all species ($R^2 = 0.67$; $P = 0.002$; Supplementary Figure S2). $\delta^{13}C$ values increased to a mean of − 6.9‰ and 261‰ in the dark and light incubations, respectively (ANOVA; F = 252, $P < 0.001$; Figure 2). In most species, ^{13}C enrichments in the light treatment were similar between the host and symbiont fractions (Figure 2a), but the light bottle $\delta^{13}C_{symbiont}$ was a better predictor of holobiont P/R than the corresponding $\delta^{13}C_{host}$ ($R^2 = 0.84$ vs 0.67, respectively; Supplementary Figure S2). Fragments incubated in the dark took up less ^{13}C owing to a lack of photosynthesis (Figure 2a). Yet, significant ^{13}C assimilation was observed in the host fractions of many species (Figure 2b). The $\delta^{13}C_{host}$ of *P. anceps*, *A. acerosa* and *B. asbestinum* incubated in the dark were all $<10‰$, greater than initial values, but much lower than the $\delta^{13}C_{host}$ values from the light bottle treatment. In fact, all host fractions from the dark treatment were, on average, +11.8‰ enriched relative to their *Symbiodinium*.
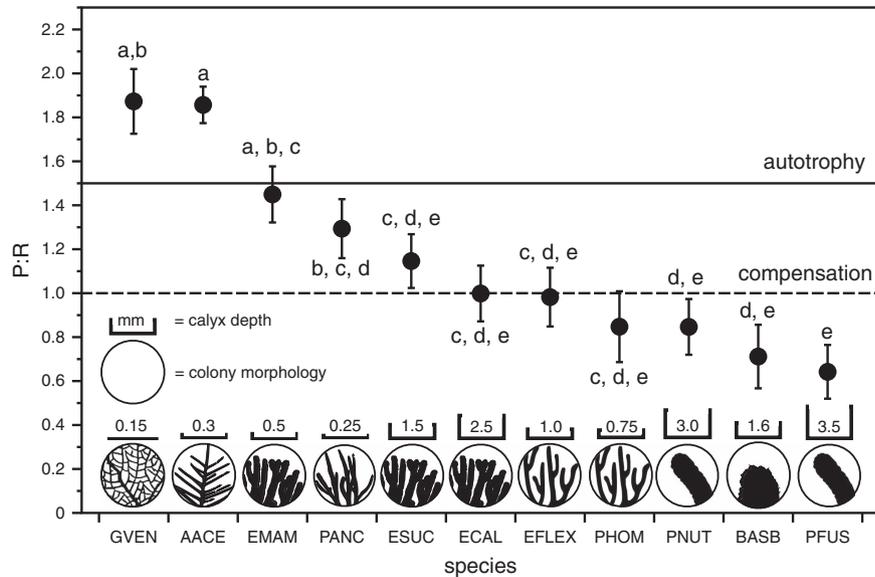
Figure 1 Least-squared mean P/R values from 11 species of symbiotic Caribbean gorgonians ordered from highest to lowest values. Dashed line defines compensation (where P/R = 1.0), and the solid line a conservative threshold for autotrophy (P/R > 1.5; (Wilkinson, 1983)). P/R was calculated from at least four pairs (one light, one dark treatment) of similarly sized clonal fragments from independent colonies ($n \geq 4$). Error bars represent s.e.m. and letters indicate significant differences between groups as determined by Tukey's *post hoc* test ($P < 0.05$). For species abbreviations refer to the Methods section. Colony morphology (not to scale) and calyx depth (to scale in mm) are shown for reference.
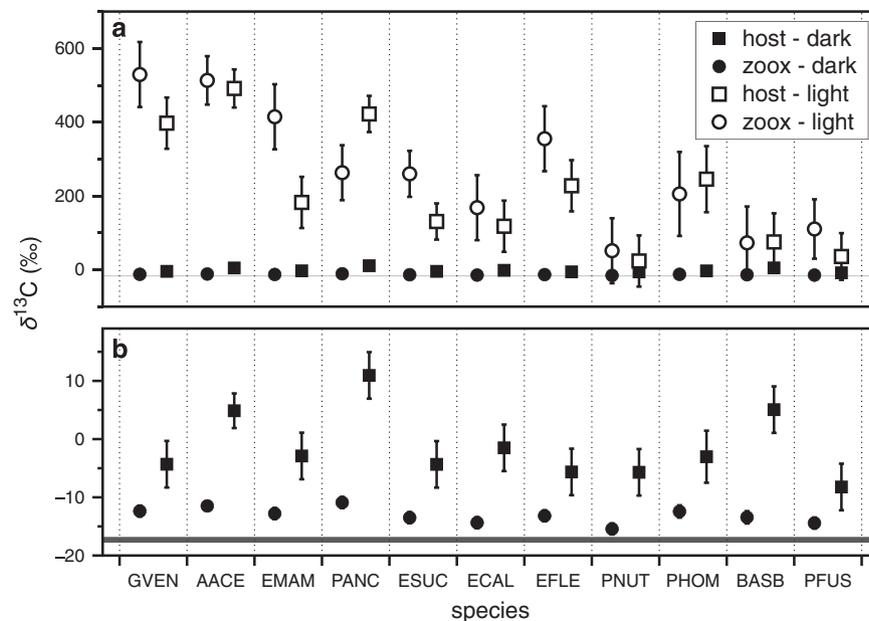

Figure 2 (a) Mean $\delta^{13}C$ of 11 species of gorgonians and their *Symbiodinium* after a ~ 7.5 h incubation in dark bottles (filled shapes) or light bottles (open shapes). (b) The same data for host and symbiont fractions incubated in darkness and with enlarged scale to illustrate differences. Gray horizontal lines indicate the upper and lower 95% confidence intervals of the mean natural abundance $\delta^{13}C$ values for all species (initial samples). Species abbreviations are listed in the Methods section and are presented in order of decreasing P/R. Error bars represent the 95% confidence interval of the mean.

Phylogenetic signals in metabolic traits

The phylogeny of Octocorallia used in this study is a subset of the phylogeny reported by McFadden *et al.* (2006) based on *ND2* and *msh1* sequences. We found strong support for clades representing the genera *Plexaurella* and *Eunicea*, as well as a clade containing *Antillogorgia* and *Gorgonia* (Figure 3). Four traits displayed significant phylogenetic signal, including

the enriched $\delta^{13}C_{host}$ and $\delta^{13}C_{symbiont}$ values, P:R and calyx depth (Supplementary Table S3). The two most significant traits, $\delta^{13}C_{host}$ and calyx depth, demonstrated a significant negative correlation under both models of trait covariance (BM: $r = -0.450$, $P = 0.044$; OU: $r = -0.83$, $P < 0.001$; Figure 3). Calyx depth was negatively correlated with P/R ($R^2 = 0.65$; $P = 0.005$; Figure 4).
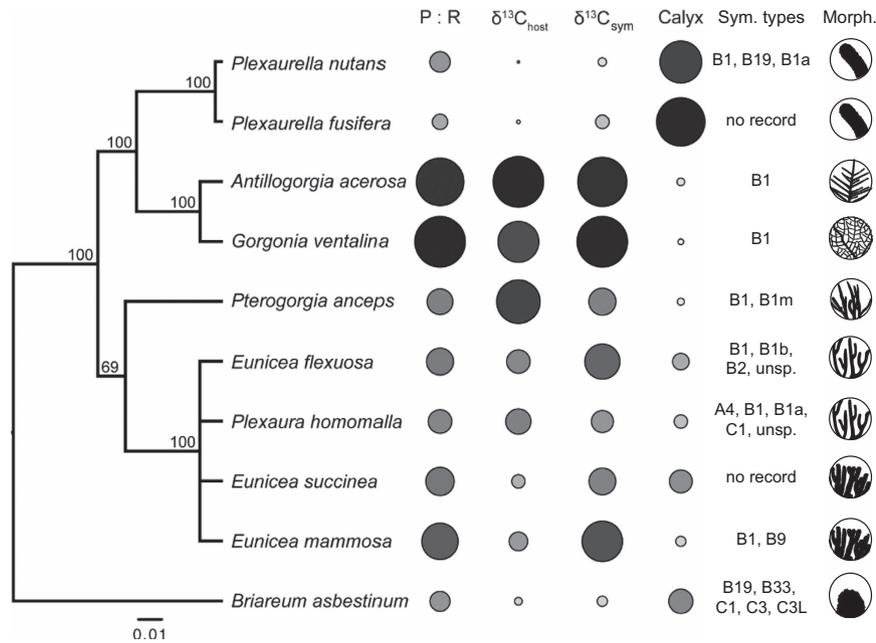
Figure 3 Phylogeny of Octocorallia used in this study, derived from sequences reported by McFadden *et al.* (2006). The scale bar indicates number of substitutions per site, while numbers at nodes indicate percentage Bayesian posterior probabilities. To the right of the phylogeny, four columns of circles represent values of photosynthesis to respiration ratios (P/R), $\delta^{13}C_{host}$, $\delta^{13}C_{symbiont}$ and calyx depth (used as a proxy for polyp size). The size and shading of each circle are proportional to the values reported in Supplementary Table S1. In addition, the known *Symbiodinium* associations are shown as well as the colony morphology (not to scale). 'unsp.' Indicates a unique *Symbiodinium* genotype with no clade designation.

A posteriori tests – specificity and bleaching
Using quality-controlled records obtained from the GeoSymbio database (Franklin *et al.*, 2012), we found that the gorgonian species studied herein host one to five unique *Symbiodinium* types throughout the Caribbean Sea (Figure 3; Supplementary Table S1). *G. ventalina* and *Antillogorgia* have only been found in association with clade B1 suggesting a highly specific partnership. In contrast, records from all sea rods suggest more generalist associations with low specificity for a given *Symbiodinium* type, ranging from two reported species in *Pterogorgia* and *E. mammosa*, up to five in *B. asbestinum* including B19, B33, C1, C3 and C3L (Figure 3; Supplementary Table S1). Taken together, 85% of the variation in holobiont P/R was explained by the number of known *Symbiodinium* genotypes associated with each host species (Figure 5).

Using data extracted from Prada *et al.* (2010) for 8 of 11 of our studied species, we found that P/R explained 33% of the variation in bleaching prevalence (Figure 6). However, when *P. anceps* was excluded from this analysis, the explained variation improved to 96%. Excluding *P. anceps* may be appropriate as this species often exhibits white polyps that could be mistaken for a bleached condition (pers. obs.). The logistic fit illustrates that species with P/R values <1.1 are highly susceptible to bleaching. Conversely, corals bearing small polyps with high P/R appear to resist bleaching.
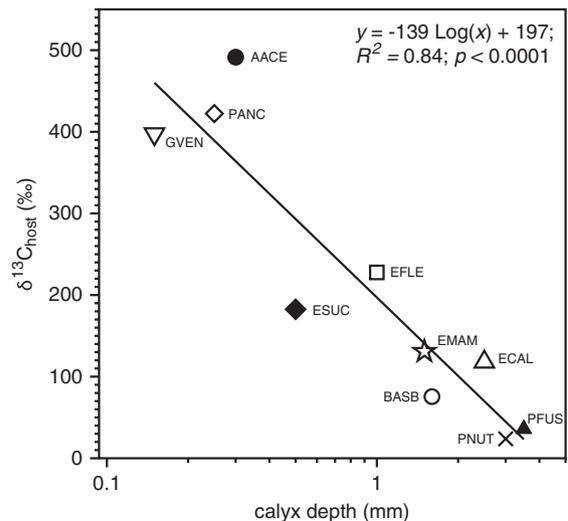

Figure 4 Enriched $\delta^{13}C_{host}$ values as a function of calyx depth (used as a proxy for polyp size) for eight species of gorgonians (data from Thibaudeau, 1983). Solid line represents a significant relationship between the variables. For species abbreviations refer to the Methods section.

Discussion

Gorgonian productivity varies with morphology
Our data show that the sea fan *G. ventalina* and plume *A. acerosa* produced >2 times their required carbon demand with P/R values similar to many scleractinian corals. As such, we conclude that these species are net autotrophs, acquiring the majority of
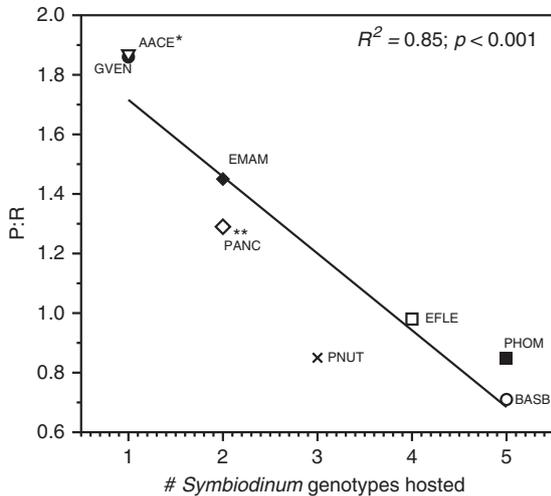
Figure 5 Observed *Symbiodinium* genotypes (based on ITS2) obtained from the GeoSymbio database, representing eight gorgonian species analyzed in this study. All $n \geq 3$. *Indicates P/R data for *A. acerosa*, using symbiont records for *A. elisabethae* and *A. bipinnata* as there are no records for *A. acerosa*. **Indicates P/R for *P. anceps* and symbiont records for all *Pterogorgia* as there was only one record for *P. anceps*. For species abbreviations refer to the methods section.
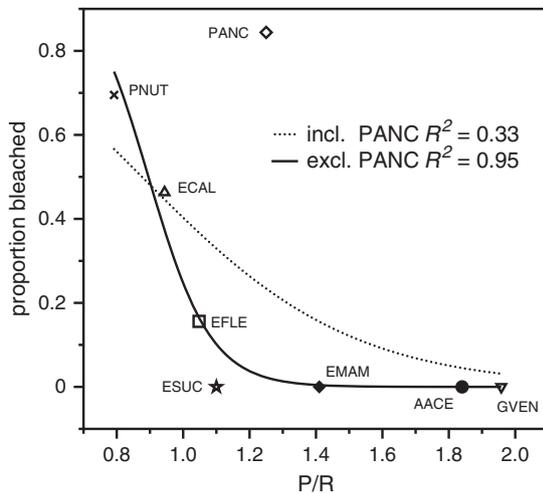

Figure 6 Observed prevalence of bleaching of eight gorgonian species during the 2005 mass bleaching in Puerto Rico (data from Prada *et al.* (2010)) as a function of gorgonian P/R. The dashed line represents a logistic function fit to all data, whereas the solid line represents a logistic function fit to all data excluding *P. anceps*. For species abbreviations refer to the Methods section.

their nutrition from *Symbiodinium* photosynthesis. This contrasts with all sea rod species that had P/R < 1.5 and all Indo-Pacific alcyonaceans that have negative P/R values (Fabricius & Klumpp, 1995). Species with fan or plume morphologies and small polyps might maximize light exposure and optimize symbiont densities and nutrient exchange via increased SA:V. Similar observations have been made for Indo-Pacific sponges, which are more productive than their Caribbean counterparts with highly autotrophic species exhibiting flattened

morphologies (encrusting, fan, dish) that are convergent traits in corals (Wilkinson, 1983).

In one of the first studies of gorgonian metabolism, Cary (1918) measured respiration in 12 Caribbean species and found that the highest rates of respiration were observed in species with the highest SA:V (that is, *Gorgonia, Antillogorgia* and *Pterogorgia*). Thus, we hypothesize that some gorgonian species are more metabolically active than others, as positive NPP is maintained by high rates of photosynthesis, despite substantial respiration. Therefore, observed variations in P/R are due to overall metabolic rates rather than variation in either photosynthesis or respiration alone. Like Kanwisher and Wainwright (1967), we found a wide range of P/R values for Caribbean gorgonians, which followed a general pattern of increasing P/R with SA:V. We stress, however, that comparisons among disparate studies should be interpreted with caution, as the method of oxygen measurement and environmental conditions can vary considerably between studies. Nevertheless, it is interesting that Caribbean gorgonians have a similar range of P/R values to some Indo-Pacific hard corals, although our data are lower than previous reports (Roffman, 1968).

Porter (1976) put forth several hypotheses about functional morphology of corals that are consistent with our observations on gorgonians. First, the parallels drawn between tree morphology (*sensu* Horn (1971)) are particularly striking as the most autotrophic species (*G. ventalina* and *A. acerosa*) have morphologies similar to the broad leaves and needles of trees, respectively, which are plant adaptations for enhancing photosynthesis while moderating transpiration. In sea plumes, alternating branches reduce self-shading and permit light transmission to lower portions of the colony. Sea fans have a reticulate and planar morphology with high surface area. Over time, sea fans twist their axial skeleton to a position perpendicular to water flow (Wainwright and Dillon, 1969). It has been suggested that fan morphologies aid in particle capture (Leversee, 1976). While this may be true for deep-water sea fan species, for *G. ventalina* found in high energy, shallow environments this orientation facilitates the oscillation of the colony from full illumination to shade in synchrony with the wave period. This also permits the photosynthetic centers to recover from light saturation and maximize photosynthetic efficiency over the course of the day. Colonies uprooted or twisted from this position often show heavy melanization (purpling) in response to excessive light stress to one face of the fan, whereas adjacent colonies with normal skeletal development/positioning exhibit normal coloration (pers. obs.).

Alternatively, the cylindrical or bladed, digitate and branching forms common to the various genera of sea rods may be more suited to capturing plankton and particulate matter, especially those with larger polyp sizes. Thick, rounded colony branches may function to generate eddies on the side of the branch opposite an oncoming current. Such eddies can

cause plankton and particulate matter to linger, affording a polyp more opportunity for capture (Leversee, 1976). There is limited evidence to suggest that larger polyps facilitate prey capture. Lasker et al. (1983) found that two sea rods, Plexaura homomalla and P. nina, found on the shallow and deep forereef, respectively, differed in their prey capture abilities. With twice the polyp size, P. nina was able to capture more particles. It is interesting that the difference in polyp size and distribution of these congeners occurs over a depth gradient, which suggests that larger polyps are advantageous in deeper habitats where light is limiting.

$\delta^{13}C$ as an indicator of trophic position

The natural abundance isotope values of gorgonians are useful indicators of both diet and localized variation in the base of the marine food web. Our data show a wide range in $\delta^{13}C$ values (nearly 3.5‰) and a strong correlation between the $\delta^{13}C_{host}$ and $\delta^{13}C_{symbiont}$, indicating either a common carbon source or tight cycling of carbon between host and symbiont (Supplementary Figure S1). Interestingly, there is little support for utilization of POM in the gorgonian diet. POM from Bocas del Toro had a $\delta^{13}C$ of − 23‰ (Freeman and Thacker, 2011), which is a reflection of a large terrestrial contribution to Almirante Bay (Aronson et al., 2014). The average $\delta^{13}C$ of gorgonians (−17.4‰) greatly exceeded the 1‰ trophic enrichment expected between a consumer and its diet. Instead, these values may be enriched via photosynthetic assimilation of dissolved inorganic carbon (DIC; $\delta^{13}C_{HCO_3-} \approx 0$‰). As such, gorgonian $\delta^{13}C$ is a weighted average of DIC and POM contributions to the auto- and heterotrophic diet, respectively. Ultimately, we limit our interpretation of these data as different environmental sources and conditions among the three collection sites preclude a detailed view of the trophic position of gorgonians based on natural abundance $\delta^{13}C$. In this regard, the ^{13}C tracer study was effective in highlighting the relative utilization of DIC via photosynthesis among these species.

^{13}C Tracer reveals the benefits of symbiosis

We have shown that Caribbean gorgonians receive important carbon resources from their associated Symbiodinium, which drives significant variation in productivity among species. The autotrophic assimilation of $H^{13}CO_3^-$ in the light bottles was evident in $\delta^{13}C_{host}$ and $\delta^{13}C_{symbiont}$, suggesting a tightly coupled and rapid translocation of ^{13}C-labeled carbohydrates and other biomolecules during the brief incubation (Figure 2a). The higher R^2 observed between $\delta^{13}C_{symbiont}$ and P/R (Supplementary Figure S2) may indicate superior precision of direct quantification of ^{13}C assimilation by the holobiont versus the relatively indirect calculation of productivity based on oxygen metabolism from clonal fragments within separate bottles.

The relative enrichment of $\delta^{13}C_{host}$ attained versus $\delta^{13}C_{symbiont}$ reflects the variation in photosynthate translocated from source (symbiont) to sink (host). Eunicea mammosa and E. succinea had the lowest relative $\delta^{13}C_{host}$ enrichment. Such low $\delta^{13}C_{host}$ could result from several factors including (1) higher symbiont metabolism relative to the host, (2) limited translocation of photosynthate due to lower host SA:V, (3) differential host and symbiont biomass, and similarly, (4) a relatively larger structural pool of carbon in the host fraction, which 'dilutes' any translocated ^{13}C. However, if these factors were important in determining the final ^{13}C assimilation in the host, then similar patterns would have been observed in the Plexaurella, which have the largest polyps, lowest SA:V, and thickest host tissues among the species studied. Given that this is not the case, an alternative explanation is that Symbiodinium within E. mammosa and E. succinea are less mutualistic, retaining more of their photosynthates for their own metabolism. Such observations of cheating have been reported for a variety of microbial symbioses (Bronstein, 2001; Porter and Simms, 2014). This contrasts with species like P. anceps that achieved higher $\delta^{13}C_{host}$ than $\delta^{13}C_{symbiont}$, suggesting a larger carbon subsidy to the host and therefore a more mutualistic relationship. Interestingly, P. anceps had the highest natural abundance $\delta^{13}C$ values reported in this study (Supplementary Figure S1), suggesting a large contribution of autotrophically derived DIC.

Although ^{13}C assimilation was not observed in any dark-incubated Symbiodinium, it was curiously evident in all host fractions (Figure 2b). The former is easily explained by a lack of light to drive photosynthesis. In the dark, host enrichment in the absence of symbiont photosynthesis must be proceeding by anaplerotic pathways, such as fatty-acid synthesis and oxidation (DeNiro and Epstein, 1977) or by associated microbiota. Microbes other than Symbiodinium could contribute to ^{13}C enrichment through similar biosynthetic pathways, as their signal is inseparable from the host fraction. Quantifying the contributions of associated chemoautotrophic microbes such as cyanobacteria and archaea to holobiont productivity and biogeochemical cycling warrants further study. However, the large differences in observed $\delta^{13}C_{host}$ enrichments (>3 to 30-fold) between the light and dark treatment suggest that associated microbe ^{13}C assimilation was minimal relative to Symbiodinium.

We found that not all hosts benefit from Symbiodinium autotrophy. Interestingly, there was no effect of light on Plexaurella or Briareum $\delta^{13}C_{host}$ and $\delta^{13}C_{symbiont}$ (except P. fusifera $\delta^{13}C_{symbiont}$). Given that marginal enrichments of the $\delta^{13}C_{host}$ can be attributed to light-independent assimilation (Figure 2b), we conclude that Symbiodinium hosted by P. fusifera, P. nutans and B. asbestinum did not translocate carbon to their hosts. Indeed, the three species were among the lowest with regards to P/R with mean values <1.0. As such, the interaction between

Symbiodinium and large polyp gorgonian hosts may be more commensal than mutualistic, at least as it pertains to carbon metabolism. However, even though observed ^{13}C assimilation was low in many sea rod species, the role of *Symbiodinium* may still be critically important for obtaining growth-limiting resources such as nitrogen (Ribes *et al.*, 1998).

Symbiont identity and gorgonian productivity

Caribbean gorgonians generally associate with members of *Symbiodinium* clade B (Baker *et al.*, 2013), which is common on Caribbean reefs (Pettay and Lajeunesse, 2007). Certain sub-clade types such as B1 are especially common, leading to the conclusion that this is a generalist symbiont associated with many hosts (LaJeunesse, 2002). However, Finney *et al.* (2010) revealed that this clade contains substantial genetic variation with evidence of highly specialized symbionts among various host species. Such specialization could occur during divergence among host species, such has been reported for depth partitioned lineages of *E. flexuosa* and co-evolution of their *Symbiodinium* (Prada *et al.*, 2014). Our comparison of known *Symbiodinium* types found evidence of symbiont specificity increasing with holobiont productivity (Figure 5). Generalist hosts had lower productivities in comparison with specialists, which exhibited a higher degree of autotrophy. The mechanism underlying the enhanced metabolism afforded by specialized symbioses, including the functional significance of octocoral-associated *Symbiodinium* clade B warrants further investigation (Baker *et al.*, 2013; Pernice *et al.*, 2014).

Bleaching and gorgonian productivity

During the 2005 mass coral bleaching in Puerto Rico only 8 out of 24 gorgonian species showed visible signs of bleaching among four monitored reefs. Remarkably, with the exception of *Muricea*, no species suffered subsequent mortality (Prada *et al.*, 2010). However, the corals that readily bleached were species with larger polyps and, as we have reported here, lower rates of productivity (Figure 6). Considering the observed specificity among highly productive species, we predict that such resistance to bleaching is reflective of the obligate nature of these symbioses. Essentially, bleaching may be disadvantageous to an obligate host if nutrition is derived exclusively from symbionts. This explains the difficulty in experimentally bleaching *G. ventalina* by elevated temperatures (Kirk *et al.*, 2005), prolonged incubation in darkness, or with the herbicide DCMU (Baker, unpublished data). Conversely, in more facultative interactions hosts are more likely to expel symbionts when the costs (such as nutrient retention, increased respiration, or oxidative damage) outweigh the nutritional benefits, especially if the host can alternatively acquire resources easily by heterotrophy. Further work is needed to test these hypotheses among other *Symbiodinium* – cnidarian symbioses.

Gorgonian metabolism and the evolution of symbiosis

Across the diversity of gorgonians, there is a strong negative correlation between traits associated with heterotrophy and autotrophy, reflecting strong evolutionary and ecological trade-offs between their divergent life-history strategies. Indeed, we found a link between productivity and the evolutionary trajectories of host species, which may be driven by habitat specialization and niche partitioning within highly competitive coral reef environments. The strong negative phylogenetic correlation observed between polyp size and carbon translocated from symbiont to host suggests that there is an evolutionary trade-off between heterotrophic and autotrophic modes of nutrition. Species like *G. ventalina* and *A. acerosa* possess very small polyps compared with some sea rods like *Eunicea* and *Plexaurella*, whose polyps are up to an order of magnitude larger (Thibaudeau 1983). As a consequence, the density and surface area of each polyp, relative to the overall colony size is much higher for the sea fans and plumes than the sea rods we studied. Thus, selection may favor the reduction of feeding structure SA:V if it increases the amount of light exposure to productive symbionts in the host polyp tissues (Kanwisher and Wainwright, 1967; Wainwright and Dillon, 1969). The converse appears to be true for most sea rods, where larger and fewer polyps and the thick, rod shaped colony morphology would serve to limit productivity per unit of biomass but ensure that feeding structures are ready for heterotrophy during changing environmental conditions.

Over deep time, gorgonian diversification in the Caribbean was less inhibited by competitive interactions with scleractinians than in the Pacific. With more niche space available, any species that acquired mutualistic *Symbiodinium* could boost its ability to compete for space. In doing so, selection may have favored more obligate associations with *Symbiodinium* with more efficient metabolisms leading to greater productivity and growth (and thus, competitive abilities) in oligotrophic environments. Such divergence is obvious within the *Gorgoniidae*, which display a stark contrast between clades formed by *Gorgonia* and *Antillogorgia* versus the *Plexaurella* (Figure 3). These two host species are apparent specialists with *Symbiodinium* clade B1 as reported by substantial sampling efforts throughout the Caribbean Sea (Andras *et al.*, 2011). Moreover, this specialization may have accelerated the selection for reduced polyp size and colony morphologies to enhance light capture (Figure 3). A shared trajectory toward symbiont specificity and autotrophy is further supported by the observation that *Gorgonia* and *Antillogorgia* are capable of hybridization (McFadden *et al.*, 2010). In contrast, we have shown that *Plexaurella* do not benefit substantially

from *Symbiodinium* and hosts are less specific in their *Symbiodinium* associations (Figures 2 and 3). This may reflect a divergence from the *Gorgonia/Antillogorgia* sister group to avoid competition for space in high light environments.

While we have examined the benefits of the gorgonian-*Symbiodinium* symbiosis, further work must address the potential costs of these interactions. For example, with changing environmental conditions (higher SST, eutrophication, acidification), do the costs of symbiosis outweigh the benefits? We hypothesize that host energetics and related physiological mechanisms (such as immunity to pathogens) could be a key indicator of the cost of symbiosis. For example, productive and obligate hosts like *G. ventalina* were nearly extirpated from the Caribbean by disease (Bruno *et al.*, 2011), whereas other gorgonian species were not as impacted. The severity of this disease has been attributed to eutrophication (Baker *et al.*, 2007). Thus, the cost of symbiosis, including the pairing of auto- and heterotrophic metabolisms could become an Achilles' heel when the environment changes.

For other species, there are benefits to being flexible in a symbiotic relationship particularly during periods of environmental change. The relatively heterotrophic sea rods (that is, *Plexauridae* including *Eunicea*) are Caribbean endemics undergoing rapid speciation. In our study, this group displayed variable metabolic performance (Figure 3). Indeed, *Eunicea* is the most diverse anthozoan genus with 16 described species (Sánchez *et al.*, 2003). They are phenotypically plastic and can adapt to different habitat types along a reefscape, which contributes to their high rate of speciation (Prada and Hellberg, 2013; Prada *et al.*, 2014). In the process of speciation, the association with *Symbiodinium* may weaken from an obligate mutualism to a commensal or parasitic relationship. In the Anthropocene, selection may favor facultative symbioses as the coastal oceans become more eutrophic and nitrogen limitation is alleviated (Baker *et al.*, 2010). We predict that generalist and facultative hosts may be more resilient in the face of environmental stressors that specifically impact symbionts (reduced light) and capitalize on the increasing abundance of heterotrophic resources in the form of plankton and particulate organic matter to meet their nutritional needs.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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