

Biochemical variability in sponges across the Caribbean basin

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Abstract

Sponges are a diverse phylum of sessile filter-feeding invertebrates that are abundant on Caribbean reefs and provide essential ecological services, including nutrient cycling, reef stabilization, habitat, and food for a variety of fishes and invertebrates. As prominent members of the benthic community, and thus potential food resources, factors determining the biochemical and energetic content of sponges will affect their trophic contributions to coral reef ecosystems. In order to evaluate the influence of geographic variation on biochemical composition and energetic content in the tissue of sponges, we collected several common and widespread species (*Agelas conifera*, *Agelas tubulata*, *Amphimedon compressa*, *Aplysina cauliformis*, *Niphates amorphia*, *Niphates erecta*, and *Xestospongia muta*) from multiple shallow reefs in four countries across the Caribbean Basin, including Belize, Curaçao, Grand Cayman, and St. Croix, U.S. Virgin Islands. In addition, we correlated inherent species-level traits, including the production of antipredator chemical defenses and the relative abundance of microbial symbionts, with biochemical and energetic content. We found that energetic content was higher in sponges with antipredator chemical defenses, and was significantly correlated with the concentration of chemical extracts from these sponges. We also noted that sponges with high microbial abundance contained significantly more soluble protein than sponges with low microbial abundance. Finally, both biochemical and energetic content varied significantly among sponges from different locations; sponges from Grand Cayman had the highest lipid and energetic content, whereas sponges from Belize had the highest carbohydrate content but lowest energetic content. Despite similar environmental conditions at these sites, our results demonstrate that biochemical and energetic content of sponges exhibits geographic variability, with potential implications for the trophic ecology of sponges throughout the Caribbean Basin.

KEYWORDS

biogeography, chemical defenses, environmental stress, holobiont, nutritional defenses

1 | INTRODUCTION

Sponges are one of the most prominent taxa on Caribbean reefs (van Soest et al., 2012). Within sites across the region, sponges are often the most abundant phylum in terms of biomass, and they also represent the largest source of biodiversity (Diaz & Rützler, 2001; Loh

et al., 2015; Norström et al., 2009; Slattery & Lesser, 2012; van Soest et al., 2012). Sponges provide reefs with critical ecosystem services, including substrate stabilization (Wulff & Buss, 1979, 1984), habitat for various fishes and invertebrates (Herrnkind et al., 1997; Ismet et al., 2019; Koukouras et al., 1996), nutrient cycling (e.g., Fiore et al., 2013), the transfer of energy to higher trophic levels (de Goeij et al.,

2013; Lesser, 2006; Rix et al., 2016), and food for generalist and specialist predators, including many fish species (Randall & Hartman, 1968), invertebrates (Waddell & Pawlik, 2000; Wulff, 2006a), and the endangered hawksbill sea turtle (Meylan, 1988).

As sessile organisms susceptible to predation (Chanas & Pawlik, 1995; Freeman & Gleason, 2010; McClintock, 1987; Uriz et al., 1996), many sponge species produce unpalatable secondary metabolites as a defensive strategy against predators (Pawlik et al., 1995). Production of these antipredator metabolites, however, may be energetically costly, and chemically defended species recruit and grow at slower rates than undefended species (Leong & Pawlik, 2010; Loh et al., 2015; Loh & Pawlik, 2014; Wulff, 2006a). Theories on optimal foraging posit that animals should select prey to maximize their net intake of energy and vital nutrients, such as protein (Emlen, 1966; Krebs & Davies, 1993; MacArthur & Pianka, 1966). The corresponding optimal defense theory (ODT; Cronin, 2001; López-Legentil et al., 2006; Rhoades, 1979; Skogsmyr & Fagerström, 1992) predicts that prey may avoid predation by employing defensive strategies, such as production of chemical defenses (Rhoades, 1979), or by producing nutritionally poor tissue (Duffy & Paul, 1992).

Nutritional defense strategies have been demonstrated in multiple systems (e.g., plants, Haukioja et al., 1991; sponges, Duffy & Paul, 1992; nudibranchs, Penney, 2002), and there is evidence that this may also occur in sponges throughout much of their biogeographic range (i.e., Antarctica, McClintock, 1987; Indo-Pacific, Duffy & Paul, 1992; Mediterranean, Uriz et al., 1996), but not within the Caribbean (Chanas & Pawlik, 1995, 1996; Freeman & Gleason, 2010).

Sponges are often differentiated based on characteristics of their prokaryotic microbiome. Specifically, sponges with high microbial abundance (HMA sponges) contain $\geq 10^8$ prokaryotic cells/g of tissue and tend to have higher overall microbial diversity than sponges with low microbial abundance (LMA sponges) sponges, which contain $\leq 10^6$ prokaryotic cells/g of tissue (Gloeckner et al., 2014). These differences in symbiont communities have implications for feeding strategies, in that HMA sponges tend to have denser tissue, slower filtration rates, and preferentially consume dissolved organic matter (DOM; Weisz et al., 2007). By contrast, LMA sponges tend to have less dense tissue, faster filtration rates, and prefer particulate organic matter (POM; Weisz et al., 2007). These nutrient pools vary spatially and temporally (e.g., Corredor & Morell, 2001; D'Croz et al., 2005; Sellner, 1981; Westrum & Meyers, 1978), and could potentially influence the biochemical and energetic content (i.e., nutritional quality) of the HMA or LMA sponges using these resources. Sponges also exhibit variation in the composition of their microbiomes, including the ability to host photoautotrophic microbial symbionts (Erwin & Thacker, 2007; Freeman et al., 2014). Since these photoautotrophs fix carbon in a light-dependent manner, there may be differences in carbon reserves between sponges that host photosymbionts collected from different regions, because the rate of photosynthesis can vary due to local environmental conditions (Freeman et al., 2020).

Additionally, site-specific environmental stressors, including pollution or sedimentation from terrestrial runoff (Biggerstaff et al.,

2017), turbidity (Reiswig, 1971), extreme storm activity (Gochfeld et al., 2020), and/or biotic processes, such as predation (Leong & Pawlik, 2010; Loh et al., 2015; Loh & Pawlik, 2014; Wulff, 2006a) or disease (Gochfeld et al., 2012; Olson et al., 2014), can affect either the host sponges or their microbial symbionts. Individuals subjected to these stressors may require access to energetic reserves in the form of carbohydrates and lipids, and proteins for tissue repair (Sokolova, 2013), or they may produce tissue with a lower energetic content compared to unstressed counterparts (Cronin, 2001).

Here we evaluated whether inherent species-specific traits, such as the production of antipredator chemical defenses, microbial abundance, the presence of photosymbionts, or geographic location, impact biochemical composition and total energetic content of sponge tissue. We tested whether biochemical and energetic content of sponges was associated with antipredator chemical defenses, the abundance and type of sponge microbial symbionts, and local to regional variability relative to environmental conditions. We hypothesized that: (1) Nutritional quality of sponge tissue would vary regionally, with reduced energetic content in sponges exposed to greater environmental stress, regardless of species; (2) microbial abundance or relative abundance of photosymbionts would modulate this response; and (3) regardless of the regional environment, chemically defended sponges would produce more energetically dense tissue than undefended sponges. Addressing these questions will provide further insights into the role of sponge nutritional value to coral reef community dynamics.

2 | METHODS

2.1 | Sample collection

To assess the effect of the environment on sponge biochemical and energetic content, we collected individuals (2–21 individuals of each species) of *Agelas conifera*, *Agelas tubulata*, *Amphimedon compressa*, *Aplysina cauliformis*, *Niphates amorphia*, *Niphates erecta*, and *Xestospongia muta* at a depth of 15 m from two to four reef sites within four regions across the broader Caribbean Basin (Figure 1; Table S1). Specifically, we collected our samples in Belize (Carrie Bow Cay, Southwater Cay, Curlew Cay); Curaçao (Blue Wall, Pescadero Bay, Buoy 1, Double Reef); Grand Cayman (Kittiwake Anchor Chain, Sentinel Rock, Slaughterhouse Wall, Hepp's Pipeline); and St. Croix, U.S. Virgin Islands (Cane Bay, Eagle Ray, Salt River). Target species were selected based on their broad distribution and abundance throughout the Caribbean, although not every species was found in every region or at every site (Figure 1; Table S1). These species were also selected to represent a range of antipredator chemical defenses (i.e., “defended” species, which constitutively express chemical defenses to deter predators; “undefended” species; and “variably defended” species, in which feeding deterrence varied among sites; based on data from Pawlik et al., 1995), differences in microbial abundance (i.e., HMA or LMA species), and differences in the presence or

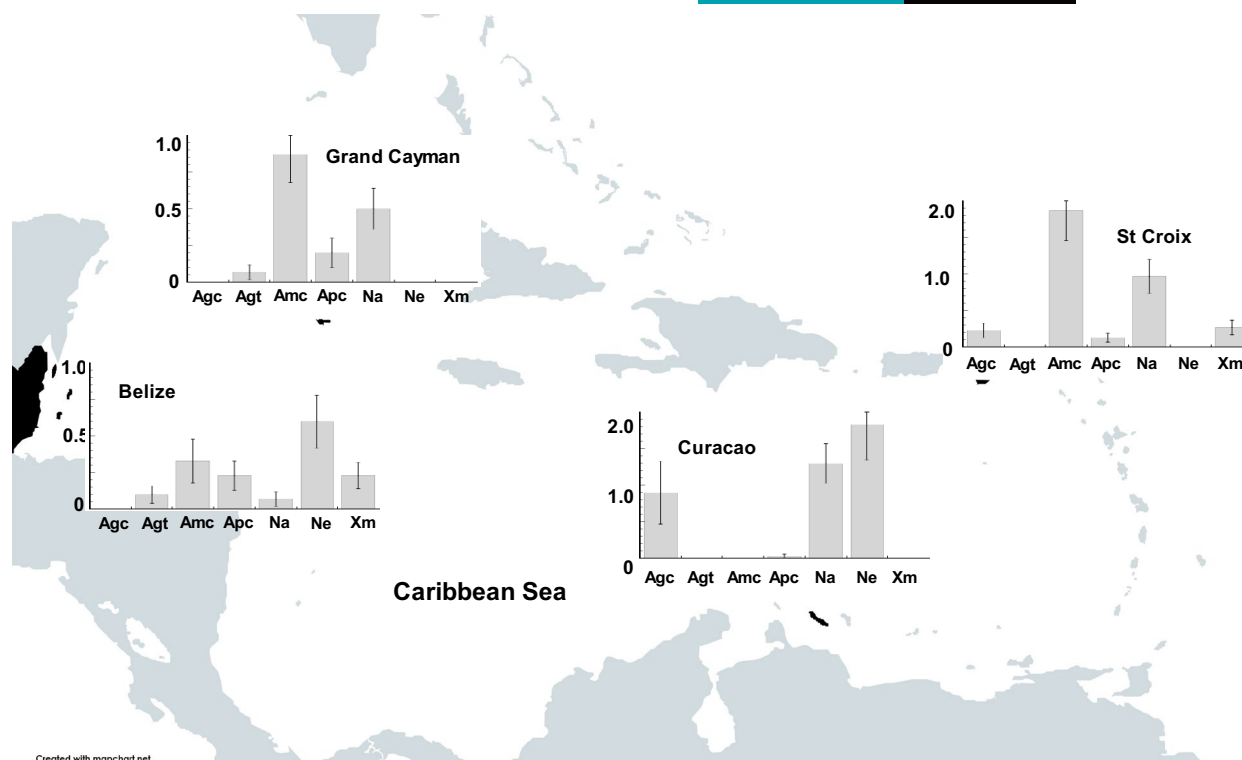


FIGURE 1 Map of sampling regions and relative abundance of seven target sponge species. Sampling occurred in regions highlighted in black. For each graph, the y-axis represents the number of individuals/m² (mean ± SE) from replicate 1-m² quadrats ($n = 30$) within each region. The codes on the x-axis refer to our target sponges: Agc, *Agelas conifera*; Agt, *Agelas tubulata*; Amc, *Amphimedon compressa*; Apc, *Aplysina cauliformis*; Na, *Niphates amorphia*; Ne, *Niphates erecta*; Xm, *Xestospongia muta*

TABLE 1 Summary of traits of target sponge species. Sponge species were classified as chemically defended if they deterred fish feeding, undefended if they did not, and variable if defense levels varied by site of collection. Species were classified as high microbial abundance (HMA; $\geq 10^8$ prokaryotic cells/g of tissue) or low microbial abundance (LMA; $\leq 10^6$ prokaryotic cells/g of tissue). Relative abundance of photosymbionts is based on chlorophyll concentration. Because the species pairs *Agelas conifera* and *A. tubulata*, and *Niphates amorphia* and *N. erecta*, are sometimes considered to be the same species, data from each genus were combined.

Target species	Antipredator chemical defenses ^a	Microbial abundance ^b	Relative abundance of photosymbionts
<i>Aplysina cauliformis</i>	Yes	HMA	High ^c
<i>Xestospongia muta</i>	Variable	HMA	Intermediate ^c
<i>Agelas conifera/tubulata</i>	Yes	HMA	Low ^d
<i>Amphimedon compressa</i>	Yes	LMA	Low ^c
<i>Niphates amorphia/erecta</i>	No	LMA	Low ^c

^aPawlik et al. (1995).

^bGloeckner et al. (2014).

^cErwin and Thacker (2007).

^dFreeman and Easson (2014).

absence of photoautotrophic symbionts (Table 1). Abundance of the target species at each site was determined by counting the number of individuals of each species within ten 1-m² quadrats at each of three sites per region. The mean abundance of each species was calculated from the 30 quadrats from each region.

The sponges *A. conifera*, *A. tubulata*, *N. amorphia*, and *N. erecta* were not present in every region we sampled (Figure 1; Table S1). Despite being nominally defined as separate species, it has been suggested that *A. conifera* and *A. tubulata* may represent conspecific morphotypes, rather than individual species (M. S. Pankey, unpubl.

data, July 2018). Furthermore, they express the same combination of characteristics, in that they are both chemically defended, are HMA sponges, and do not host photosymbionts (Table 1). Likewise, *N. amorphia* and *N. erecta* share the same combination of traits with each other, and there is an ongoing debate as to whether they represent encrusting and erect morphotypes, rather than distinct species (Kobluk & van Soest, 1989). We therefore considered *A. conifera* and *A. tubulata*, and *N. amorphia* and *N. erecta*, to represent ecologically equivalent congeners and collected these sponges based on species presence or relative abundance within a particular region.

Tissue samples ranging 60–225 g were excised from individual sponges in situ using scissors. They were placed into individual resealable plastic bags, kept submerged in seawater in a shaded cooler, and returned to shore, where they were frozen at -20°C for transport. Once at the University of Mississippi, sample wet mass and volume were recorded, samples were freeze-dried to determine the dry mass, and they were ground to a powder.

Quantification of dissolved organic material at each site was performed on water samples collected in 180ml, acid-washed syringes at 15 m depth, ~1 m above the reef substrate. Aliquots (40 ml) were then filtered through a 0.2- μm GF/F filter and immediately frozen at -20°C for transport to the University of New Hampshire (UNH). The filtrates were analyzed at the UNH Water Quality Analysis Laboratory. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were quantified using high-temperature catalytic oxidation and high-temperature oxidation with chemiluminescent detection, respectively. HOBO pendant light and temperature data loggers were deployed at ~15 m for 1–15 days, depending on site. The raw data were converted from lux to photosynthetically active radiation (PAR) using a standard conversion: $(\text{lux}/1000) \times 18$. The maximum irradiance at midday for PAR reported represents the mean ($\pm\text{SD}$) irradiances between 12:00 and 13:00 h over the days of data collection.

2.2 | Analysis of proximate biochemical composition

The proximate biochemical composition (PBC) of sponge tissue was quantified as follows. Carbohydrates were extracted from 10 mg of ground freeze-dried tissue in 5 ml of 5% trichloroacetic acid (TCA) for 4 h. Carbohydrate concentration was then determined using the phenol-sulfuric acid method in microplate format described in Masuko et al. (2005). Briefly, 50 μl of TCA-digested sample solution or glucose standards, 150 μl of sulfuric acid, and 30 μl of 5% phenol were pipetted into triplicate wells in a 96-well plate, and incubated for 10–15 min at 90°C . Absorbance was then measured at 490 nm using a BioTek Synergy HT Multi-Detection Microplate Reader. The concentration of carbohydrates in samples was calculated relative to a glucose standard curve.

Protein was extracted from 10 mg of ground freeze-dried tissue in 5 ml of 1 M sodium hydroxide (NaOH) for 24 h. The soluble protein concentration was then analyzed using the Bradford Method (Bradford, 1976). Briefly, 40 μl of NaOH-digested sponge sample or a Bio-Rad bovine serum albumin (BSA) standard, was mixed with 2 ml of Bio Rad Quick StartTM Bradford reagent, and 1 ml was transferred to a cuvette. Absorbance at 600 nm was measured using an Eppendorf Biophotometer. Soluble protein concentration in sponge samples was calculated relative to a standard curve using BSA.

Lipids were extracted using a modified version of the protocol described by Freeman et al. (1957). Briefly, 50 mg of ground freeze-dried tissue was sonicated for 15 min in a 2:1 chloroform:methanol solution, and filtered into a 50-ml conical tube containing 20 ml of distilled water. The organic layer was then pipetted into a pre-weighed scintillation vial. This process was repeated three times per

sample. The organic solvent was then evaporated over a period of 12 h via vacuum centrifugation, and the final mass of dry lipid was recorded on a Mettler Toledo microbalance.

Ash was measured using methods described by McClintock et al. (1991). Briefly, 100 mg of ground freeze-dried sponge tissue was placed into a pre-weighed foil weigh boat and ashed at 500°C in a muffle furnace for 5 h, and the final mass of ash was recorded on a Mettler Toledo microbalance.

Refractory material was calculated by subtracting the combined masses of all of the measured components (carbohydrates, soluble protein, lipids, and ash) from the total sponge tissue mass to obtain ash-free dry weight (AFDW). Although this fraction is technically composed of additional biochemical constituents, including nucleic acids and some secondary metabolites, their mass contributions within the refractory material are likely to be limited; thus, for the purpose of energetic calculations, refractory material is assumed to consist predominantly of insoluble protein (Chanas & Pawlik, 1995; Lawrence, 1973; McClintock et al., 1991).

The proportion of each individual biochemical constituent within the sponge tissue was calculated by dividing the mass of each individual constituent by the total mass of dry tissue, excluding ash. The total energetic content of sponge tissue was calculated by multiplying the proportional dry mass of each biochemical constituent by the kilojoule (kJ) coefficients detailed in Gnaiger and Bitterlich (1984).

To quantify the concentration of total sponge extract obtained from each sample, ~300 mg of ground freeze-dried sponge tissue was extracted in 10 ml of 1:1 methanol: methylene chloride and sonicated for 15 min. This was repeated twice, and the three extracts were combined in a pre-weighed vial. The solvent was removed via vacuum centrifugation, and the extract mass was recorded. Natural extract concentrations were calculated by converting tissue dry mass into volume.

The chemical defense category of the target species was obtained from data published in Pawlik et al. (1995). Information on the relative microbial abundance for these species was obtained from Gloeckner

TABLE 2 Interactions between the effects of species and region on composition and energy in two-way mixed model ANOVAs including only the three species (*Aplysina cauliformis*, *Niphates amorphosa*, and *Xestospongia muta*) found in all four regions (Grand Cayman, Curaçao, St. Croix, or Belize). Response variables included individual biochemical constituents, energy, and extract concentrations.

Response variable	df	F	p
Carbohydrates	6	24.29	<0.0001
Lipids	6	11.61	<0.0001
Soluble protein	6	24.11	<0.0001
Refractory material	6	31.13	<0.0001
Ash	6	4.763	0.0003
Energy (total mass)	6	11.79	<0.0001
Energy (ash-free dry weight)	6	6.226	<0.0001
Total extract	6	36.88	<0.0001

et al. (2014), and the relative abundance of photosymbionts was found in Erwin and Thacker (2007) and Freeman and Easson (2014). For each of the target species, these characteristics are summarized in Table 1.

2.3 | Data analysis

For all sponges, seven response variables (milligrams of carbohydrates, lipids, soluble, and refractory material (hereafter referred to as

insoluble protein) per gram AFDW; milligrams of ash per gram total mass; and kilojoules per gram total mass and AFDW; Table 2) were analyzed via mixed model ANOVAs using the R package lmerTest, and perMANOVA or perANOVA using the adonis2 function in the package vegan. Because not every species occurred in each region (Figure 1; Table S1), a two-way ANOVA was conducted on the subset of species that was found in every region (*A. cauliformis*, *N. amorphia*, and *X. muta*). This model consisted of the predictor variables species, sampling region, and their interaction, while sites within regions were treated as a

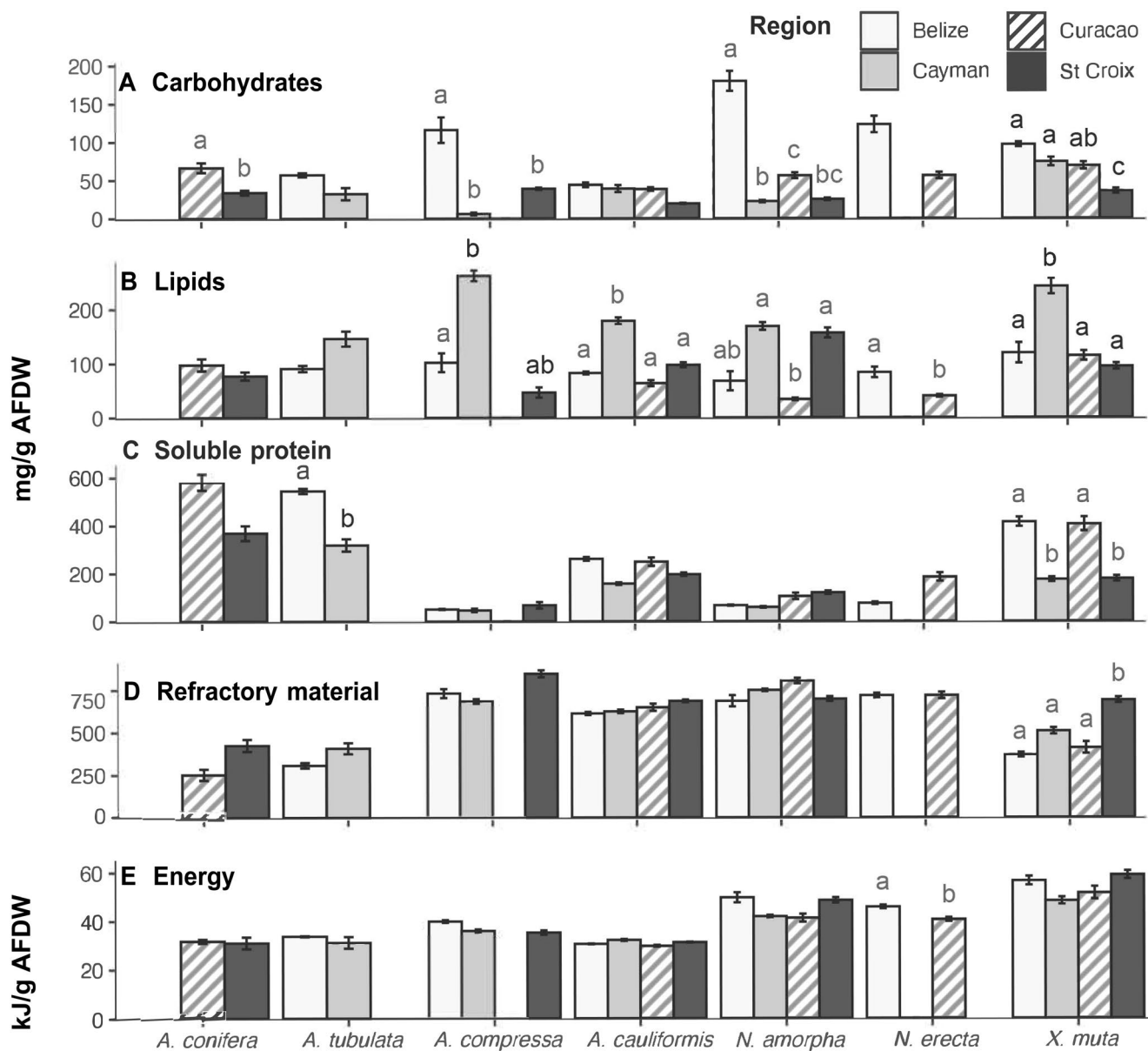


FIGURE 2 Sponge biochemical and energetic content by species. Barplots represent the mean concentration (±SE) in milligrams per gram of ash-free dry weight (AFDW) of carbohydrates (A), lipids (B), soluble protein (C), and refractory material (D) by species (*Agelas conifera*, *Agelas tubulata*, *Amphimedon compressa*, *Alpysina cauliformis*, *Niphates amorphia*, *Niphates erecta*, and *Xestospongia muta*) and region. Sample sizes ranged 2–21 individuals per species. The total energetic content of each species within each region (kilojoules per gram AFDW) is also presented (E). Note: for purposes of energetic calculations, refractory material is assumed to consist of insoluble protein. Within a species, regions with different lower-case letters are significantly different, as indicated by Tukey's post-hoc tests ($p < 0.05$); the absence of letters indicates no significant differences between regions

random factor. Additionally, four one-way ANOVAs were used to evaluate the main effect of region, with site as random factor, on response variables in *A. conifera*, *A. tubulata*, *A. compressa*, and *N. erecta* (Figure 1; Table S1). Subsequent pairwise comparisons following ANOVAs were conducted using the R package *emmeans*. Factors in the perMANOVA and perANOVA models included chemical defense (defended, undefended, or variably defended), microbial abundance (HMA or LMA), the relative abundance of photosymbionts (Table 1), sampling region, and the two-way interactions between region and the other three predictor variables. Species was not included as a predictor variable because of a lack of independence between combinations of traits and species, which confounds the analysis. Factors in the initial perMANOVA model included the predictor variables chemical defense, microbial abundance, the relative abundance of photosymbionts, region, and all possible two-, three-, and four-way interactions. However, only the main effects and two-way interactions between chemical defense, microbial abundance, and the relative abundance of photosymbionts with region were found to significantly predict response variables, and as a result, all other tested interactions (i.e., three-way and four-way interactions) were eliminated from consideration and compressed into a model with multiple two-way interactions. Proportions were arcsine-square root transformed, and normality of each response variable was assessed by plotting histograms of the residuals; homoscedasticity of the response variables was assessed by graphing the residuals versus the predicted values (Kozak & Piepho, 2017). Pairwise comparisons between the interactions of species, chemical defense, microbial abundance, or relative abundance of photosymbionts with region were conducted using the function *pairwise PermutationMatrix* from the package *rcompanion*. All *p*-values from mixed models and perANOVAs were Bonferroni adjusted for multiple testing. Plots were generated with packages *dplyr*, *ggplot2*, *RColorBrewer*, and *gridExtra*.

To determine whether chemical defense is proportional to energetic content, we used the sample extract concentration as a proxy for overall chemical defense (Thompson et al., 1987). Our extraction method typically yields multiple secondary metabolites with defensive roles and often biosynthetic precursors and breakdown

products, and these extracts exhibit defensive properties that can be correlated with field observations of feeding deterrence (e.g., Slattery et al., 2008; Slattery & Lesser 2014). Regression analysis was conducted using total sponge extract concentration (milligrams total sponge extract per milliliter) as the predictor variable and total energetic content (kilojoules per gram) as the response variable. Cook's distances were calculated and did not identify any outliers that were overly influencing the model. The figure was generated with *ggplot2*. All statistical analyses were conducted in R version 4.0.2.

3 | RESULTS

3.1 | Target species abundance and variance in abiotic factors between regions

Although we attempted to select target species that were broadly distributed throughout the Caribbean, we were unable to collect every species at our selected sites, because species distribution and abundance varied across regions (Figure 1; Table S1; all values are reported as means $\pm$ SE). At each site, LMA species represented the most abundant target species (i.e., *A. compressa* in Grand Cayman and St. Croix, and *N. erecta* in Belize and Curaçao; Figure 1). Ambient irradiance between sites ranged 180.5–377.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and temperatures ranged 27.1–29.6°C (Table S2). DOC and DON did not vary significantly among regions (Table S2).

3.2 | Geographic variation in proximate biochemical composition and energetic content across species

We found a significant interaction between the effects of species and region on PBC and on energetic content in *A. cauliformis*, *N. amorphia*, and *X. muta* (Figure 2; Figure S1; Table 2; Table S3). In the other four species, there was significant regional variation in

TABLE 3 Summary of one-way mixed model ANOVAs comparing biochemical constituents, energetic content, and extract concentration of sponges across regions (for *Agelas tubulata* from Belize and Grand Cayman; *Agelas conifera* from Curacao and St. Croix; *Amphimedon compressa* from Belize, Grand Cayman, and St. Croix; and *Niphates erecta* from Belize and Curacao).

Response variable	<i>Agelas tubulata</i>			<i>Agelas conifera</i>			<i>Amphimedon compressa</i>			<i>Niphates erecta</i>		
	df	F	p	df	F	p	df	F	p	df	F	p
Carbohydrates	1	0.4557	1	1	25.82	0.0002	2	64.93	<0.0001	1	5.891	0.6531
Lipids	1	6.655	0.5870	1	1.273	1	2	14.94	0.0657	1	14.02	0.0066
Soluble protein	1	35.81	0.0462	1	8.99	0.6098	2	0.3851	1	1	30.64	0.0968
Refractory material	1	9.072	0.3515	1	13.92	0.4653	2	15	0.0888	1	0.0014	0.4653
Ash	1	0.2411	1	1	1.478	1	2	10.34	1	1	44.82	<0.0001
Energy (total mass)	1	1.497	1	1	0.1409	1	2	48.33	<0.0001	1	9.976	0.3178
Energy (ash-free dry weight)	1	0.4596	1	1	3.865	1	2	5.983	0.3762	1	20.53	0.0639
Total extract	1	0.0740	1	1	206.2	<0.0001	2	1.163	1	1	0.1279	1

different biochemical components for each species; only in *A. compressa* was there significant regional variation in total energetic content, and only in *A. conifera* was there variation in total extract concentration (Figure 2; Table 3). Overall, sponges from Belize had the highest carbohydrate content (Figure 2A), whereas sponges from Grand Cayman had the highest lipid content (Figure 2B).

3.3 | Variation in proximate biochemical composition and energetic content with chemical defense

In order to further investigate factors driving these differences in PBC and energetic content, we performed a perMANOVA, using

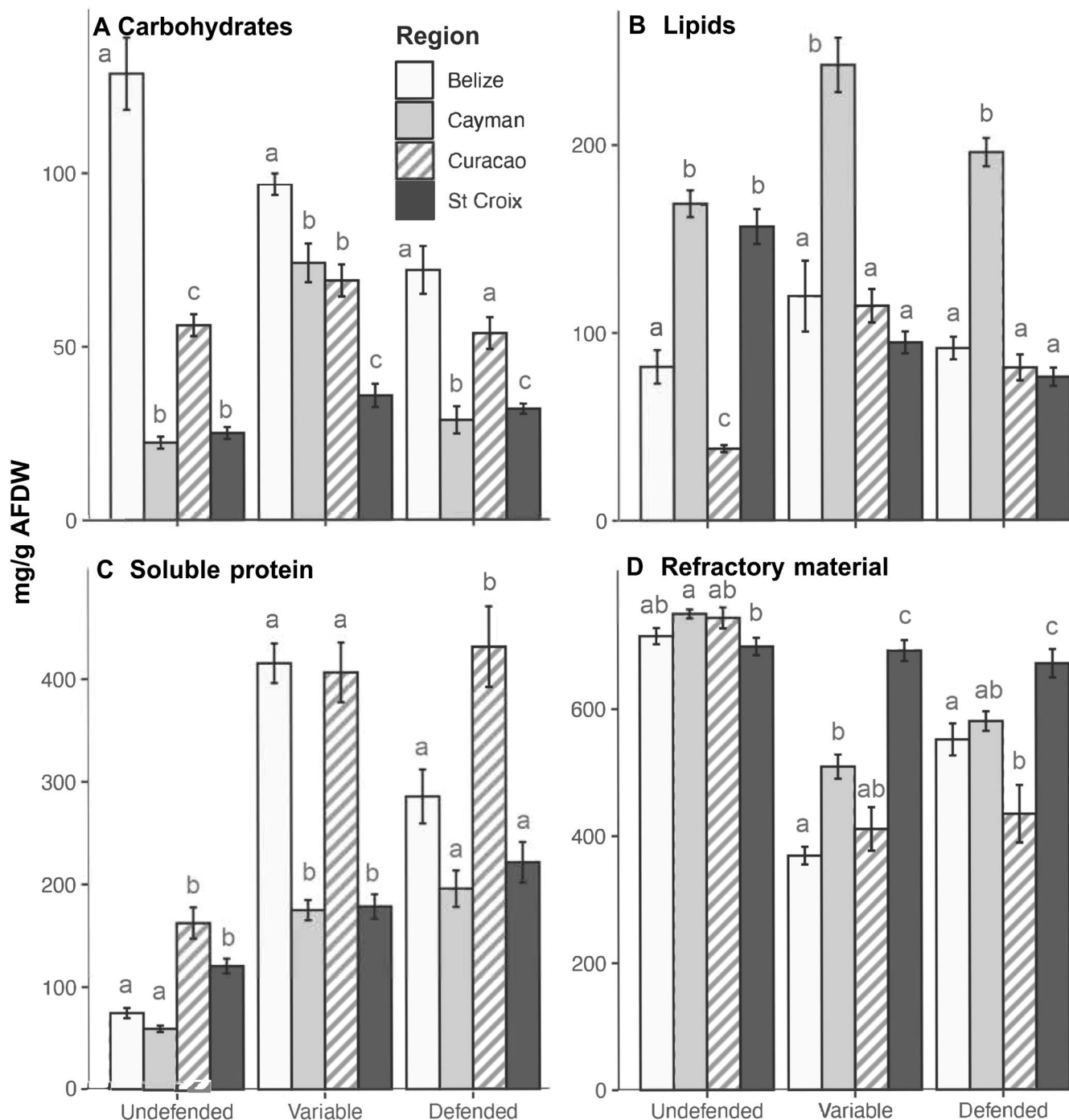


FIGURE 3 Biochemical content among sponges categorized by the presence of antipredator chemical defenses. Barplots represent the mean concentration ($\pm$ SE) in milligrams per gram of ash-free dry weight (AFDW) of carbohydrates (A), lipids (B), soluble protein (C), and refractory material (D) by chemical defense and region. Sample sizes ranged 10–61 individuals per species. Chemical defense categories are from Pawlik et al. (1995). Regions with different lower-case letters are significantly different ($p < 0.05$) by pairwise comparisons within a particular chemical defense status; the absence of letters indicates no significant differences between regions

TABLE 4 Summary of perMANOVAs for all proximate biochemical constituents, and perANOVAs comparing biochemical constituents and energetic content across levels of chemical defense (based on Pawlik et al., 1995), microbial abundance (based on Gloeckner et al. 2014), presence of photosymbionts (based on Erwin & Thacker, 2007; Freeman & Easson, 2014), and region (Grand Cayman, Curaçao, St. Croix, or Belize).

Response variable	Predictor variable	df	F	p	R ²
All constituents	Chemical defense	2	27.6963	0.0001	0.02847
	Microbial abundance	1	746.0122	0.0001	0.38343
	Photosymbionts	1	274.8377	0.0001	0.14126
	Region	3	125.9021	0.0001	0.19413
	Chemical defense × Region	5	19.8885	0.0001	0.05111
	Microbial abundance × Region	3	14.8718	0.0001	0.02293
	Photosymbionts × Region	3	6.2144	0.0004	0.00958
Carbohydrates	Chemical defense	2	38.95	0.0007	0.0758
	Microbial abundance	1	13.08	0.007	0.0127
	Photosymbionts	1	12.36	0.0007	0.0120
	Region	3	113.8	0.0007	0.3321
	Chemical defense × Region	5	18.75	0.0007	0.0912
	Microbial abundance × Region	3	44.85	0.0007	0.1309
	Photosymbionts × Region	3	9.372	0.0007	0.0273
Lipids	Chemical defense	2	10.60	0.0007	0.0261
	Microbial abundance	1	30.33	0.0007	0.0374
	Photosymbionts	1	0.0059	1	<0.0001
	Region	3	95.65	0.0007	0.3541
	Chemical defense × Region	5	17.84	0.0007	0.1101
	Microbial abundance × Region	3	15.47	0.0007	0.0573
	Photosymbionts × Region	3	2.413	0.360	0.0089
Soluble protein	Chemical defense	2	66.97	0.0007	0.0578
	Microbial abundance	1	1302	0.0007	0.5617
	Photosymbionts	1	241.5	0.0007	0.1042
	Region	3	54.77	0.0007	0.0709
	Chemical defense × Region	5	8.522	0.0007	0.0184
	Microbial abundance × Region	3	32.39	0.0007	0.0419
	Photosymbionts × Region	3	2.483	0.336	0.0032
Refractory material	Chemical defense	2	2.244	0.643	0.0034
	Microbial abundance	1	426.8	0.0007	0.3234
	Photosymbionts	1	292.5	0.0007	0.22156
	Region	3	39.30	0.0007	0.0893
	Chemical defense × Region	5	10.22	0.0007	0.0387
	Microbial abundance × Region	3	16.54	0.0007	0.0376
	Photosymbionts × Region	3	16.16	0.0007	0.0367
Ash	Chemical defense	2	599.5	0.0007	0.6403
	Microbial abundance	1	161.6	0.0007	0.0863
	Photosymbionts	1	74.09	0.0007	0.0396
	Region	3	16.74	0.0007	0.0268
	Chemical defense × Region	5	5.498	0.0007	0.0147
	Microbial abundance × Region	3	9.585	0.0007	0.0154
	Photosymbionts × Region	3	0.8600	1	0.0014

(Continues)

TABLE 4 (Continued)

Response variable	Predictor variable	df	F	p	R ²
Energy (total mass)	Chemical defense	2	313.8	0.0007	0.4809
	Microbial abundance	1	24.61	0.0007	0.0189
	Photosymbionts	1	17.38	0.0007	0.0133
	Region	3	48.72	0.0007	0.1120
	Chemical defense × Region	5	12.00	0.0007	0.0460
	Microbial abundance × Region	3	33.25	0.0007	0.0765
	Photosymbionts × Region	3	0.1279	1	0.00029
Energy (ash-free dry weight)	Chemical defense	2	773.0	0.0007	0.7306
	Microbial abundance	1	72.94	0.0007	0.0345
	Photosymbionts	1	28.19	0.0007	0.0133
	Region	3	18.88	0.0007	0.02676
	Chemical defense × Region	5	13.45	0.0007	0.0318
	Microbial abundance × Region	3	5.056	0.0133	0.0072
	Photosymbionts × Region	3	0.2705	1	0.0004

geographic region and species traits (Table 1) as predictor variables. We found significant interaction between the effects of chemical defense and the region where the sponges were collected on PBC ($F = 19.89$, $df = 5$, $p < 0.0001$, $R^2 = 0.05$; Figure 3; Table 4). Post-hoc perANOVAs revealed that carbohydrates, lipids, and soluble protein also varied significantly by the main effect of chemical defense (Table 4).

Differences in total energetic content, both in total tissue dry mass and in AFDW (Figure 2E; Figure S1), appeared to be strongly influenced by the main effect of chemical defenses (Table 1), as evidenced by high R^2 values (Table 4). Linear regressions of energetic content (kJ/g of dry tissue mass) and natural volumetric concentration (mg/ml) of total sponge extracts (Figure S2), which we used as a proxy for chemical defense production across species, showed no significant relationship among undefended species ($F = 0.92$, $p = 0.34$, $R^2 < 0.01$; Figure 4A). By contrast, extract concentration explained 16% of the variance in energetic content among variably defended species ($F = 14.27$, $p < 0.0004$), and 4% of the variance in energetic content when variably defended and defended sponges were combined ($F = 11.16$, $p < 0.001$; Figure 4B). Despite these modest effect sizes, this analysis provides additional support for the positive relationship between the level of chemical defense and sponge energetics, as indicated in Table 4.

3.4 | Variation in proximate biochemical composition and energetic content with microbial abundance

There were significant interactions between the effects of microbial abundance and region on sponge PBC (perMANOVA, $F = 14.87$, $df = 3$, $p < 0.0001$, $R^2 = 0.02$; Figure 5; Table 4). All PBC constituents

and energetic content varied significantly, as revealed by perANOVA analysis (Figure 3; Table 4). HMA sponges had a significantly higher soluble protein content (318.29 ± 10.40 mg/g AFDW) than their LMA counterparts (84.91 ± 4.81 mg/g AFDW; Figure 5C). Additionally, there were significant interactions between the effects of region and relative abundance of photosymbionts on PBC ($F = 6.21$, $df = 3$, $p < 0.0004$, $R^2 = 0.009$; Figure 5; Table 4), largely due to variation in carbohydrates and lipids (Figure 6; Table 4). However, soluble protein, ash, and energetic content varied significantly with relative photosymbiont abundance, independent of region (Table 4).

4 | DISCUSSION

We found regional variability in sponge PBC, particularly in carbohydrate and lipid content (Figure 2A,B; Tables 2 and 3), and these differences were largely consistent between sponge species. Notably, we found that sponges collected from Grand Cayman had the highest average energetic and lipid content, whereas St. Croix had intermediate levels, and Belize and Curacao had lowest levels. Why the sponges collected from Grand Cayman appear to store more carbon in the form of lipids, compared to conspecifics collected elsewhere in the Caribbean, is unclear because there were no significant differences in DOC between regions (Table S2). One possible explanation is that elevated lipid content may reflect individuals preparing to reproduce. Sponge embryos are lecithotrophic (Maldonado & Bergquist, 2002), and therefore increased lipid reserves would be consistent with egg production. Our sampling in Grand Cayman occurred in early January, and although little is known about the frequency and timing of the reproductive cycles of our target species (Fell, 1993), a spawning event was previously documented in March in *X. muta* at Carrie Bow Cay, Belize (Ritson-Williams et al., 2005). Additionally, in the Florida Keys, Leong and Pawlik (2011)

demonstrated that larval brooding for the viviparous species *A. compressa* and *N. erecta* peaked between May and September. If the timing of these reproductive cycles is consistent across the remaining target species throughout the Caribbean Basin, then our collection in Grand Cayman could have occurred during a pre-spawning or brooding period when lipid-rich eggs would have been present in the sponge tissue. In contrast to Grand Cayman, sponges collected from Belize and Curaçao had among the lowest average lipid concentration (Figure 2B). This difference may reflect the timing of the sampling period, because these individuals were collected in April and June, which could represent individuals that had already spawned or were brooding larva and were therefore no longer bearing lipid-rich eggs.

Alternatively, the depletion of energetic reserves is a common stress biomarker of benthic invertebrates relative to temperature and pollution (Sokolova, 2013). The Caribbean Coastal Marine Productivity Program (CARICOMP) recorded a long-term trend (1993–2015) of increased turbidity at Carrie Bow Cay (Chollet et al., 2017). Increased turbidity may be caused by increased runoff and sedimentation due to human activities or rainfall, and can be associated with a reduction in photosynthetically active radiation (PAR) available to sponge photosymbionts (Deregibus et al., 2016; Edmunds et al., 2019), which could result in lower overall photosynthetic performance. However, all sponges collected in Belize had relatively high carbohydrate, and lower lipids contents (Figure 2A), compared to conspecifics in other regions, regardless of the relative abundance of photosymbionts associated with each species (Figure 2A,B). Additionally, PAR levels in Belize were similar to those in Grand Cayman (203 ± 139 vs. $204 \pm 38 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). Therefore, it is unlikely that the depleted lipid content in the Belize sponges was associated with reduced PAR, although this may be associated with the presence of an as yet unidentified stressor in the region.

Sponges collected from St. Croix had intermediate lipid and lower carbohydrate contents (Figure 2A,B) relative to those from Grand Cayman. As a more heavily developed island, these lower carbon reserves in St. Croix may be due to anthropogenic impacts on nearshore benthic populations (Smith et al., 2008). In addition, our collection in St. Croix occurred 11 months after the landfall of Hurricanes Irma and Maria, two intense storms that significantly reduced sponge cover on reefs in nearby St. Thomas (Gochfeld et al., 2020). Thus, reduced lipid reserves may reflect the effects of stressors associated with those storm events, including tissue damage due to wave energy or scouring, increased sedimentation, turbidity, and pollution from runoff (sensu Gochfeld et al., 2020; Leong & Pawlik, 2010). Regeneration of tissue due to storm damage may also explain the increased insoluble protein content among sponges collected in St. Croix (Figure 2D). The insoluble protein fraction largely reflects structural proteins, such as spongin and collagen, which provide sponges with tissue rigidity (Wulff, 2006b), and increased investment in structural proteins may reflect recovery from, or adaptation to, assaults from high wave activity (sensu Slattery et al., 2016). Interestingly, the only species that did not show decreased lipid and

increased insoluble protein content was *N. amorpha*. Unlike the other species we sampled, which occur as emergent phenotypes, *N. amorpha* is an encrusting sponge that is less susceptible to damage from wave energy because of its low profile on the reef (Gochfeld et al., 2020). Hence, individuals of *N. amorpha* in St. Croix likely evaded the tissue damage and the need for subsequent repair experienced by the other species following the impact of the hurricanes.

We also found that throughout the Caribbean Basin, chemically defended species had higher energetic content than undefended species (Figure 4; Figure S1). Secondary metabolites are complex organic molecules that may be energetically rich; thus, the correlation between secondary metabolites and energetic density in variably defended and defended sponges could be due to the presence of chemical defense compounds themselves. Alternatively, these data are consistent with nutritional defenses, whereby chemically

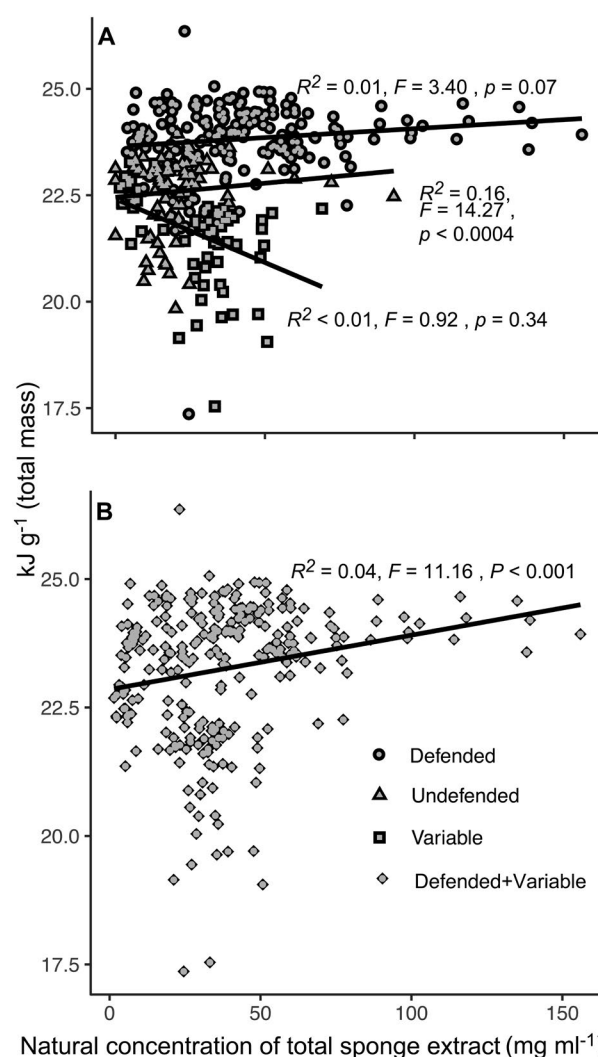


FIGURE 4 Linear regression of sponge tissue energetic content as a function of natural concentration of total sponge extract for three categories of antipredator chemical defense. **A.** Sponges known to be chemically defended (circles, $n = 200$), variably defended (squares, $n = 69$), or undefended (triangles, $n = 81$) (based on Pawlik et al., 1995). **B.** Chemically defended and variably defended sponges combined (diamonds, $n = 269$)

undefended sponges may gain protection from predators by producing nutritionally poor tissue (Duffy & Paul, 1992). Uriz et al. (1996) previously reported higher energetic content in the chemically defended Mediterranean sponge *Dysidea avara* (14.35 ± 1.43 kJ/g) than its undefended counterpart *Crambe crambe* (8.59 ± 0.61 kJ/g).

However, our data are inconsistent with other reports on Caribbean sponges (Chanas & Pawlik, 1995; Freeman & Gleason, 2010). These differences may be due to site-specific variability in biochemistry, as we noted across our sampling regions, or to differences in analytical methodologies. For example, Chanas and Pawlik (1995) reported

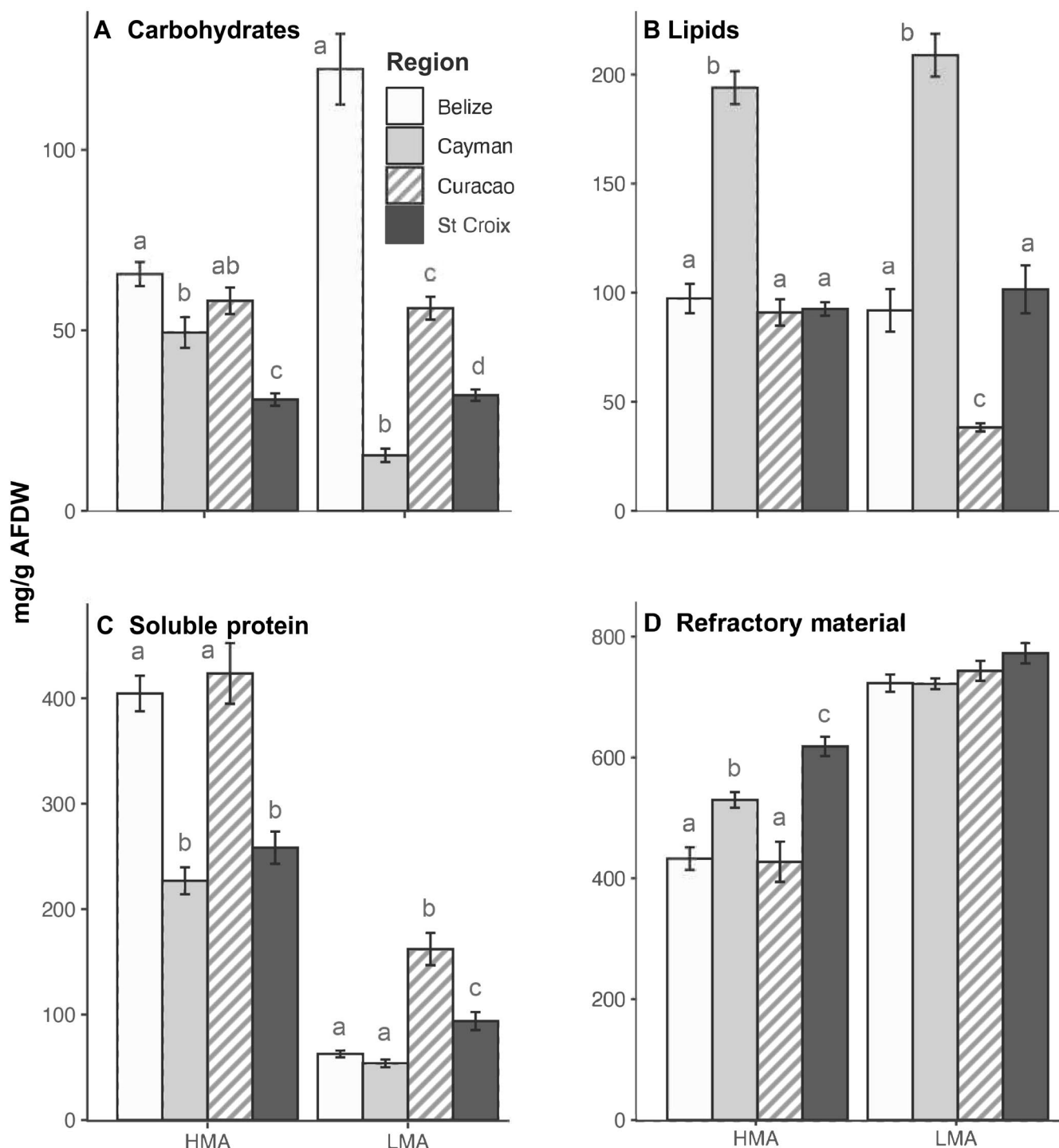


FIGURE 5 Sponge biochemical content in sponges with high and low microbial abundance (HMA and LMA, respectively). Barplots represent the mean concentration ($\pm$ SE) of carbohydrates (A), lipids (B), soluble protein (C), and refractory material (D) in milligrams per gram ash-free dry weight (AFDW) by microbial abundance and region. Sample sizes ranged 21–61 individuals per species. HMA and LMA designations based on Gloeckner et al. (2014). Within a particular microbial abundance category, regions with different lower-case letters are significantly different in pairwise comparisons ($p < 0.05$); the absence of letters indicates no significant differences between regions

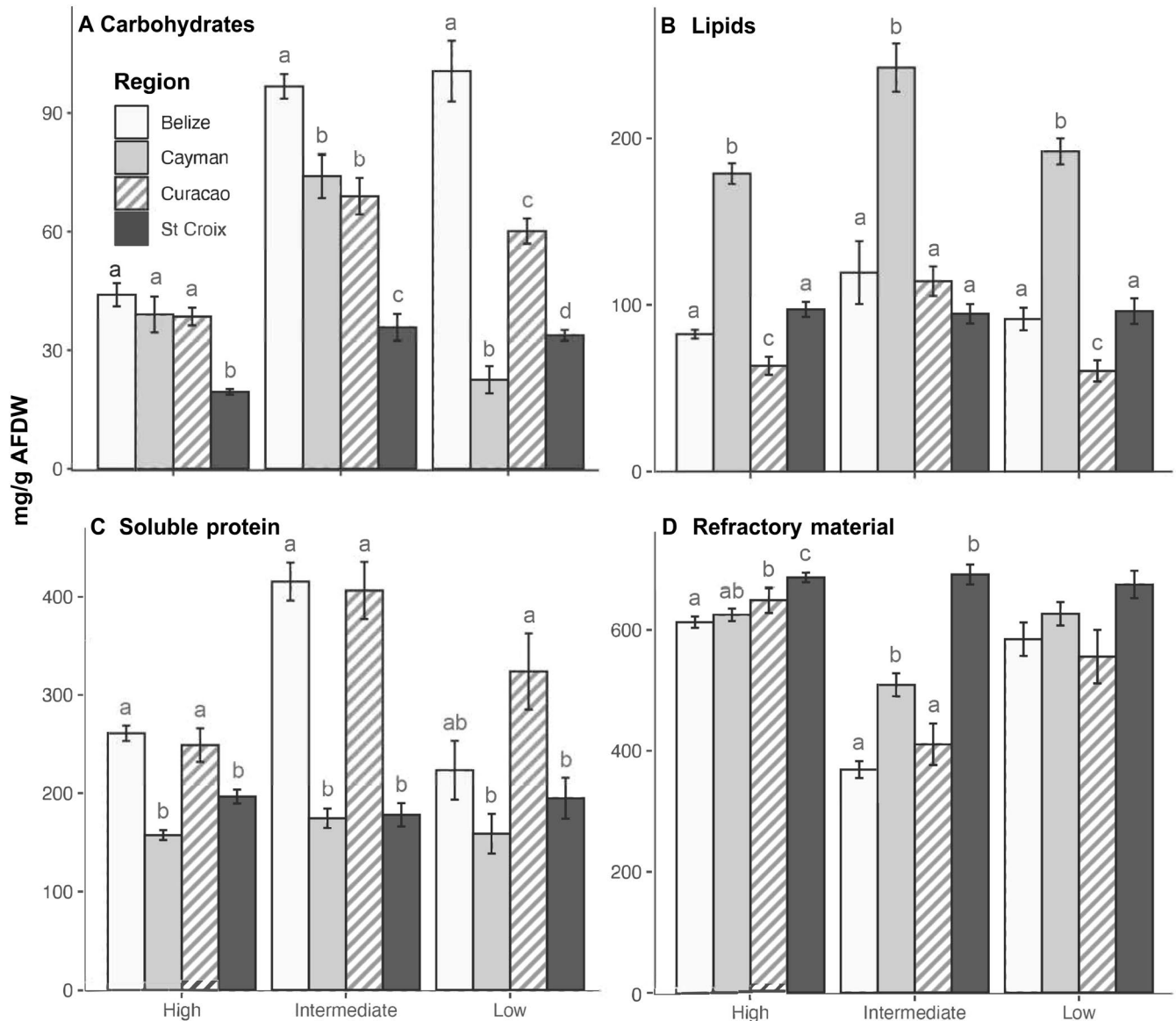


FIGURE 6 Sponge biochemical content by relative abundance of photosymbionts. Barplots represent the mean concentration ($\pm$ SE) of carbohydrates (A), lipids (B), soluble protein (C), and refractory material (D) in milligrams per gram ash-free dry weight (AFDW) by region and by relative abundance of photosymbionts (high, intermediate, or low). Sample sizes ranged 10–60 individuals per species. Photosymbiont abundance based on Erwin and Thacker (2007) and Freeman and Easson (2014). Within a particular relative abundance of photosymbionts, regions with different lower-case letters are significantly different in pairwise comparisons ($p < 0.05$); the absence of letters indicates no significant differences between regions

their measurements of energetic content in units of kilojoules per milliliter; however, we report our data in kilojoules per gram. This is because carbon and nitrogen energetic budgets that are most often used to quantify metabolic scaling within tissues, are mass based (Lesser, 2013). Thus, differences in tissue density between species are not reflected in the volumes reported by Chanas and Pawlik (1995), and may mask differences in energy content compared to normalization based on mass. In fact, when we convert the average energetic content of individual species to kJ/ml (using tissue mass-to-volume ratios from the sponge samples in the present study), our values are roughly in agreement with those estimated from figure 10 in Chanas and Pawlik (1995): 3.06 kJ/ml versus ~3.00 kJ/ml for

A. conifera/tubulata (our data vs. Chanas & Pawlik, 1995); 2.02 kJ/ml versus ~2.00 kJ/ml for *A. compressa*; 3.36 kJ/ml versus ~3.00 kJ/ml for *A. cauliformis*; 1.27 kJ/ml versus ~2.00 kJ/ml for *N. amorpho/erecta*; and 1.57 kJ/ml versus ~2.00 kJ/ml for *X. muta*.

Both microbial abundance and the relative abundance of photosymbionts were predictive of sponge PBC and energetic content (Figures 5 and 6; Table 4). Specifically, HMA sponges produced more soluble protein than did LMA sponges (Figure 5C). This may reflect the higher density of bacterial cells in HMA sponges, which can account for up to 35% of the total sponge biomass (Hentschel et al., 2012; Reisswig, 1981; Webster et al., 2001), and their contribution to the sponge biochemical constituents. An average of 50% of dry

bacterial mass is composed of soluble protein (Stickland, 1951), which is 20%–45% greater than observed here (Figure 5) or reported elsewhere (Freeman & Gleason, 2010; McClintock, 1987) for whole sponge tissue. As bacteria produce a higher proportion of soluble protein than sponge tissue, we can extrapolate that the symbiotic bacteria produce more soluble protein than sponge cells and that the greater concentration of soluble protein found in HMA sponges is largely bacterial in origin. Sponge PBC varied with the relative abundance of photosymbionts, and there was a significant interaction between relative photosymbiont abundance and region. This interaction may be explained by differences in local environmental factors that impact photosynthesis, such as water turbidity (Klein, 1992) and low pH (Mangan et al., 2016).

In summary, we found that sponge PBC and energetic content is influenced by inherent biological traits, including the production of antipredator chemical defenses, microbial abundance, and the relative abundance of photosymbionts. Additionally, we provide evidence to suggest that lower energetic content among sponge species represents an alternative strategy to avoid predation, in lieu of antipredator chemical defenses. We also found regional differences in sponge PBC and energetic content, particularly energy stores in the form of carbohydrates and lipids. We speculate that these energy reserves are impacted by seasonal reproductive cycles and the incidence of potential stressors, including terrestrial runoff and storm activity. Our results suggest that the plasticity of sponge nutritional content may influence Caribbean coral reef trophic dynamics, with implications for reef structure, function, and resilience.

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REFERENCES

- Biggerstaff, A., Smith, D. J., Jompa, J., & Bell, J. J. (2017). Metabolic responses of a phototrophic sponge to sedimentation supports transitions to sponge dominated reefs. *Scientific Reports*, 7, 2725. <https://doi.org/10.1038/s41598-017-03018-y>
- Bradford, M. M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye bonding. *Analytical Biochemistry*, 72, 248–254.
- Chanas, B., & Pawlik, J. R. (1995). Defenses of Caribbean sponges against predatory reef fish. II. Spicules, tissue toughness, and nutritional quality. *Marine Ecology Progress Series*, 127, 195–211. <https://doi.org/10.3354/meps127195>
- Chanas, B., & Pawlik, J. R. (1996). Does the skeleton of a sponge provide a defense against predatory reef fish? *Oecologia*, 107, 225–231. <https://doi.org/10.1007/bf00327906>
- Chollett, I., Collin, R., Bastidas, C., Cróquer, A., Gayle, P. M. H., Jordán-Dahlgren, E., Koltjes, K., Oxenford, H., Rodriguez-Ramirez, A., Weil, E., Alemu, J., Bone, D., Buchan, K. C., Creary Ford, M., Escalante-Mancera, E., Garzón-Ferreira, J., Guzmán, H. M., Kjerfve, B., Klein, E., ... Cortés, J. (2017). Widespread local chronic stressors in Caribbean coastal habitats. *PLoS ONE*, 12, e0188564. <https://doi.org/10.1371/journal.pone.0188564>
- Corredor, J. E., & Morell, J. E. (2001). Seasonal variation of physical and biogeochemical features in Eastern Caribbean surface water. *Journal of Geophysical Research: Oceans*, 106, 4517–4525. <https://doi.org/10.1029/2000jc000291>
- Cronin, G. (2001). Resource allocation in seaweeds and marine invertebrates: Chemical defense patterns in relation to defense theories. In J. B. McClintock & J. B. Baker (Eds.), *Marine chemical ecology* (pp. 325–353). CRC Press.
- D'Croz, L., Del Rosario, J. B., & Góndola, P. (2005). The effect of fresh water runoff on the distribution of dissolved inorganic nutrients and plankton in the Bocas del Toro Archipelago, Caribbean Panama. *Caribbean Journal of Science*, 41, 414–429.
- de Goeij, J. M., van Oevelen, D., Vermeij, M. J. A., Osinga, R., Middelburg, J. J., de Goeij, A. F. P. M., & Admiraal, W. (2013). Surviving in a marine desert: The sponge loop retains resources within coral reefs. *Science*, 342, 108–110. <https://doi.org/10.1126/science.1241981>
- Deregisbus, D., Quartino, M. L., Campana, G. L., Momo, F. R., Wiencke, C., & Zacher, K. (2016). Photosynthetic light requirements and vertical distribution of macroalgae in newly ice free areas in Potter Cove, South Shetland Islands, Antarctica. *Polar Biology*, 39, 153–166. <https://doi.org/10.1007/s00300-015-1679-y>
- Diaz, M. C., & Rützler, K. (2001). Sponges: An essential component of Caribbean coral reefs. *Bulletin of Marine Science*, 69, 535–546.
- Duffy, J. E., & Paul, V. J. (1992). Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. *Oecologia*, 90, 333–339. <https://doi.org/10.1007/bf00317689>
- Edmunds, P. J., Tsounis, G., Boulon, R., & Bramanti, L. (2019). Acute effects of back-to-back hurricanes on the underwater light regime of a coral reef. *Marine Biology*, 166, 20. <https://doi.org/10.1007/s00227-018-3459-z>
- Emlen, J. M. (1966). The role of time and energy in food preference. *The American Naturalist*, 100, 611–617. <https://doi.org/10.1086/282455>
- Erwin, P. M., & Thacker, R. W. (2007). Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. *Journal of the Marine Biological Association of the United Kingdom*, 87, 1683–1692. <https://doi.org/10.1017/S0025315407058213>
- Fell, P. (1993). Porifera. In K. G. Adiyodi & R. G. Adiyodi (Eds.), *Reproductive biology of invertebrates: Asexual propagation and reproductive strategies*, Vol. VI, Part A (pp. 1–44). John Wiley and Sons.
- Fiore, C. L., Baker, D. M., & Lesser, M. P. (2013). Nitrogen biogeochemistry in the Caribbean sponge, *Xestospongia muta*: A source or sink of dissolved inorganic nitrogen? *PLoS ONE*, 8, e72961. <https://doi.org/10.1371/journal.pone.0072961>

- Freeman, C. J., Easson, C. G., Matterson, K. O., Thacker, R. W., Baker, D. M., & Paul, V. J. (2020). Microbial symbionts and ecological divergence of Caribbean sponges: A new perspective on an ancient association. *The ISME Journal*, 14, 1571–1583. <https://doi.org/10.1038/s41396-020-0625-3>
- Freeman, C. J., Easson, G. C., & Baker, D. M. (2014). Metabolic diversity and niche structure in sponges from the Miskito Cays, Honduras. *PeerJ*, 2, e695. <https://doi.org/10.7717/peerj.695>
- Freeman, C. J., & Gleason, D. (2010). Chemical defenses, nutritional quality, and structural components in three sponge species: *Ircinia felix*, *I. campana*, and *Aplysina fulva*. *Marine Biology*, 157, 1083–1093. <https://doi.org/10.1007/s00227-010-1389-5>
- Freeman, N. K., Lindgren, F. T., Ng, Y. O., & Nichols, A. V. (1957). Infrared spectra of some lipoproteins and related lipids. *Journal of Biological Chemistry*, 203, 293–304.
- Gloeckner, V., Wehrl, M., Moitinho-Silva, L., Gernert, C., Schupp, P., Pawlik, J. R., Lindquist, N. L., Erpenbeck, D., Wörheide, G., & Hentschel, U. (2014). The HMA-LMA dichotomy revisited: An electron microscopical survey of 56 sponge species. *Biological Bulletin*, 227, 78–88. <https://doi.org/10.1086/bblv227n1p78>
- Gnaiger, E., & Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: A stoichiometric concept. *Oecologia*, 62, 289–298. <https://doi.org/10.1007/BF00384259>
- Gochfeld, D. J., Kamel, H. N., Olson, J. B., & Thacker, R. W. (2012). Trade-offs in defensive metabolite production but not ecological function in healthy and diseased sponges. *Journal of Chemical Ecology*, 38, 451–462. <https://doi.org/10.1007/s10886-012-0099-5>
- Gochfeld, D. J., Olson, J., Chaves-Fonnegra, A., Smith, T., Ennis, R. S., & Brandt, M. E. (2020). Impacts of Hurricanes Irma and Maria on coral reef sponge communities in St. Thomas, U.S. Virgin Islands. *Estuaries and Coasts*, 43, 1235–1247. <https://doi.org/10.1007/s12237-020-00694-4>
- Haukioja, E., Ruohomäki, K., Suomela, J., & Vuorisalo, T. (1991). Nutritional quality as a defense against herbivores. *Forest Ecology and Management*, 39, 237–245. [https://doi.org/10.1016/03781127\(91\)90179-y](https://doi.org/10.1016/03781127(91)90179-y)
- Hentschel, U., Piel, J., Degnan, S. M., & Taylor, M. W. (2012). Genomic insights into the marine sponge microbiome. *Nature Reviews Microbiology*, 10, 641–654. <https://doi.org/10.1038/nrmicro2839>
- Herrnkind, W. F., Butler, M. J. V. I., Hunt, J. H., & Childress, M. (1997). Role of physical refugia: Implications from a mass sponge die-off in a lobster nursery in Florida. *Marine and Freshwater Research*, 48, 759–770. <https://doi.org/10.1071/MF97193>
- Ismet, M. S., Bengen, D. G., Setianingsih, W. A., Yudhani, B. A., & Natih, N. M. N. (2019). Associative-mesofauna abundance and its correlation with sponges antibacterial activity in seagrass ecosystem. *IOP Conference Series: Earth and Environmental Science*, 404, 012007. <https://doi.org/10.1088/1755-1315/404/1/012007>
- Klein, R. M. (1992). Effects of green light on biological systems. *Biological Reviews*, 67, 199–284. <https://doi.org/10.1111/j.1469-185x.1992.tb01019.x>
- Kobluk, D. R., & Van Soest, R. W. M. (1989). Cavity-dwelling sponges in a southern Caribbean coral reef and their paleontological implications. *Bulletin of Marine Science*, 44, 1207–1235.
- Koukouras, A., Russo, A., Voultsiadou-Koukoura, E., Arvanitidis, C., & Stefanid, D. (1996). Macrofauna associated with sponge species of different morphology. *Marine Ecology*, 17, 569–582. <https://doi.org/10.1111/j.1439-0485.1996.tb00418.x>
- Kozak, M., & Piepho, H. (2017). What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. *Journal of Agronomy and Crop Science*, 204, 86–98. <https://doi.org/10.1111/jac.12220>
- Krebs, J. R., & Davies, N. B. (1993). *An introduction to behavioral ecology* (3rd ed.). Blackwell Scientific Publications.
- Lawrence, J. M. (1973). Level, content, and energy equivalents of the lipid, carbohydrate and protein in the body components of *Luidia clathrata* (Echinodermata: Asteroidea: Platyasterida) in Tampa Bay. *Journal of Experimental Marine Biology and Ecology*, 11, 263–274.
- Leong, W. L., & Pawlik, J. R. (2010). Evidence of a resource trade-off between growth and chemical defenses among Caribbean coral reef sponges. *Marine Ecology Progress Series*, 406, 71–78. <https://doi.org/10.3354/meps08541>
- Leong, W. L., & Pawlik, J. R. (2011). Comparison of reproductive patterns among 7 Caribbean sponge species does not reveal a resource trade-off with chemical defenses. *Journal of Experimental Marine Biology and Ecology*, 401, 80–84. <https://doi.org/10.1016/j.jembe.2011.02.043>
- Lesser, M. P. (2006). Benthic–pelagic coupling on coral reefs: Feeding and growth of Caribbean sponges. *Journal of Experimental Marine Biology and Ecology*, 328, 277–288. <https://doi.org/10.1016/j.jembe.2005.07.010>
- Lesser, M. P. (2013). Using energetic budgets to assess the effects of environmental stress on corals: Are we measuring the right things? *Coral Reefs*, 32, 25–33. <https://doi.org/10.1007/s00338-012-0993-x>
- Loh, T.-L., McMurray, S. E., Henkel, T. P., Vicente, J., & Pawlik, J. R. (2015). Indirect effects of overfishing on Caribbean reefs: Sponges overgrow reef-building corals. *PeerJ*, 3, e901. <https://doi.org/10.7717/peerj.901>
- Loh, T.-L., & Pawlik, J. R. (2014). Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 4151–4156. <https://doi.org/10.1073/pnas.1321626111>
- López-Legentil, S., Bontemps-Subielos, N., Turon, X., & Banaigs, B. (2006). Temporal variation in the production of four secondary metabolites in a colonial ascidian. *Journal of Chemical Ecology*, 32, 2079–2084. <https://doi.org/10.1007/s10886-006-9148-2>
- Macarthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, 100, 603–609. <https://doi.org/10.1086/282454>
- Maldonado, M., & Bergquist, P. R. (2002). Phylum Porifera. In C. M. Young, M. A. Sewell, & M. E. Rice (Eds.), *Atlas of marine invertebrate larvae* (pp. 21–50). Academic Press.
- Mangan, N. M., Flamholz, A., Hood, R. D., Miloc, R., & Savage, D. F. (2016). pH determines the energetic efficiency of the cyanobacterial CO₂ concentrating mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, 113, E5354–E5362. <https://doi.org/10.1073/pnas.1525145113>
- Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C. (2005). Carbohydrate analysis by phenol sulfuric acid method in microplate format. *Analytical Biochemistry*, 339, 69–72.
- McClintock, J. B. (1987). Investigation of the relationship between invertebrate predation and biochemical composition, energy content, spicule armament and toxicity of benthic sponges at McMurdo Sound, Antarctica. *Marine Biology*, 94, 479–487. <https://doi.org/10.1007/BF00428255>
- McClintock, J. B., Heine, J., Slattery, M., & Weston, J. (1991). Biochemical and energetic composition, population biology, and chemical defense of the Antarctic ascidian *Cnemidocarpa verrucosa* Lesson. *Journal of Experimental Marine Biology and Ecology*, 147, 163–175. [https://doi.org/10.1016/0022-0981\(91\)90180-5](https://doi.org/10.1016/0022-0981(91)90180-5)
- Meylan, A. (1988). Spongivory in Hawksbill turtles: A diet of glass. *Science*, 239, 393–395. <https://doi.org/10.1126/science.239.4838.393>
- Norström, A. V., Nyström, M., Lokrantz, J., & Folke, C. (2009). Alternative states on coral reefs: Beyond coral–macroalgal phase shifts. *Marine Ecology Progress Series*, 376, 295–306. <https://doi.org/10.3354/meps07815>
- Olson, J. B., Thacker, R. W., & Gochfeld, D. J. (2014). Molecular community profiling reveals impacts of time, space, and disease status on the bacterial community associated with the Caribbean sponge *Aplysina cauliformis*. *FEMS Microbiology Ecology*, 87, 268–279.

- Pawlik, J. R., Chanas, B., Toonen, R. J., & Fenical, W. (1995). Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrence. *Marine Ecology Progress Series*, 127, 183–194. <https://doi.org/10.3354/meps127183>
- Penney, B. K. (2002). Lowered nutritional quality supplements nudibranch chemical defense. *Oecologia*, 132, 411–418. <https://doi.org/10.1007/s00442-002-0993-6>
- Randall, J. E., & Hartman, W. D. (1968). Sponge-feeding fishes of the West Indies. *Marine Biology*, 1, 216–225. <https://doi.org/10.1007/BF00347115>
- Reiswig, H. M. (1971). *In situ* pumping activities of tropical Demospongiae. *Marine Biology*, 9, 38–50. <https://doi.org/10.1007/BF00348816>
- Reiswig, H. M. (1981). Partial carbon and energy budgets of the bacteriosponge *Verongia fistularis* (Porifera: Demospongiae) in Barbados West-Indies. *Marine Biology*, 2, 273–294.
- Rhoades, D. F. (1979). Evolution of plant chemical defense against herbivores. In G. A. Rosenthal (Ed.), *Herbivores: Their interaction with secondary plant metabolites* (pp. 3–54). Academic Press.
- Ritson-Williams, R., Becerro, M. A., & Paul, V. J. (2005). Spawning of the giant barrel sponge *Xestospongia muta* in Belize. *Coral Reefs*, 24, 160. <https://doi.org/10.1007/s00338-004-0460-4>
- Rix, L., de Goeij, J. M., Mueller, C. E., Struck, U., Middelburg, J. J., van Duyl, F. C., Al-Horani, F. A., Wild, C., Naumann, M. S., & van Oevelen, D. (2016). Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems. *Scientific Reports*, 6, 18715. <https://doi.org/10.1038/srep18715>
- Sellner, K. G. (1981). Primary productivity and the flux of dissolved organic matter in several marine environments. *Marine Biology*, 65, 101–112. <https://doi.org/10.1007/bf00397073>
- Skogsmyr, I., & Fagerström, T. (1992). The cost of anti-herbivory defense: An evaluation of some ecological and physiological factors. *Oikos*, 64, 451–457.
- Slaterry, M., Gochfeld, D. J., Diaz, M. C., Thacker, R. W., & Lesser, M. P. (2016). Variability in chemical defense across a shallow to mesophotic depth gradient in the Caribbean sponge *Plakortis angulospiculatus*. *Coral Reefs*, 35, 11–22. <https://doi.org/10.1007/s00338-015-1324-9>
- Slaterry, M., Kamel, H. N., Ankisetty, S., Gochfeld, D. J., Hoover, C. A., & Thacker, R. W. (2008). Hybrid vigor in a tropical Pacific soft coral community. *Ecological Monographs*, 78, 423–443.
- Slaterry, M., & Lesser, M. P. (2012). Mesophotic coral reefs: A global model of community structure and function. *Proceedings of the 12th International Coral Reef Symposium*, 1, 9–13.
- Slaterry, M., & Lesser, M. P. (2014). Allelopathy in the tropical alga *Lobophora variegata*: Mechanistic basis for a phase shift on mesophotic coral reefs. *Journal of Phycology*, 50, 493–505.
- Smith, T. B., Nemeth, R. S., Blondeau, J., Calnan, J. M., Kadison, E., & Herzlieb, S. (2008). Assessing coral reef health across onshore to offshore stress gradients in the US Virgin Islands. *Marine Pollution Bulletin*, 56, 1983–1991. <https://doi.org/10.1016/j.marpolbul.2008.08.015>
- Sokolova, I. M. (2013). Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. *Integrative and Comparative Biology*, 53, 597–608. <https://doi.org/10.1093/icb/ict028>
- Stickland, L. H. (1951). The determination of small quantities of bacteria by means of the biuret reaction. *Journal of General Microbiology*, 5, 698–703. <https://doi.org/10.1099/00221287-5-4-698>
- Thompson, J. E., Murphy, P. T., Bergquist, P. R., & Evans, E. A. (1987). Environmentally induced variation in diterpene composition of the marine sponge *Rhopaloeides odorabile*. *Biochemical Systematics and Ecology*, 15, 595–606. [https://doi.org/10.1016/0305-1978\(87\)90111-6](https://doi.org/10.1016/0305-1978(87)90111-6)
- Uriz, M.-J., Turon, X., Becerro, M. A., & Galera, J. (1996). Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage. *Journal of Experimental Marine Biology and Ecology*, 205, 187–204.
- van Soest, R. W. M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N. J., Santodomingo, N., Vanhoorne, B., Kelly, M., & Hooper, J. N. A. (2012). Global diversity of sponges (Porifera). *PLoS ONE*, 7, e35105.
- Waddell, B., & Pawlik, J. R. (2000). Defenses of Caribbean sponges against invertebrate predators. II. Assays with sea stars. *Marine Ecology Progress Series*, 195, 133–144. <https://doi.org/10.3354/meps195133>
- Webster, N. S., Webb, R. I., Ridd, M. J., Hill, R. T., & Negri, A. P. (2001). The effects of copper on the microbial community of a coral reef sponge. *Environmental Microbiology*, 3, 19–31. <https://doi.org/10.1046/j.1462-2920.2001.00155.x>
- Weisz, J. B., Lindquist, N., & Martens, C. S. (2007). Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia*, 155, 367–376. <https://doi.org/10.1007/s00442-007-0910-0>
- Westrum, B. L., & Meyers, P. A. (1978). Organic carbon content of sea water from over three Caribbean reefs. *Bulletin of Marine Science*, 28, 153–158.
- Wulff, J. L. (2006a). Ecological interactions of marine sponges. *Canadian Journal of Zoology*, 84, 146–166. <https://doi.org/10.1139/z06-019>
- Wulff, J. L. (2006b). Resistance vs recovery: Morphological strategies of coral reef sponges. *Functional Ecology*, 20, 699–708. <https://doi.org/10.1111/j.1365-2435.2006.01143.x>
- Wulff, J. L., & Buss, L. W. (1979). Do sponges help hold coral reefs together? *Nature*, 281, 474–475. <https://doi.org/10.1038/281474a0>
- Wulff, J. L., & Buss, L. W. (1984). Sponge-mediated coral reef growth and rejuvenation. *Coral Reefs*, 3, 157–163. <https://doi.org/10.1007/BF00301960>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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