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Biochemical Variation in Caribbean Sponges of the Genus Aplysina

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Biochemical Variation in Caribbean Sponges of the Genus *Aplysina*

by
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Abstract

Sponges are among the simplest multicellular organisms, consisting of groupings of cells with similar functions rather than true tissues. Nonetheless, sponges are ecologically significant in many marine environments, including coral reefs. They are sedentary organisms that feed by filtration from the water column, which contributes to the numerous roles they play on coral reefs, including upkeep of the reef, production of usable energy, and recycling of nutrients.

Like all multicellular organisms, sponges are made up of biologically significant macromolecules. These macromolecules provide the energy for the sponge, and their relative concentrations determine how sponges can store and access the energy necessary to perform required functions. These macromolecules include proteins, which are often structural, lipids, which function in long-term storage of energy, and carbohydrates, which allow easy access to energetically favorable breakdown pathways. Anything not contained in these categories is considered energetically inert, but may serve other purposes within the sponge, such as providing physical structure.

This study evaluated the distribution of these biochemical components within three distinct morphotypes of Caribbean sponges: *Aplysina fulva, Aplysina cauliformis* thick morph, and *Aplysina cauliformis* thin morph. It was hypothesized that these morphotypes within the same genus would present differently in terms of biochemical components. The study also assessed the distribution of these biochemical components

within the morphotypes at differentially impacted sites from St. Thomas, US Virgin Islands, and the Bahamas. It was again hypothesized that sponges would vary in their biochemical constituents due to variability in water quality among these sites.

Results showed that the distribution of these biochemical components varied significantly, but not consistently, between both sites and morphotypes. Protein and carbohydrates were present at high concentrations at the most environmentally impacted site, while protein and lipid were present at high concentrations at the least impacted site, suggesting a relationship between assimilation of nutrients into macromolecules and water quality. Sites with an intermediate level of impact had an inverse relationship between percent carbohydrates and percent lipids in *A. cauliformis* and a proportional relationship in *A. fulva*, suggesting that these two components could be related in a site with limited resources.

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Introduction

Every multicellular organism is made up of a variety of macromolecules that enable it to complete its life cycle and functions by providing energy and structure. These compounds include various proteins, lipids, and carbohydrates, as well as inorganic material. Proteins are macromolecules formed from individual amino acids. There are 20 different amino acids that can be combined in thousands of ways to make a diverse group of proteins that are thought to define the function of an organism. Carbohydrates are formed from monosaccharide precursors, and are involved in the energy yielding process of glycolysis, which is a primary energy-producing pathway in organisms that cannot carry out photosynthesis. Lipids are macromolecules that are hydrophobic, not consisting of a unified set of base units. They often function in energy storage and the formation of biological membranes (Nelson and Cox, 2008). Because these macromolecules are created and used for various purposes within an organism, it is surmised that differences in the relative proportion of these molecules could indicate differences in lifestyle or condition of the organism. In all organisms, there is likely a trade-off of resource usage between the different functions an organism must perform, including reproduction, growth, and general survival. These trade-offs determine how energy is stored (Ben-David-Zaslow & Benayahu, 1999).

Sponges, while very simple multicellular organisms, possess these same characteristic macromolecules. Depending upon the function the sponge is trying to carry

out, the energy requirements differ. For instance, growth typically has a very high demand for energy, which could play a role in determining what types and relative amounts of macromolecules are stored (Thomassen & Riisgård, 1995). McClintock & Pearce (1986) suggest that reproduction in marine invertebrates correlates with an increase in energy demands as well, as reproduction coincides with an increase in the biochemical components used for energy production. The energy requirements likely differ between stressed and un-stressed sponges, as trade-offs occur in order to produce secondary metabolites for defense in stressed sponges (Gochfeld et al., 2012). Quantifying differences in the relative proportions of these molecules in a sponge can help determine total energy content (Lawrence, 1973; Lawrence & Guille, 1982). For instance, on a per mass basis, lipids contain a higher energy content than carbohydrates. Storage of energy as lipids could indicate a requirement for a larger amount of energy. On the other hand, carbohydrates can be broken down quickly and efficiently in comparison to lipids and can be used to rapidly generate energy for the organism. Carbohydrates can therefore be considered a more temporary storage of energy (McClintock & Pearse, 1986). In addition to providing an energy source, protein serves a structural and connective function, suggesting that high levels of protein may not be directly related to a large energy requirement (Slattery & McClintock, 1995).

Sponge physiology

Sponges are composed of groups of cells rather than true tissues or organs, and do not possess any comprehensive organ systems (Thomassen & Riisgård, 1995). However, while they are physiologically relatively simple, they are one of the most diverse

invertebrate phyla worldwide, and occur in an enormous variety of morphologies (Wulff, 2001). Typically, sponges feed by filtration. Filtration makes use of choanocytes, which are flagellated cells that channel water into and through the sponge (Thomassen & Riisgård, 1995). Filtered particles often consist of bacterial species, both autotrophic cyanobacteria and heterotrophic bacteria, and plankton, and are usually no larger than 5-10 μm (Pile et al., 1996; Bell et al., 2015). While choanocytes can cause some movement of water within the sponge using their flagellae, sponges have very little control of what enters their bodies (Bell et al., 2015). They do retain some control over what remains in their body however, and can select based on nutritional quality or symbiont propensity (Werhl et al., 2007; Hanson et al., 2009). The diet of the sponge relies heavily on the constituents of the water column surrounding it, including the microbial community that varies with geography, weather and time (Pile et al., 1996). While filter feeding is the main source of nutrition, some of a sponge's nutrients can come from photosynthetic symbionts. These symbionts can include photosynthetic dinoflagellates known as zooxanthellae or photosynthetic bacteria such as some forms of cyanobacteria (Bell et al., 2015).

Functional Roles of Sponges

Sponges play a variety of roles in the coral reef ecosystem. For example, they act as both consumers and food in their respective food chains. They also stabilize coral reef structures, where they can assist in the regeneration of a damaged reef by "gluing" the reef together (Bell, 2008). A primary role of sponges is the functional connection they make between the benthic and pelagic regions by their filtration of the water column

(Bell, 2008; Bell et al., 2015). They play a role in nutrient recycling, especially for carbon, nitrogen, and silica in which they both break down the sources of these nutrients, such as inorganic nitrogen compounds and organic carbon matter, to release them into the environment and intake the nutrients, playing some role in their transformation into usable substances. In terms of nitrogen cycling, sponges act as a host for a huge diversity of microbial species that can perform nitrification, denitrification, and other associated processes (Bell, 2008; Hoffmann et al., 2009). The sponges can function as energy producers as well, in collaboration with the photosynthetic symbionts living inside them, contributing a significant portion to the productivity of coral reefs. In this primary production, they have a role in recycling carbon (Wulff, 2001; Bell, 2008).

Sponges of the genus *Aplysina* are among the most abundant sponges found on Caribbean coral reefs. Among these are several branching species or morphotypes, which vary in physical appearance and in their production of secondary metabolites (Stockton, 2016). However, their biochemical composition has yet to be quantified to determine whether they are also distinct from each other biochemically. This is considered likely given that sponges produce the largest diversity of secondary metabolites, or natural products, of any plant or animal phylum (Mehbub et al., 2014), and *Aplysina* species are well known for their diversity of secondary metabolites (Gochfeld et al., 2012; Puyana et al., 2015). Since secondary metabolites are potentially energetically costly to produce, variability in the production of these defenses could result in tradeoffs between sources of energetic macromolecules within the sponge (Coley et al., 1985; Stowe et al., 2000). Furthermore, it has been demonstrated that secondary metabolite production varies geographically (Sacristan-Soriano et al., 2011; Stockton, 2016), in which case, one could

hypothesize that the energetic components that make up the sponge could differ based on location. Sponges included in this study represent three branching morphotypes of Caribbean *Aplysina*: the thick ("brown erect") and thin ("violet creeping") morphotypes of *Aplysina cauliformis,* along with *Aplysina fulva* (Zea et al., 2014). The locations from which the sponges were collected (a remote site in the Bahamas and several sites in St. Thomas, US Virgin Islands) have varying degrees of pollution and impacted water quality. Discovering whether the biochemical composition of these sponges differ in relation to water quality could elucidate the impacts that urbanization and coastal development have on marine sponges in the Caribbean. This study tested the following hypotheses: (1) that different morphotypes of *Aplysina* have different biochemical constituents, and (2) that different locations (and associated differences in water quality) have an effect on the biochemical constituents of sponges.

Methods

The sponges were collected by hand using SCUBA at approximately 5 m of depth from five different locations, one in the Exuma Cays, Bahamas and four near St. Thomas in the US Virgin Islands. The sponges were frozen, and approximately 1-2 cm pieces of frozen sponge were freeze-dried and pulverized. A total of 134 samples was analyzed, broken down into groups based upon morphotype and site. Morphotypes included *Aplysina cauliformis* thick (AC-TK), *Aplysina cauliformis* thin (AC-TN), and *Aplysina fulva* (AF) (Figure 1). Sites include the Bahamas (BAH) and St. Thomas (STT), which is further broken down into Flat Cay (FC), Brewer's Reef (BR), Saba Island (SB), and Savana Island (SV) (Figure 2). Each group contained 10 replicates, except for AC-TK from Saba Island, which only contained four replicates. There were no AF samples from Savana Island.

Protein Analysis

Analysis of total protein concentration was based on the Bradford assay protocol (Bradford, 1976). Briefly, pulverized sponge tissue (10.0-10.4 mg) was extracted in 1 M NaOH for 18-24 hours. The protein extracts were reacted with Bio Rad Quick Start™ Bradford 1x Dye Reagent in a ratio of 0.2:1, and the concentrations were quantified by comparing absorbance values against a standard curve generated with the use of seven Bio Rad protein standards ranging from 0.125 to 2.000 mg/mL. Absorbances of each

sample were measured at a wavelength of 595 nm in an Eppendorf BioPhotometer. To calculate the protein concentration in the tissue of the sponge, the concentration of protein in the NaOH solution, measured in μg/mL, was multiplied by a conversion factor based on the volume of sponge extract (5 mL NaOH/original weight of sponge in mg).

Lipid Analysis

Total lipid analysis was based on the procedure of Freeman et al. (1957). For this analysis, 50.0 – 50.4 mg of dried pulverized sponge was extracted with 2:1 chloroform:methanol for 15 minutes on a sonicator. The extract was filtered through 70 mm filter paper into a separatory funnel and separated over water. The filtrate was kept and underwent the same procedure a second time. After the second separation and subsequent removal of filtrate, the aqueous portion was mixed with 2:1 chloroform:methanol to remove any trace amount of lipids, and after separation, the hydrophobic extract was added to the filtrate. The extract was dried in a SpeedVac and weighed. The mass of lipids was calculated by subtracting the initial weight of the pre-weighed vial from its final weight, and this value was then divided by the initial sponge weight.

Carbohydrate Analysis

Carbohydrate analysis was based on the methods of Dubois et al. (1953). Between 10.0 – 10.4 mg of pulverized sponge were extracted using 5 mL of 5% trichloroacetic acid for 3 hours. 50 μL of the sponge extract were mixed with 150 μL of concentrated sulfuric acid and 30 μL of 5% phenol. The samples were run in triplicate in 96-well plates. The plates were placed in a 90˚C water bath for 5 minutes, then they were placed in a room

temperature water bath for 5 minutes. Absorbance was measured at a wavelength of 490 nm on a BioTek Synergy HT Multi-Detection Microplate Reader, and the percentage of carbohydrates in the original sample was quantified against a standard curve developed using four glucose standards ranging from 0.01-0.225 mg/mL.

Ash Content

Between 15-30 mg of sponge was placed into small foil dishes that had been dried for 30 minutes in a muffle furnace at 500˚C and then weighed on a microbalance. The samples were placed into the furnace for 4 hours at 500˚C and then reweighed. The ash content (i.e., inorganic components of the sponge) was determined by subtraction of the final weight from the initial weight of the sample and determination of the percent remaining.

Statistical Analysis

For each of the biochemical components (protein, lipid, carbohydrate, ash), a 1-way analysis of variance (ANOVA) was performed to evaluate whether they differed among morphotypes at each site. In addition, 1-way ANOVAs were performed on each biochemical component to determine whether there were differences among sites for each morphotype. A p-value of < 0.05 was considered to be significant. For ANOVAs that showed significant differences among groups, the post-hoc Fisher's Protected Least Significant Difference (PLSD) test identified the significant differences between the sites or morphotypes.

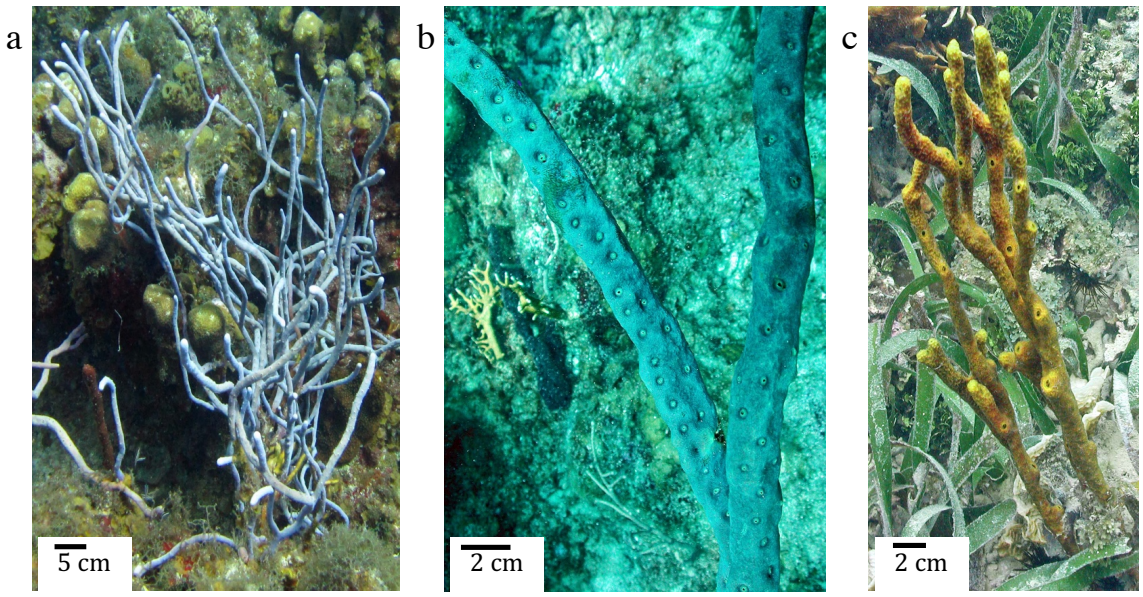

Figure 1. Photographs of the three morphotypes of *Aplysina* used in this study. a) *Aplysina cauliformis* thin morphotype; b) *Aplysina cauliformis* thick morphotype; c) *Aplysina fulva.* (Photos by D. Gochfeld, from Stockton, 2016)

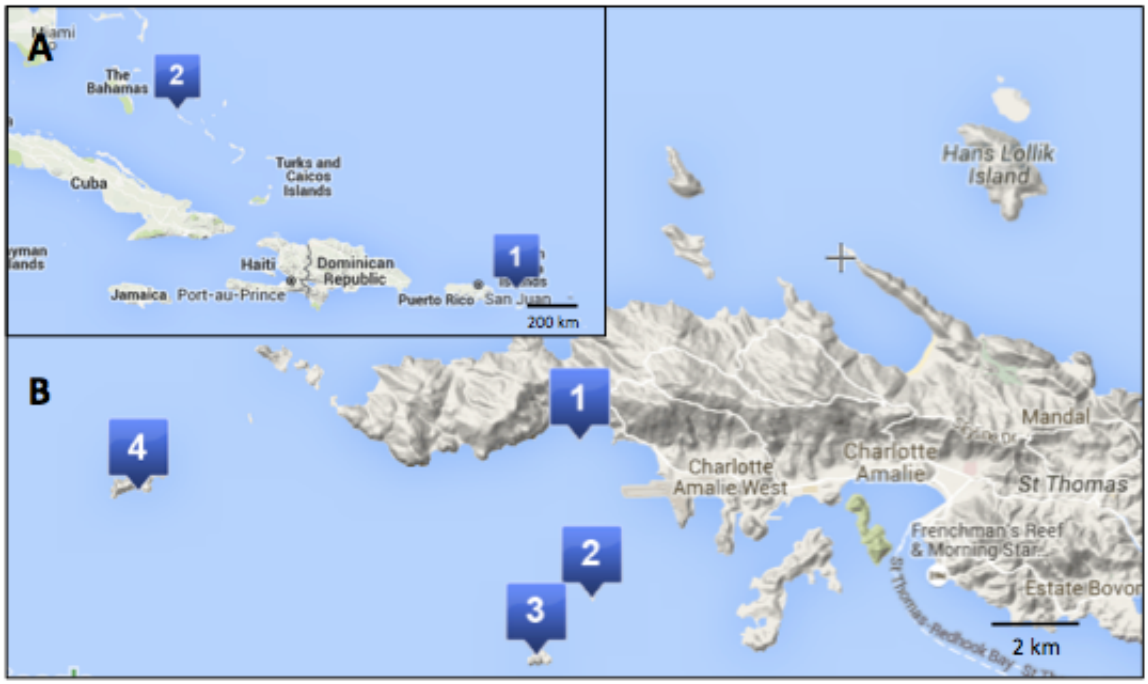

Figure 2. Map of sponge collection sites. A) number 1 represents St. Thomas, US Virgin Islands, and number 2 represents the Bahamas; B) sites within St. Thomas (1 = Brewer's Reef, 2 = Flat Cay, 3 = Saba Island, and 4 = Savana Island (from Stockton, 2016).

Results

Comparison among Morphs

As seen in Figure 3**,** sponges from Flat Cay differed significantly among morphotypes in terms of percent ash, percent lipid, and protein concentration (ANOVA, $p = 0.0218$, $p = 0.0182$, $p < 0.0001$, respectively). The samples did not differ in percent carbohydrate between morphs (ANOVA, $p > 0.05$). Levels of lipid for AF were dramatically lower than those found in the AC-TK and AC-TN morphs.

Sponges from Saba Island (Figure 4) did not differ significantly in percentage ash or percentage carbohydrates among morphs (ANOVA, $p > 0.05$). The morphs did differ significantly in percent lipids and protein concentration (ANOVA, $p = 0.0002$, $p = 0.006$, respectively). AF had very high levels of lipids as compared to AC-TK and AC-TN.

The sponges from Brewer's Reef differed significantly in terms of percent ash, percent lipid, and protein concentration (ANOVA, $p = 0.0001$, $p = 0.0003$, $p = 0.0182$, respectively), as can be seen in Figure 5. Percent ash was lower in AC-TN samples than in the other morphs, whereas percent lipid was higher and protein concentration was lower in AF than in the other two morphs. The samples from Brewer's Reef did not differ significantly in percent carbohydrates (ANOVA, $p > 0.05$).

As seen in Figure 6**,** sponges from Savana Island differed significantly in terms of percent ash and percent carbohydrate (ANOVA, $p = 0.0071$, $p = 0.0285$, respectively). They did not differ significantly among morphotypes in terms of percent lipid or protein

concentration (ANOVA, $p > 0.05$). The difference between AC-TK and AC-TN in percentage carbohydrates was dramatic.

Sponges from the Bahamas, pictured in Figure 7, did not differ significantly among morphotypes in percent ash, percent carbohydrate, or protein concentration (ANOVA, $p > 0.05$). The samples did differ significantly in terms of percent lipid (ANOVA, $p < 0.0001$), with the thin morphotype having significantly more lipid that the other morphs.

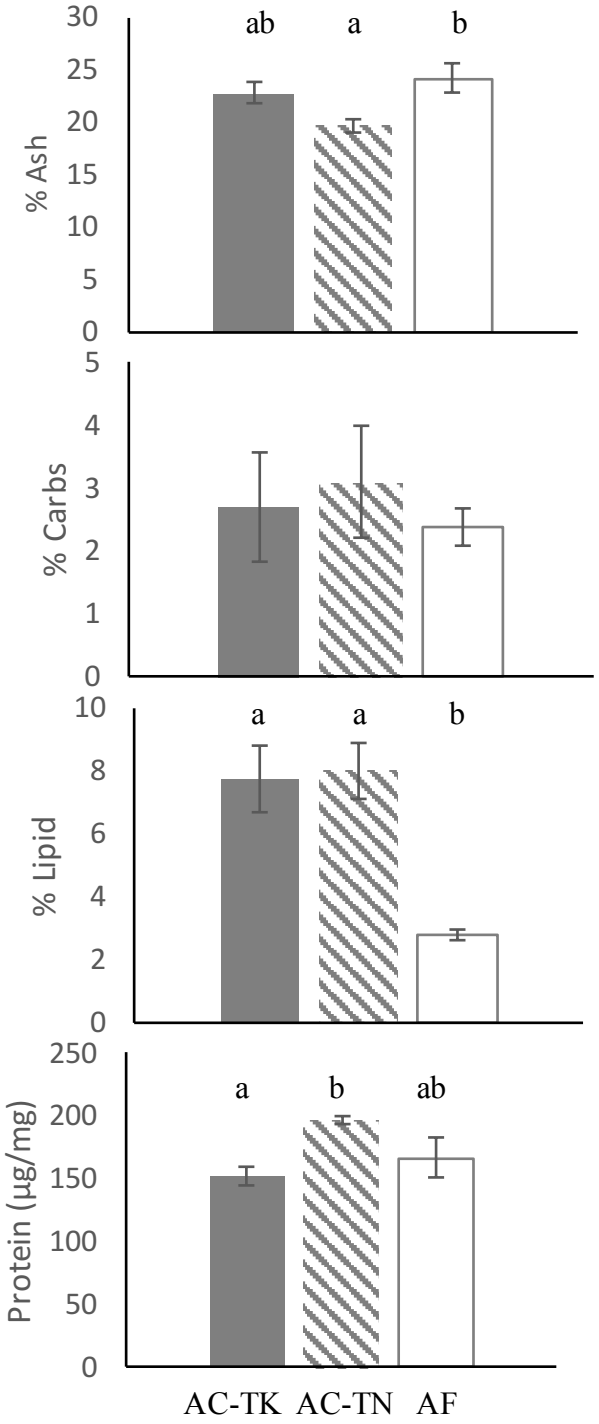

Figure 3. Proximate biochemical composition of *Aplysina* morphotypes from Flat Cay, St. Thomas, including *A. cauliformis* thick (AC-TK), *A. cauliformis* thin (AC-TN), and *A. fulva* (AF). Histograms represent mean ± 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

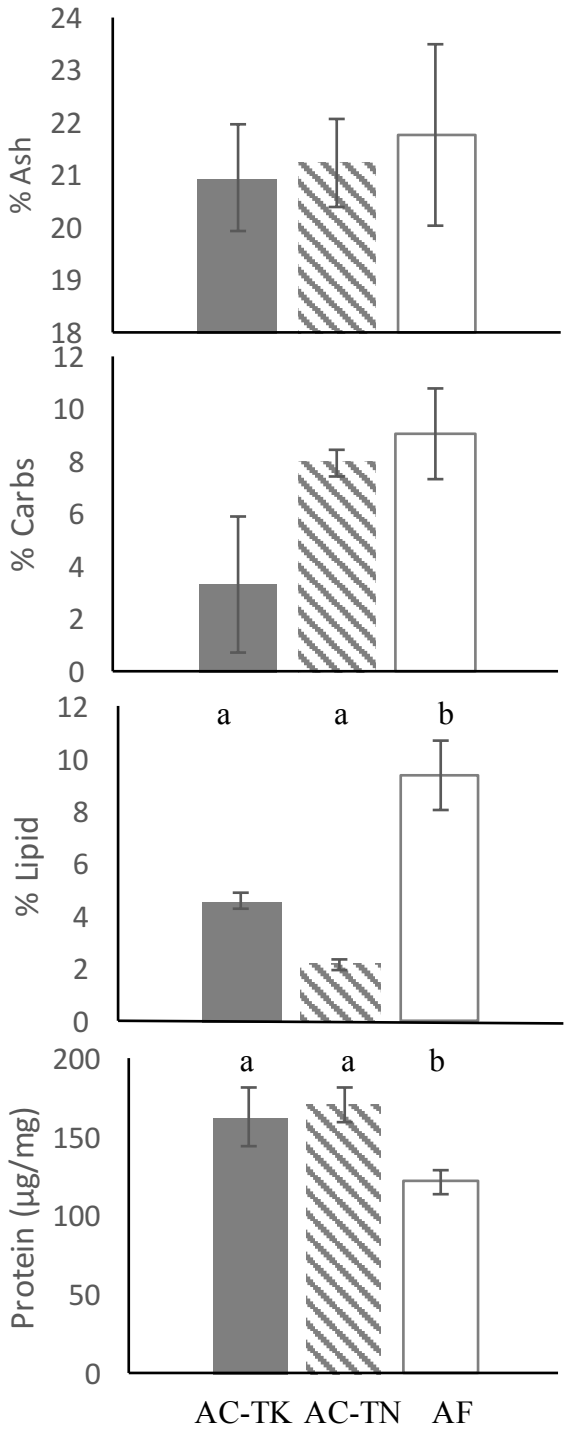

Figure 4. Proximate biochemical composition of *Aplysina* morphotypes from Saba Island, St. Thomas, including *A. cauliformis* thick (AC-TK), *A. cauliformis* thin (AC-TN), and *A. fulva* (AF). Histograms represent mean $\pm$ 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

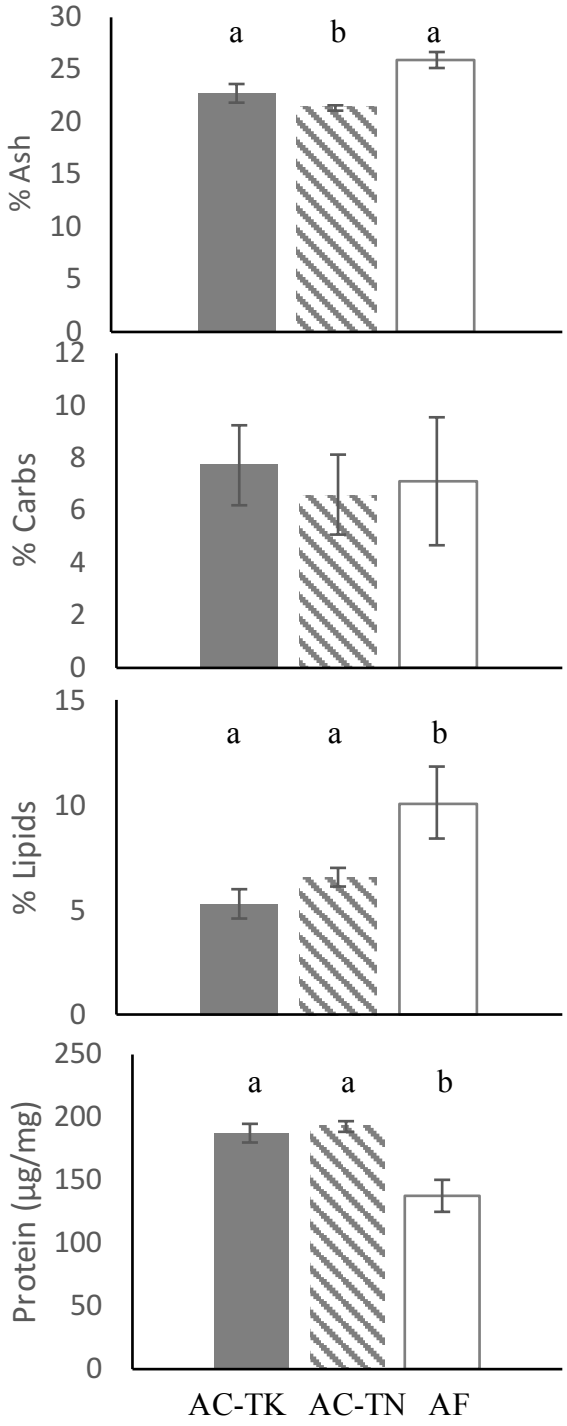

Figure 5. Proximate biochemical composition of *Aplysina* morphotypes from Brewer's Reef, St. Thomas, including *A. cauliformis* thick (AC-TK), *A. cauliformis* thin (AC-TN), and *A. fulva* (AF). Histograms represent mean $\pm$ 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

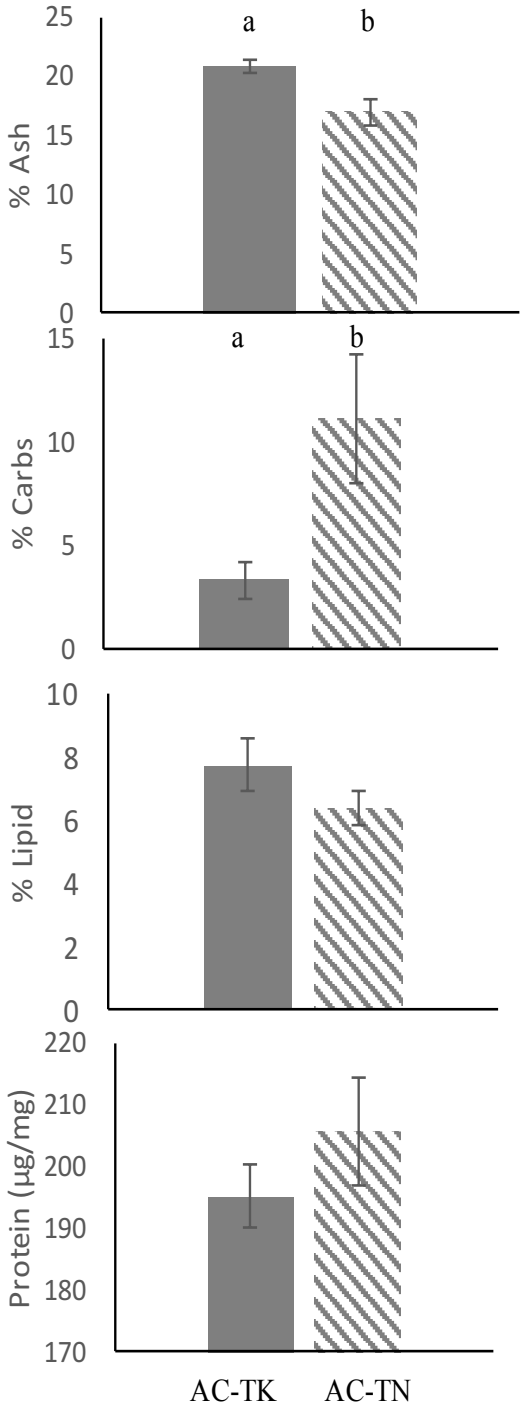

Figure 6. Proximate biochemical composition of *Aplysina* morphotypes from Savana Island, St. Thomas, including *A. cauliformis* thick (AC-TK), *A. cauliformis* thin (AC-TN), and *A. fulva* (AF). Histograms represent mean ± 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

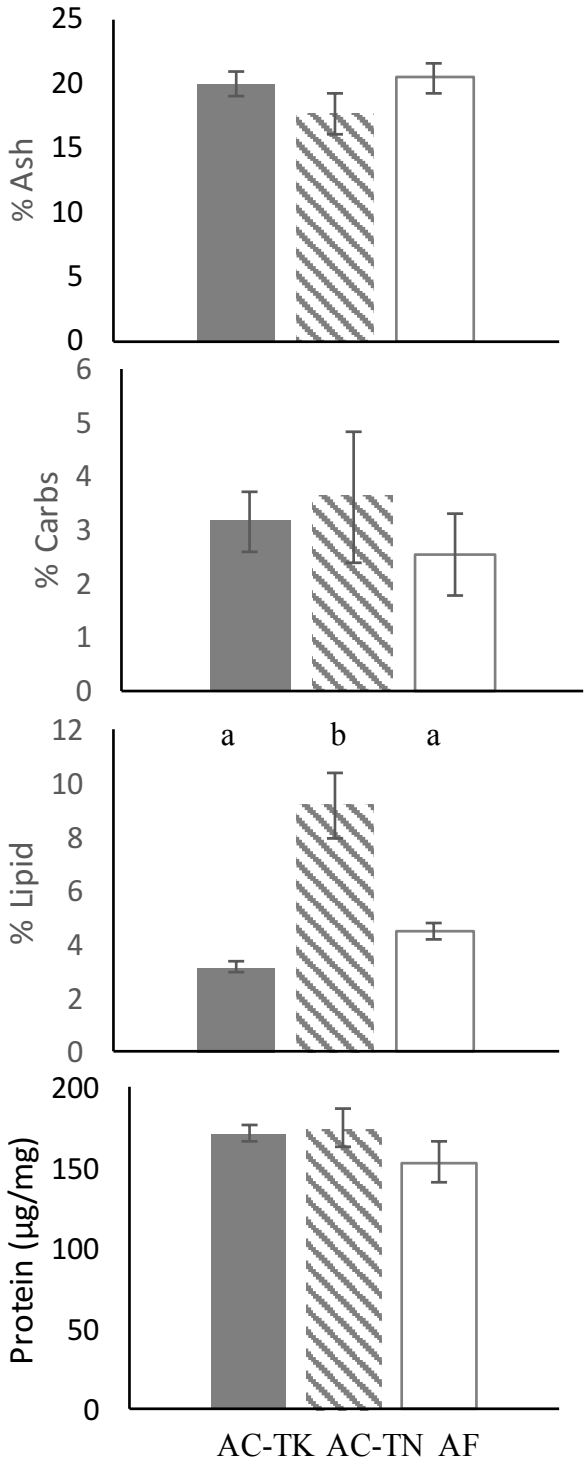

Figure 7. Proximate biochemical composition of *Aplysina* morphotypes from the Bahamas, including *A. cauliformis* thick (AC-TK), *A. cauliformis* thin (AC-TN), and *A. fulva* (AF). Histograms represent mean ± 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

Comparison among Sites

As shown in Figure 8, *A. fulva* differed significantly in percent ash, percent carbohydrates, and percent lipids (ANOVA, $p = 0.0295$, $p = 0.0066$, $p < 0.0001$, respectively) among the study sites. Sponges did not differ significantly in protein concentration (ANOVA, $p > 0.05$). Sponges from Brewer's Reef and Saba Island had significantly higher carbohydrate and lipid levels than sponges from Flat Cay and the Bahamas.

Among sites, the thick morphotype of *A. cauliformis* did not differ significantly in percent ash (ANOVA, $p > 0.05$), but did differ significantly in carbohydrate, lipid, and protein (ANOVA, $p = 0.0152$, $p = 0.0002$, $p = 0.0011$, respectively), as shown in Figure 9. Most notably, sponges from Brewer's Reef had significantly higher levels of carbohydrates.

The thin morphotype of *A. cauliformis* differed significantly in percent ash, percent carbohydrate, percent lipid, and protein concentration among sites (ANOVA, $p = 0.0062$, $p = 0.0149$, $p < 0.0001$, $p = 0.0356$, respectively), as seen in Figure 10. Sponges from Saba Island had very low levels of lipids. Sponges from Flat Cay and the Bahamas had high levels of lipids and low levels of carbohydrates.

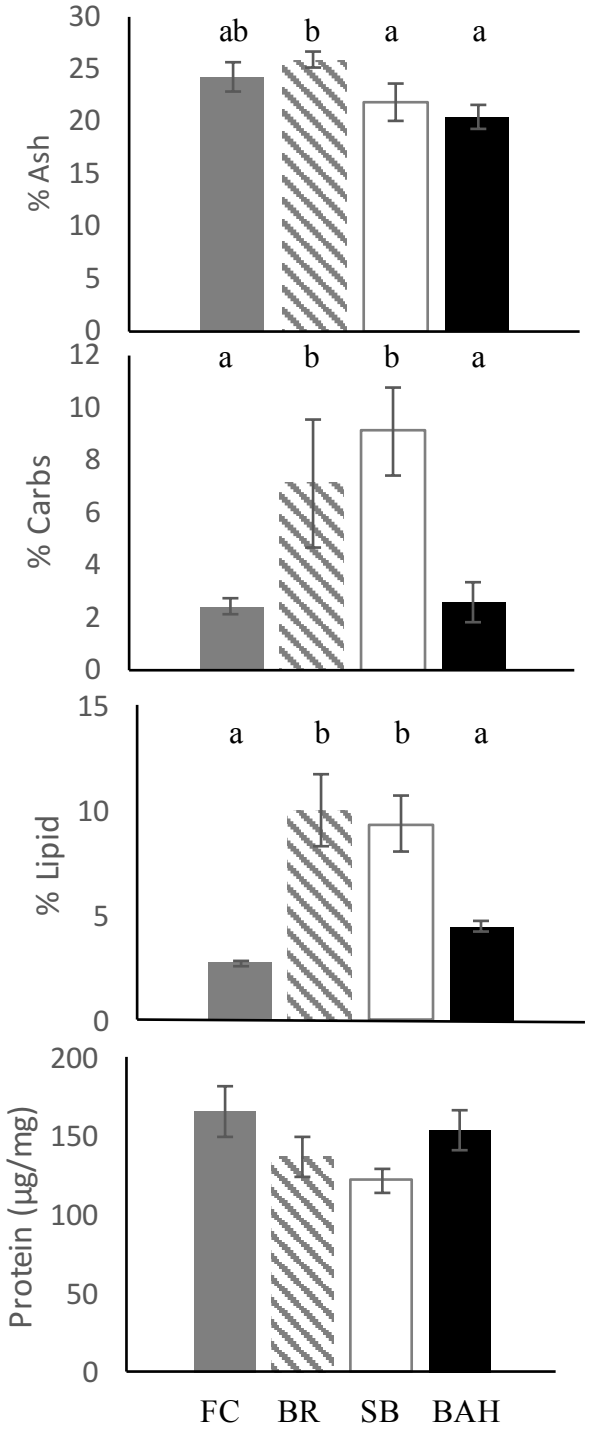

Figure 8. Proximate biochemical composition of *Aplysina fulva* at Flat Cay (FC), Brewer's Reef (BR), and Saba Island (SB), St. Thomas, and the Bahamas (BAH). Histograms represent mean $\pm$ 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

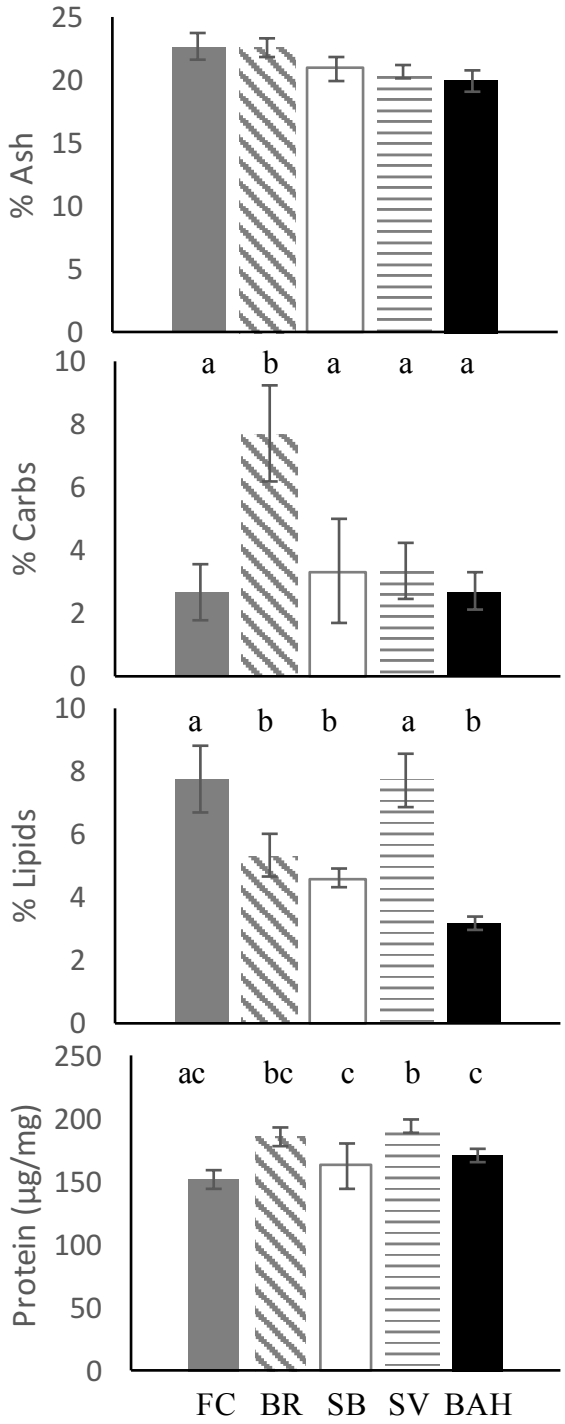

Figure 9. Proximate biochemical composition of the *Aplysina cauliformis* thick morphotype at various sites, including Flat Cay (FC), Brewer's Reef (BR), Saba Island (SB), and Savana Island (SV), St. Thomas, and the Bahamas (BAH). Histograms represent mean $\pm$ 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

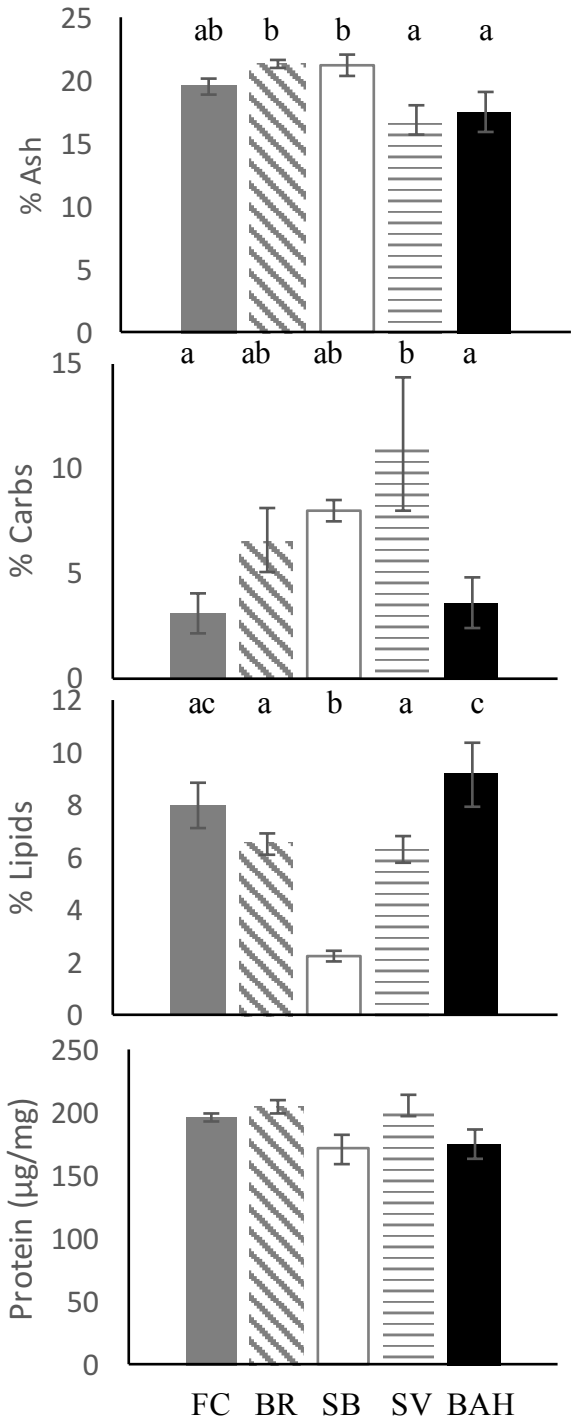

Figure 10. Proximate biochemical composition of the *Aplysina cauliformis* thin morphotype at various sites, including Flat Cay (FC), Brewer's Reef (BR), Saba Island (SB), and Savana Island (SV), St. Thomas, and the Bahamas (BAH). Histograms represent mean $\pm$ 1 SE for a) percent ash, b) percent carbohydrates c) percent lipids, and d) concentration of protein (µg/mg). Different letters above each bar indicate significant differences found with Fisher's PLSD.

Discussion

Variation in Biochemical Compounds among Aplysina *Morphotypes*

There were significant differences in biochemical constituents among all three morphotypes; however, differences among the morphotypes varied among sites as well. The thick morphotype of *A. cauliformis* was at some sites greater than the thin morphotype in lipids and carbohydrates and at other sites less, suggesting there are not necessarily definable differences in storage of energy between the morphs. There are more identifiable differences in the structural components of the two morphs. The thick morph is considered more rigid and is harder to break physically (Gochfeld, personal communication), which would suggest a higher concentration of protein, the primary structural macromolecule. However, the thin morphotype of *A. cauliformis* had consistently greater protein concentrations than the thick morphotype, which is contrary to the degree of rigidity of the two morphs. The thick morphotype has greater percent ash than the thin morph, which could potentially contribute to structure or rigidity, supporting the physical observation of rigidity for the thick morphotype.

While there are few consistently definable differences between *A. fulva* and the morphotypes of *A. cauliformis*, *A. fulva* does trend towards lower protein concentration than *A. cauliformis.* The morphologies are visibly different, as shown in Figure 1, and *A. fulva* tends to be the most flexible morph (Gochfeld, personal communication), which could be a reflection of this difference in protein content. *A. cauliformis* and *A. fulva* have

been shown to both use chitin as their primary structural protein, so differences in structure are likely related to the concentration and not the type of protein (Erlich et al., 2010).

Variability of Biochemical Composition among Sites

The samples under analysis were collected from areas in the Bahamas and St. Thomas in the US Virgin Islands that differ greatly in the amount of anthropogenic impacts and potential stressors to which the sponges were exposed. Level of impact for the different sites was determined by distance from human population centers and from shore, as well as through evidence of sedimentation and other negative indicators of water quality, such as concentration of chlorophyll *a*, nitrogen, and phosphorous found in the water (Ennis et al., 2016). By this estimation, Brewer's Reef would be the most impacted of the sample sites, followed by Flat Cay and Saba Island with a medium level of impact, while Savana Island and the Bahamas would be the two most pristine sites. Both the Bahamas and the Virgin Islands rely heavily on tourism and have undergone drastic urbanization and coastal development in recent years (Buchan, 2000; Ennis et al., 2016), although the site from which sponges were collected in the Bahamas is at least 25 miles from the nearest population center and therefore is relatively pristine (Gochfeld et al. 2012). The impacts of run-off and sedimentation have been studied in relation to their effects on water quality and coral reef health (Ennis et al., 2016), but a comprehensive study on the effects of pollution and decreasing water quality on the energetics of sponges has not been undertaken (Bell et al., 2015). There is evidence that water quality has diminished near population centers in the Bahamas, as opposed to further away from potential pollutants (Buchan, 2000). The effects of development have been characterized

more fully near the island of St. Thomas. The effects of this have mostly been qualified in terms of the health of corals and coral reef ecosystems, but increased runoff and sedimentation has noticeably decreased water quality, which in turn can negatively impact the corals and potentially the sponges (Ennis et al., 2016). Decreased water quality can expose coral reefs to an influx of nutrients that promote the growth of algal species that can overtake corals, and sedimentation can introduce disease into the population by carrying pathogens into the vicinity of the sponges, as well as decreasing light available for photosynthetic production by coral symbionts (Bell et al., 2015; Ennis et al., 2016). Some of these same effects may apply to sponges that inhabit affected areas. There is evidence that increases in sedimentation can damage the filtering capability of sponges, as well as physically damage the external surface of the sponge (Bannister et al., 2012; Bell et al., 2015). While the specific effects of sedimentation on sponge health and reproduction have not been fully elucidated, it has been suggested that increased sediment is associated with a decrease in sponge diversity (Bell et al., 2015).

When looking at percentage carbohydrates in *A. fulva,* the sites with the lowest percentage are Flat Cay and the Bahamas. This same pattern is reflected in both the *A. cauliformis* thick and thin morphotypes. These sites differ in their distance from coastal development, suggesting that run-off impact is not the sole cause of decreased carbohydrate production. Since carbohydrate production provides a rapid turnover of energy, this suggests that these sponges are either not producing a large amount of easily usable energy, or they are using those stores quickly (McClintock & Pearse, 1986). To differentiate between these two possibilities, the percentage of lipids is also considered. A high percentage of lipids would support the low energy production theory, as the

organism would not be expending large amounts of energy, explaining the low requirement for transitory energy sources. Echinoderms, for example, have been found to possess similar reserves of lipid in times of high and low productivity (Lawrence & Guille, 1982). A low percentage of lipids would suggest the opposite; the organism probably has a high energy demand (e.g., for growth or reproduction) and has converted most of its lipid stores to more readily usable carbohydrates. Lipid concentration has been connected to reproductive state and nutrient production or intake in gorgonians, and likely plays similar roles in sponges (Shirur et al., 2014).

Site-specific patterns of lipid and carbohydrate levels are similar in *A. fulva,* suggesting that the stressors present at the sites of collection affect all forms of energy storage and are not selective. Protein concentration presents an opposite pattern to lipids and carbohydrates, with lower concentrations of protein in sponges that have higher lipid and carbohydrate. As mentioned before, protein is often structural (Lawrence & Guille, 1982). Potentially, more energy may be used to produce structural components when there is less nutrient storage, but not when high levels of carbohydrate and lipid are being stored. An increased or decreased protein concentration could be a reflection of the physical stress to which the sponges are exposed. Sponges that are exposed to force from waves and water motion, which varies based upon location, could have a higher need for structural proteins, while those in relatively calm waters could have comparatively low levels of protein.

The thick morph of *Aplysina cauliformis* has low levels of carbohydrates across all sites, barring Brewer's Reef, which is significantly different than the rest. Brewer's Reef is considered the most impacted site due to proximity to the shoreline, as shown in

Figure 2**,** as well as a higher concentration of nitrogen present in the water (Ennis et al., 2016). The higher levels of carbohydrates at this site could be a response to nutrient enrichment or the presence of other sources of pollution in the water. Across all morphotypes, sponges collected from Brewer's Reef had high levels of all biochemical components in comparison to the other four collections sites, suggesting that differences in the levels of these components may be strongly related to collection site. These high levels of biochemical components suggest that impact is not necessarily detrimental to the growth and energetics of a sponge; in fact, it might be just the opposite. There has been some evidence of increased prevalence of *A. caulformis* and *A. fulva* at highly impacted sites in Panama (Easson et al., 2015), which could also be the case at Brewer's Reef. While high prevalence does not necessarily relate to healthy sponges, sponges with these higher levels of macromolecules could be more viable and exist in higher abundance, even in impacted areas.

However, samples from Flat Cay, Saba Island, and the Bahamas do not represent this same continuity over biochemical components. In the thin morph of *A. cauliformis* especially, the levels of carbohydrates and lipids are the inverse of each other. Potentially, since these sites have an undefined level of impact, as they are offshore but still might be exposed to sedimentation or other sources of reduced water quality due to water circulation patterns, they may have more variable effects on sponge biochemical composition. However, these observations point to a likely relationship between levels of carbohydrate and lipid, as is discussed earlier.

Savana Island, the site with the least probable impact in St. Thomas, as evidenced by Figure 2, also presents with high concentrations of the biochemical components across

both the thick and thin morphotypes of *A. cauliformis.* This is in direct contrast to the findings from Brewer's Reef. However, this is supported by the work by Ennis et al. (2016) demonstrating the detrimental effect of pollution on the entire coral reef ecosystem. A pristine site should allow normal and abundant growth of sponges, although *Aplysina fulva* was not found at this site, suggesting that sponges have different environmental requirements. Savana Island is farther offshore and likely exposed to higher wave action than the other St. Thomas sites, which may limit which species can occur there. The levels of protein, lipid, and ash in both the thick and thin morphotypes of *A. cauliformis* found at Savana Island are comparable to those found in Brewer's Reef.

Comparison to Other Sponges

As a whole, levels of lipid and carbohydrate reported in both *A. cauliformis* morphs and in *A. fulva* in this study were higher than those found in *Chondrilla nucula,* a Caribbean sponge collected at the same site in the Bahamas, which had lipid levels below 5% of the sponge and carbohydrate levels below 1% of the sponge (Lee, 2012), as compared to around 5-8% lipids and carbohydrates found in this study. Levels of ash were lower in *A. cauliformis* and *A. fulva* in comparison to *C. nucula,* which had levels of ash ranging from 20 to 45% (Lee, 2012) in comparison to values in this study of under 25%. These differences in ash could result from sand contained within the body of *C. nucula* (Gochfeld & Slattery, personal communication). These differences in lipids and carbohydrates could be due to both species differences, including differential symbiont populations, production of defense molecules, or energy usage, or environmental differences. Gochfeld et al. (2012) found similar levels of protein in the thin morph of *A.*

cauliformis from the Bahamas as in this study, and found no significant effect of experimental nutrient enrichment. In contrast, Easson et al. (2014) found a decline in protein concentration at higher nutrient concentrations for *A. cauliformis.* These two studies with contrasting results suggest that, at least with respect to protein concentration, biochemical components may be highly variable in *Aplysina* sponges in relation to environmental nutrient concentrations. Clearly, responses of other biochemical components to other water quality impacts need to be assessed in sponges.

Conclusion

In conclusion, the study showed that biochemical components can vary based upon both the sponge morphotype and the site from which the sponge was collected. The variance in morphology is significant, but inconsistent, as the samples do not vary consistently based upon the morphotype. There are some differences found in protein concentration between all three morphotypes that could relate to the structural makeup of the sponges. There are more consistent differences between the biochemical components within sponges collected at the different sites. The site with the highest level of impact, Brewer's Reef, has high levels of protein and carbohydrate when compared to the other sites. Similarly, Savana Island, a St. Thomas site with a very low amount of impact, has high levels of protein and lipid. This suggests that these sponges thrive best in sites of low or high impact, with intermediate impact sites appearing not as lucrative energetically for the sponges. To fully understand this phenomenon, more conclusive data on the impact levels and sources at all five sites need to be obtained, including run-off volumes, water quality including levels of nitrogen, phosphorous, and chlorophyll *a*, and turbidity. The exact affect of these particular stressors on sponge health could be tested to determine variability between morphotypes. This information would provide a better determination of pollution effects on the bioenergetics of the sponges. In general, however, while there are differences in the production of bioenergetic constituents in certain morphotypes at certain sites, these three types of *Aplysina* do not appear to be

consistently strongly or differentially affected by potential stressors at the levels found at the study sites in St. Thomas or the Bahamas.

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