On a reef far, far away: Offshore transport of floodwaters following extreme storms impacts sponge health and associated microbial communities

Running title: Offshore sponge microbiomes after extreme storms

Amanda N. Shore[1*], Jordan A. Sims[1], Michael Grimes[2], Lauren I. Howe-Kerr[1], Lauren Stadler[3], Jason B. Sylvan[4], Kathryn E.F. Shamberger[4], Sarah W. Davies[5], Lory Z. Santiago-Vázquez[2], Adrienne M.S. Correa[1]

1. BioSciences, Rice University, Houston, TX, USA.

2. Biology and Biotechnology, University of Houston, Clear Lake, TX, USA.

3. Civil and Environmental Engineering, Rice University, Houston, TX, USA

4. Oceanography, Texas A&M University, College Station, TX, USA.

5. Biology, Boston University, Boston, MA, USA.

* Corresponding author: Dr. Amanda N. Shore amanda.n.shore@gmail.com

Keywords: *Agelas clathrodes*, coral reef, flood, Flower Garden Banks National Marine Sanctuary (FGBNMS), Gulf of Mexico, Hurricane Harvey, microbial community, *Xestospongia muta*

Originality-Significance Statement

Stressors associated with terrestrial runoff have contributed to substantial population declines in nearshore marine ecosystems worldwide over the last three decades. It has been assumed that offshore marine ecosystems (>100 km from land) are largely unaffected by terrestrial runoff. Our findings, however, suggest that flooding events can significantly impact offshore marine organisms, based on the detection of shifted microbiomes and human pathogens in offshore sponges after extreme storm events across two separate years, and lack of detection in a non-flooding year.

Summary

Terrestrial runoff can negatively impact marine ecosystems through stressors including excess nutrients, freshwater, and contaminants. Severe storms, which are increasing with global climate change, generate massive inputs of runoff over short timescales (hours to days); such runoff impacted offshore reefs in the northwest Gulf of Mexico (NW GoM) following severe storms in 2016 and 2017. Several weeks after coastal flooding from these events, NW GoM reefs experienced mortality (2016 only) and/or sub-lethal stress (both years). To assess the impact of storm-derived runoff on reef filter feeders, we characterized the microbiomes of two sponges, *Agelas clathrodes* and *Xestospongia muta*, during periods of lethal stress, sub-lethal stress, and no stress over a three-year period (2016-2018). Increased anaerobes during lethal stress indicate hypoxic conditions were associated with the 2016 mortality event. Additionally, we found evidence of wastewater contamination (based on 16S libraries and quantitative PCR) in sponges 185 km offshore following storms (2016 and 2017), but not during the non-flooding year (2018). We show that water quality changes following severe storms can impact offshore benthic organisms, highlighting the need for molecular and microbial time series from near- and offshore reef ecosystems, and for the continued mitigation of stormwater runoff and climate change impacts.

INTRODUCTION

Tropical coral reef ecosystems have evolved in the context of nutrient-poor (oligotrophic) waters. Thus, when nutrient-laden (eutrophic) terrestrial runoff mixes with reef-associated waters, this can directly or indirectly stress or kill reef organisms (Knight and Fell, 1987; Kerswell and Jones, 2003; Fabricius, 2005; Humphrey *et al.*, 2008). Terrestrial runoff can also expose reef organisms to decreased salinity, and increased levels of turbidity and contaminants (e.g., microbial pathogens, chemical pollutants). Terrestrial runoff can constitute a chronic stress in areas with developed coastlines or at river outflows, or an acute stress when floodwaters generated by extreme storms move offshore. Storm-derived floodwaters originating in urban areas may constitute a greater threat, containing high nutrient and contaminant loads due, in part, to overflows of municipal and industrial wastewater management systems (Chen *et al.*, 2019; Humphrey *et al.*, 2019). Storm-associated runoff is increasingly recognized as a threat to reefs since the intensity and precipitation associated with tropical storms is increasing with climate change (Knutson *et al.*, 2010; Emanuel, 2017).

Terrestrial runoff can ultimately reduce dissolved oxygen levels and generate hypoxia in reef-associated waters through several mechanisms (reviewed in Nelson and Altieri, 2019). Increased nutrient levels can trigger bacterial, phytoplankton, and zooplankton blooms in the proximity of reefs. As these blooms die off, they are consumed by heterotrophic bacteria, whose respiration draws down oxygen within the water column, causing reef organisms to suffocate (Nelson and Altieri, 2019). Turbidity increases can reduce light penetration over reefs, limiting the ability of benthic phototrophs (or photosynthetic microbial symbionts within benthic hosts) to produce oxygen via photosynthesis (Fabricius, 2005). Hyposaline runoff can also stratify the water column above reefs, generating a freshwater lens at the air-sea interface that prevents reoxygenation of bottom waters, as oxygen below this layer is consumed by respiring benthic reef organisms (e.g., Kealoha *et al.*, 2020). The increasing potential for hypoxic conditions on reefs has recently been recognized (Nelson and Altieri, 2019; Hughes *et al.*, 2020) yet, relatively little is known regarding how often low dissolved oxygen contributes to reef decline and over what spatiotemporal scales this occurs.

Although various studies document the impacts of terrestrial runoff on coral reefs, few works specifically address runoff derived from floodwaters; all of these latter works focus on nearshore, shallow water reefs (Ostrander *et al.*, 2008; Lapointe *et al.*, 2010). It has been assumed that offshore or deep (e.g., mesophotic) reefs are unlikely to interact with terrestrial runoff (e.g., Szmant, 2002). For example, reefs of the Flower Garden Banks National Marine Sanctuary (FGBNMS, northwest Gulf of Mexico, Figure 1a) occur ~185 km offshore in relatively deep water (20-30 m), and boast some of the highest coral cover (~55%) in the wider Caribbean (Johnston *et al.*, 2016). Since 2015, the Texas coast has experienced several flooding events related to extreme storms: Memorial Day Flood of 2015; Tax Day Flood of 2016, Hurricane Harvey in 2017, and Hurricane Imelda in 2019. Each of these floods impacted Central Texas nearshore ecosystems, including salt marshes (Congdon *et al.*, 2019; Oakley and Guillen, 2019) and oyster beds (Kiaghadi and Rifai, 2019). FGBNMS reefs were not surveyed for floodwater impacts following the Memorial Day Flood of 2015 because it was assumed that any floodwaters that moved into Galveston Bay (Texas, USA) or the adjacent continental shelf would dissipate before reaching the reef system.

However, three months after the 2016 flood, a localized mortality event (LME) occurred on a portion of the East Flower Garden Bank, an offshore, relatively deep (~20 m) ecosystem. During the LME, approximately 82% of corals in a 0.06km^2 area, experienced partial or full mortality (Johnston *et al.*, 2019), and declines in many other benthic invertebrates, such as sponges, were also observed. Although data on abiotic conditions at East Bank during the 2016 LME are not available, salinity and temperature measurements from nearby sites suggest that poor water quality from floodwaters moving offshore and subsequent low dissolved oxygen levels played a critical role in the LME (Le Hénaff *et al.*, 2019; Kealoha *et al.*, 2020). Then, in late August of 2017, Hurricane Harvey released over one meter of rainfall over the course of six days in some areas of southeastern Texas (Blake and Zelinsky, 2018). Given that a smaller scale event (Tax Day Flood) triggered reef mortality, there was concern regarding the impacts that the Hurricane Harvey-derived water mass would have on FGBNMS ecosystem health. Although salinity was depressed near the FGBNMS following Hurricane Harvey, much of the water mass diverted southwest along the coast (Roffer *et al.*, 2018), and no mass mortality was observed on the reef (Wright *et al.*, 2019).

In association with declines in coral cover worldwide, the relative abundance of sponges has increased on some reefs, especially in the Caribbean, as has their overall contribution to reef ecology, structure, and biogeochemistry (Bell *et al.*, 2013; Pawlik and McMurray, 2020). Sponges harbor a diversity of microbial symbionts (e.g., bacteria, archaea, protists, fungi and viruses) that contribute to their health and nutrition but can be disrupted by environmental stress (Slaby *et al.*, 2019). Although relatively few reports document the sub-lethal effects of environmental stress on sponge microbial communities, thermal stress is known to disrupt the bacterial communities associated with many sponge species (Lesser *et al.*, 2016; Pita *et al.*, 2018). Sponge-associated bacterial communities have been also shown under experimental conditions to be resistant to some flood-associated stressors, such as elevated nutrients (Simister *et al.*, 2012; Luter *et al.*, 2014) and reduced salinity (Glasl, Smith, *et al.*, 2018). Understanding how and when environmental stressors influence sponge-microbe associations, and the subsequent implications for sponge health and function, is increasingly important as sponges increase in abundance on reefs.

This study assesses whether storm-derived coastal flooding events can perturb the microbial symbioses of offshore reef sponges. We leverage the Tax Day Flood (2016) and Hurricane Harvey (2017) as natural 'experimental treatments' applied to two sponge species (*Xestospongia muta* and *Agelas clathrodes*; Figure 1b-c) at the East Bank (EB) and West Bank (WB) of the FGBNMS. Bacterial communities were sampled from sponges at five time points: in July 2016 (at detection of the mortality event), one month after the mortality event (August 2016), immediately after Hurricane Harvey (September 2017), one month after Hurricane Harvey (October 2017), and approximately one year following Hurricane Harvey (October 2018) (Figure 1d). No flooding occurred in southeast central Texas during 2018, and thus samples from this time point function as an 'experimental baseline'. We hypothesized that: (1) sponge-associated bacterial communities shift during flood years (relative to the non-flood year); (2) flood year bacterial communities contain genetic signatures of terrestrial-derived bacteria; and (3) anaerobes have a higher relative abundance in July 2016 LME samples, reflecting hypoxic conditions that likely occurred during the LME.

RESULTS

Reduced surface salinity at the FGBNMS following floods

In the vicinity of FGBNMS, mean surface salinity is generally variable between mid-May through mid-August (shaded grey in Supplemental Figure S1). In early spring and in fall, however, salinity is more consistent (~ 35 ppt). Approximately 30 days after the 2016 Tax Day Flood impacted Texas, a period of depressed mean salinity (relative to the mean salinity for a six year period: 2013-2018) was recorded from 15 May 2016 to 15 August 2016 at Texas Automated Buoy System (TABS) Real Time Ocean Observations, Buoy V (Supplemental Figure S1b). On average, salinity was 1.2 ppt lower than the six-year mean during this period; the most significant deviation occurred around 2 July 2016 when mean salinity reached a minimum of 29.1 ppt (3.2 ppt below the six-year mean). In 2017, salinity remained > 35 ppt until early June. TABS buoy V data are unavailable for much of June to August 2017, so it is unclear how salinity changed in the months preceding Hurricane Harvey. Two abrupt reductions in salinity were recorded near FGBNMS during mid-to-late September 2017 following Hurricane Harvey, before salinity returned to 35 ppt in October (Supplemental Figure S1c). In contrast, no significant influence of freshwater was recorded in the northwest Gulf of Mexico coast in 2018 and salinity remained approximately 35 ppt after mid-June (Supplemental Figure S1d).

After the 2016 LME was discovered at EB, opportunistic sampling of debris from dead organisms, hereafter referred to as 'dead invertebrate tissue' (DIT), occurred on 27 July 2016; the mean salinity was 31.7 ppt, which was 2.1 ppt below the six-year mean for the area (Figure 1d). On this date, mean salinity had already been lower than the six-year mean for 44 days. Additional samples were collected on 6 August 2016, approximately four days after salinity had returned to 35 ppt (Figure 1d). Sampling immediately post-Hurricane Harvey on 16 September 2017 occurred when mean salinity was 34.6 ppt, or 1.3 ppt lower than the six-year mean (Figure 1b). During sampling that occurred approximately one-month post-hurricane (on 21 October 2017), mean salinity had been > 35 ppt for 19 days prior to sampling (Figure 1d). Samples for the non-flood year were collected on 23 October 2018, which had a mean salinity of 35.7 ppt; salinity had been > 35 ppt for 94 days (Figure 1d).

Acute shifts in sponge-associated bacterial communities following flood events

Paired-end Illumina MiSeq sequencing of the V4 region of the bacterial 16S rRNA gene yielded 5,975,358 high quality sequences from samples of DIT (associated with the 2016 LME, n=8) and two sponge species (*A. clathrodes*, n=54; *X. muta*, n=44) (Table 1). After removal of Mitochondrial, Chloroplast, and Unassigned reads, the total pool of 5,034,341 bacterial sequences were clustered into 9,221 Operational Taxonomic Units (OTUs). All samples clustered into 3 distinct groups, which were all significantly different from each other (ANOSIM: $p < 0.01$; Supplementary Table S1). These groups were: 1) DIT (July 2016) and diseased sponges of both species (Aug. 2016); 2) visually healthy *A. clathrodes* (Aug. 2016, Oct. 2017, Oct. 2018); and 3) visually healthy *X. muta* (Aug. 2016, Sept. 2017, Oct. 2017, Oct. 2018; Figure 2). Because healthy sponges had species-specific bacterial communities (ANOSIM: Global R = 0.688, $p = 0.01$), subsequent analyses were conducted on each sponge species individually. Additionally, within each sponge species, there were no differences between EB and WB sites (ANOSIM: $p > 0.05$) (Supplemental Table S2), therefore, sites were grouped for subsequent analyses.

Bacterial communities of *X. muta* were shifted significantly during all collection dates associated with flood events (Aug. 2016, Sept. 2017, Oct. 2017), relative to samples collected during the same season in a no flood, baseline year (Oct. 2018) (ANOSIM: $p < 0.05$, Supplemental Table S3). Bacterial communities of *X. muta* in Aug. 2016 and Sept. 2017 showed the greatest shift (Figure 3a) and had significantly higher variability (mean pairwise dissimilarity) compared to Oct. 2018 communities (ANOVA with Tukey's comparisons: $p < 0.001$) (Figure 3c). In Oct. 2017, *X. muta* bacterial community structure was still significantly different than bacterial communities under ambient conditions (Oct. 2018, Supplemental Table S3). However, Oct. 2017 communities clustered more closely to Oct. 2018 than to Sept. 2017 communities, and variability of bacterial communities in Oct. 2017 was similar to that in Oct. 2018 (ANOVA with Tukey's comparisons: $p = 0.982$, Figure 3c). Bacterial communities of *X. muta* had similar levels of Shannon Diversity across time points (ANOVA: $F = 0.83$ $p = 0.490$). However, there was a significant difference in OTU richness over time (ANOVA: $F = 6.07$ $p = 0.002$), with communities from

Sept. 2017 having lower OTU richness than communities from Oct. 2018. *X. muta* bacterial communities during Oct. 2018 of the no flood year were dominated by Gammaproteobacteria (19.9 ±0.9%), Chloroflexi (13.6 ±0.7%), Actinobacteria (13.5 ±1.2%), and Acidobacteria (11.1 ±1.0%) (Supplemental Figure S2a). Four bacterial Families in *X. muta* communities showed significant differences in abundance across sampling time points (Supplemental Table S4). Rhodobacteraceae and Nitrosopumilaceae had higher abundances in Oct. 2018; Synechococcaceae had higher abundance in Aug. 2016 and Sept. 2017; and Anaerolineaceae and Chloroflexaceae had higher abundances in Oct. 2017 (Kruskal-Wallis: $p < 0.002$).

Similar to *X. muta*-associated bacterial communities, bacterial communities of *A. clathrodes* also shifted significantly during all collection dates, relative to 'baseline' samples collected during a no flood year (Oct. 2018). *A. clathrodes* communities also differed between flooding events (Aug. 2016 vs. Oct. 2017, Figure 3b, Supplemental Table S3). Like in *X. muta*, bacterial communities in *A. clathrodes* displayed higher variability in response to the flood events (Figure 3d). *A. clathrodes* bacterial communities displayed differences in both OTU richness (ANOVA: $F = 57.13$ $p = 0.0001$) and Shannon Diversity (ANOVA: $F = 40.76$, $p = 0.0001$) across time points, with Oct. 2017 having significantly lower OTU richness and diversity. *A. clathrodes* bacterial communities during Oct. 2018 were also dominated by Gammaproteobacteria (21.2 ±0.4%), Chloroflexi (20.4 ±0.2%), Actinobacteria (16.7 ±0.9%), and Acidobacteria (13.6 ±0.8%, Supplemental Figure S2b). Eight bacterial Families in *A. clathrodes* communities showed significant differences in abundance across time points (Supplemental Table S4). Solibacteriaceae and Nitrosopumilaceae had higher abundance in Oct. 2018; Vicinamibacteraceae and Rhodospirillaceae had higher abundance in Oct. 2017; Chromatiaceae, Ectothiorhodospiraceae, and Nitrospiraceae had lower abundance in Oct. 2017; and Desulfovibrionaceae had a higher abundance in Aug. 2016 (Kruskal-Wallis: $p < 0.002$).

<u>Sponge microbiomes during the July 2016 Localized Mortality Event were dominated by anaerobes</u>

Bacterial communities from visually healthy and diseased sponges sampled immediately after the LME were dominated by taxa classified as aerobes (mean = 13.5 ± 0.6% and range = 0.95 - 27.6%, Figure

4). However, bacterial communities from DIT sampled during the LME had a significantly higher proportion of taxa classified as anaerobes (40.0 ± 8.0%), compared to bacterial communities sampled from sponges at all other time points (Mann-Whitney multiple comparisons; $p < 0.0125$, Figure 4). Within bacterial communities of DIT, there were 19 Families at > 0.1% relative abundance. Fourteen of these Families were characterized as obligate, facultative, or aerotolerant anaerobes, including several members of the Class Bacteroidetes, several members of the Class Clostridia, sulfate-reducers (Families Desulfovibrionaceae and Desulfuromonadaceae), and sulfur-oxidizers (Family Thiothrichaceae, Supplemental Figure S3a). Sponge bacterial communities in diseased individuals of both host species were primarily represented by bacterial Families categorized as 'various' (i.e. Families with Genera that have different oxygen requirements). For example, Family Rhodobacteraceae, which contains members with different oxygen requirements, dominated samples from diseased individuals of both sponge species, representing an average of 36.9 (± 4.3)% of bacterial communities in these samples (Supplemental Figure S3b). Some of the same taxa that were abundant in DIT samples (July 2016), such as Flavobacteriaceae, Peptococcaceae, and Desulfovibrionaceae, were also abundant in diseased sponge samples (Aug. 2016), but were absent (or were in minor taxa, < 0.1%) in visually healthy sponges.

<u>Sponge microbiomes show signs of wastewater contamination after flooding</u>

Bacterial OTUs classified as Family Enterobacteriaeceae were recovered from the majority of samples of both sponge species. *Xestospongia muta* bacterial communities had low abundances (< 0.1%) of Enterobacteriaceae (Figure 5a), displaying no significant differences across dates (Kruskal-Wallis: $H = 6.80$, $p = 0.146$). However, *A. clathrodes* bacterial communities in 2016 (diseased and visually healthy samples) had a significantly higher abundance of Enterobacteriaceae (Kruskal-Wallis: $H = 33.67$, $p = 0001$), relative to samples from this host in other years. Bacterial communities of *A. clathrodes* in Aug. 2016 displayed 7.92 (± 1.97)% and 1.61 (± 1.03)% abundance of Enterobacteriaceae for diseased and visually healthy samples, respectively (Figure 5b), whereas in Oct. 2017 and Oct. 2018, samples had a low abundance (< 0.1%) of Enterobacteriaceae.

To test the hypothesis that FGB sponges were exposed to wastewater-derived bacteria from storm generated floodwaters, samples were screened for seven human pathogens using quantitative PCR. Diseased and visually healthy sponge samples collected in 2016 and 2017 yielded positive detection for 2 out of 7 human pathogens screened: *Escherichia coli* and *Klebsiella pneumoniae* (Figure 5c,d). No human pathogens were detected from sponges sampled during the no flood year (Figure 5c,d). In *X. muta*, *E. coli* abundance was highest in visually healthy samples from Aug. 2016, with a mean of 1.96 $x10^3$ (± 1.40 $x10^3$) gene copies per g tissue, compared to a mean of 8.96 $x10^1$ (± 8.54 $x10^1$) and 6.90 $x10^1$ (± 2.18 $x10^1$) gene copies per g tissue for diseased samples from Aug. 2016 and visually healthy sponges from Sept. 2017, respectively (Figure 5c). In *X. muta*, *K. pneumoniae* abundance was similar across groups, averaging 1.08 $x10^2$ (± 5.78 $x10^1$), 7.84 $x10^1$ (± 7.27 $x10^1$), and 1.06 $x10^2$ (± 6.74 $x10^1$) gene copies per g tissue for diseased samples from Aug. 2016, visually healthy sponges from Aug. 2016, and visually healthy sponges from Sept. 2017, respectively (Figure 5c). In *A. clathrodes*, *E. coli* was more abundant in samples from Aug. 2016, with means of 6.47 $x10^4$ (± 5.76 $x10^4$) and 2.85 $x10^4$ (± 1.79 $x10^4$) gene copies per g tissue for diseased and visually health sponges, respectively, compared to Oct. 2017, with a mean of 6.26 $x10^2$ (± 4.17 $x10^2$) gene copies per g tissue (Figure 5d). In *A. clathrodes*, *K. pneumoniae* was less abundant (> 2 orders of magnitude difference) compared to *E. coli*, displaying similar abundance across groups where it was detected, averaging 8.22 $x10^1$ (± 7.65 $x10^1$), 8.36 $x10^1$ (± 5.68 $x10^1$), and 4.61 $x10^1$ (± 3.29 $x10^1$) gene copies per g tissue for diseased samples from Aug. 2016, visually healthy sponges from Aug. 2016, and visually healthy sponges from Oct. 2017, respectively (Figure 5d). No samples tested positive for *Enterococcus* spp., *Pseudomonas aeruginosa*, *Salmonella enterica*, *Serratia marcescens*, and *Staphylococcus aureus* (data not shown).

DISCUSSION

<u>Flooding disrupts microbiomes of offshore reef sponges</u>

It has been assumed that remote marine ecosystems (>100 km from land) are not affected by terrestrial runoff. Our findings, however, suggest that flooding events can significantly impact offshore

benthic reef organisms, based on the detection of shifted bacterial communities and human pathogens in sponges after extreme storm events across two separate years. Flooding events altered the bacterial communities of both *X. muta* and *A. clathrodes*, as shown by disruptions to their community structure in 2016 and 2017, relative to sponge bacterial communities collected during the same season in a no flood year in Oct. 2018. Furthermore, we quantified an increased relative abundance of the Enterobacteriaceae and two known human pathogens in post-flood sponge samples, indicating that floodwaters of terrestrial origin can reach and interact with offshore reefs following extreme storm events.

Bacterial communities associated with the two sponge species exhibited some differences in the strength and duration of their response to flood water stress, likely due to the fact that they harbor distinctive bacterial communities even under ambient conditions (e.g., in 2018). Larger shifts in *X. muta* bacterial communities were associated with the 2016 Tax Day Flood, which caused mortality at EB. *X. muta* bacterial communities were relatively resilient following the sub-lethal stress associated with Hurricane Harvey and were similar to baseline by Oct. 2017. Interestingly, *A. clathrodes* sponges exhibited larger shifts in its microbiota following Hurricane Harvey (sub-lethal stress) than it did following the 2016 Tax Day Flood, and *A. clathrodes* microbiomes remained disrupted in Oct 2017. Wright *et al*. (2019) similarly found that interspecific differences were the strongest driver of gene expression changes in two coral following Hurricane Harvey. Differences in host function or ability to regulate its bacterial community may explain why *X. muta* showed earlier changes (and more dispersion) in response to the 2017 flood, whereas shifts in *A. clathrodes* bacterial communities lingered.

What mechanism(s) could have produced the observed changes in sponge microbiomes during flood years? Invasion of floodwater-derived bacteria into host tissues, invasion of seawater-derived microbes into host tissues and/or shifts in the abundance of sponge-associated taxa already present on hosts all could have played a role; it may not be possible to confirm the mechanism(s) underlying natural events in the field. For example, increases in the abundance of marine cyanobacteria in the Family Synechococcaceae were a key driver of differences observed in *X. muta* bacterial communities after flooding. Synechococcaceae were enriched over EB reefs following the 2016 Tax Day Flood (Kealoha *et*

al., 2020) and were associated with higher ammonium concentrations and therefore associated with terrestrially-derived flood waters. Filtration of the water column by *X. muta* individuals could have concentrated Synechococcaceae within sponges during this period. However, observed increases in Synechococcaceae could also have been driven by the proliferation of the resident sponge-associated populations due to an increase in nutrients in the sea water column. Yet, the abundance of sponge-associated *Synechococcus* decreases in *X. mut*a following some environmental stresses, such as disease and thermal stress (Angermeier *et al.*, 2011; Lesser *et al.*, 2016). Reduced capacity of the animal host to regulate its microbiome under stress could be a contributing factor for any of these mechanisms (e.g., (Zaneveld *et al.*, 2017; Pita *et al.*, 2018). Thus, it is highly plausible that two (invasion of seawater-derived microbes, shifts in sponge-associated microbes) of the three mechanisms above contributed, albeit to different degrees, to observed sponge microbiome species following each flood.

<u>Wastewater contamination after severe flooding reaches offshore marine ecosystems</u>

The increased abundance of Enterobacteriaceae in reef sponges 185 km offshore after flooding in 2016 and, particularly, the detection of two fecal coliforms (*E. coli* and *K. pneumoniae*) during and after flood exposure strongly suggest that these reefs were exposed to wastewater contamination after severe storms. Although the Family Enterobacteriaceae is ubiquitous in nature, occupying terrestrial, aquatic, and marine habitats, especially the intestine of homeothermic animals (Whitman, 2015), members of this group are not common or abundant members of the microbiome of sponges (Moitinho-Silva *et al.*, 2017; Cleary *et al.*, 2019). It is possible that other sources of Enterobacteriaceae, such as excreted waste from reef fish and marine mammals (Wallace *et al.*, 2013) or wastewater disposal from nearby ships, explain the presence of this bacterial family across sponge samples. However, given that no human pathogens were detectable from October 2018 (no flood) samples, the most parsimonious explanation for the Enterobacteriaceae detections in this study is that FGBNMS reefs were exposed to land-based human wastewater via terrestrial runoff following the 2016 and 2017 floods.

It is unclear whether wastewater-derived bacteria contributed to mortality at EB in July of 2016, but detection of wastewater contamination at FGBNMS raises the question: do fecal coliforms pose health risks to offshore reefs? Human or animal wastewater contamination is linked to negative impacts on coral communities, particularly based on the input of excess nutrients, but chemical, bacterial, and pharmaceutical contamination are also potential issues (reviewed in Wear and Thurber, 2015). Generally, there is little information on the impacts of fecal-coliform bacteria on coral reef ecosystems. The wastewater-derived bacteria, *Serratia marcescens*, is a pathogen of *Acropora palmata* coral in the Caribbean (Sutherland *et al.*, 2010, 2011), and in Hawaii, fecal coliforms colonized coral tissues after exposure, potentially contributing to a major disease outbreak (Beurmann *et al.*, 2018). There is little information on the effect of fecal coliform exposure on marine sponge health, but some sponges may be relatively tolerant, using bacterial cells as a source of nutrition (Chaves-Fonnegra *et al.*, 2007). The surprisingly far reach of contaminated floodwaters observed here underscores the urgent need to understand how floodwaters impact the health and function of reef environments. A key question to address is whether detection of *E. coli* and *K. pneumoniae* represent detection of DNA from dead wastewater-derived bacterial cells or whether living wastewater-derived bacteria are potentially interacting with sponges (and other marine life) following extreme storms. If wastewater-derived bacteria contaminating sponges are metabolically active, then we must determine how long they persist within the sponge microbiome and the extent to which these microbes impact sponge physiology and function. A better understanding of the interactions between benthic reef hosts and terrestrial-derived bacteria will support effective management and protection of offshore reef ecosystems, such as the FGBNMS.

Previous work has shown the utility of using sponges as monitoring and potential bioremediation tools for fecal-coliform contamination in near-shore environments (Longo *et al.*, 2010; Maldonado *et al.*, 2010). This work demonstrates that sponge species can be effective tools for monitoring wastewater contamination in offshore marine ecosystems, and that the species selected for monitoring requires consideration. *E. coli* and *K. pneumoniae* were detected in both *A. clathrodes* and *X. muta* samples, but *A. clathrodes* had a higher frequency of detection and higher abundance of *E. coli*. Sponge size is a key driver

of filtering rate in marine sponges (Morganti *et al.*, 2019). *X. muta* (often exceeding 1m diameter, McMurray *et al.*, 2008) can achieve larger sizes than *A. clathrodes* (typically up to 100 cm wide, Parra-velandia *et al.*, 2014), and thus the former species could theoretically accumulate more terrestrial-derived bacteria, compared to *A. clathrodes*. However, other interspecific differences, such as rates of digestion of filtered material, may be important in selecting sponge species as biomarkers. We detected more *E. coli* and *K. pnemoniae* in *A. clathrodes*; thus, this species, as compared to *X. muta*, shows promise as a biomarker for wastewater contamination at offshore reefs, such as FGBNMS, and should be further explored.

Anaerobic taxa implicate hypoxia in the 2016 Localized Mortality Event at East Bank, FGBNMS

Although there are no direct measurements of dissolved oxygen available from the EB FGBNMS at the time of the July 2016 LME, hypoxic conditions are thought to have contributed to the mortality observed (Kealoha *et al.*, 2020). In corals, hypoxic micro-environments generated by high microbial respiration at sites of coral-algal interaction cause increased microbiome variation (community dispersion), as well as increased abundance of bacteria classified as strict anaerobes (Barott *et al.*, 2012). We report a similar pattern of increased abundance of strict anaerobes in sponge microbiomes collected in July 2016. Our data support the hypothesis that low oxygen conditions, likely generated by increased microbial and/or reef respiration, contributed to the 2016 LME at the EB of the FGBNMS.

Some anaerobes detected in DIT (July 2016) samples may be of terrestrial origin. *Cloacamonas* is found primarily in anaerobic digesters of municipal wastewater treatment plants (Pelletier *et al.*, 2008), and some Genera within the Bacteroides, Clostridiaceae, Christensenallaceae, and Ruminococcacaeae Families are abundant members of mammalian gut microbiota (Arumugam *et al.*, 2011; Waters and Ley, 2019), and are thus potential indicators of wastewater contamination on FGBNMS reefs. However, other anaerobes and microaerophiles present in DIT samples may be marine in origin. Desulfovibrionaceae and Thiotrichaceae contain sulfate-reducing and sulfur-oxidizing bacteria, respectively (Castro H F *et al.*, 2000; Garrity *et al.*, 2015), and Genera within both Families are both commonly found in anoxic and microaerophilic layers of marine sediments (Postgate and Campbell, 1966; Mußmann *et al.*, 2003; Zouch

et al., 2017). Interestingly, *Beggiatoa* (Family Thiotrichaceae) and *Desulfovibrio* (Family Desulfovibrionaceae) are both essential components of the microbial consortium that causes black band disease in corals (Sato *et al.*, 2016). *Beggiatoa* is considered an indicator of low DO conditions and thick, white, filamentous mats *in situ* can be presumptively identified as this genus (Rosenberg and Diaz, 1993; Rabalais *et al.*, 2001; Altieri *et al.*, 2017). White, filamentous mats were also observed during the LME in July 2016 (Johnston *et al.*, 2019), and thus, members of the Thiotrichaceae may be the source of the 'white bacterial mat' described by divers during DIT sample collection. Desulfovibrionaceae and Thiothrichaceae together may be opportunistically contributing to mortality initially caused by hypoxic conditions on EB in July 2016.

Comparisons of FGBNMS sponge microbiomes to those in the wider Caribbean

This study is the first to characterize sponge-associated microbial communities from the northwest Gulf of Mexico (and FGBNMS), offering the opportunity for comparisons of sponge microbial communities across regions of the GoM and the Caribbean. *X. muta* associated bacterial communities at the FGBNMS in 2018 (no storm condition) were similar between EB and WB and were dominated by Phyla also commonly reported from *X. muta* in the Florida Keys, Bahamas, and greater Caribbean (i.e. Proteobacteria, Chloroflexi, Cyanobacteria, Poribacteria, and to a lesser extent, Acidobacteria, Actinobacteria, and Thaumarchaeota (Schmitt *et al.*, 2012; Olson and Gao, 2013; Montalvo *et al.*, 2014; Fiore *et al.*, 2015; Morrow *et al.*, 2016; Villegas-Plazas *et al.*, 2019). These previous studies report regional differences due to changes in relative abundance of these shared Phyla (Fiore *et al.*, 2015; Morrow *et al.*, 2016). *X. muta* bacterial communities from the FGBNMS may also be regionally distinct, in particular containing a high abundance of Thaumarchaeota archaea (~10%) compared to what has been reported from other regions (<5%; Fiore et al 2013). Members of Thaumarchaeota, such as Nitrosomopumilaceae, play an important role in nitrogen cycling in *X. muta* via ammonia oxidation (López-Legentil *et al.*, 2010). Ammonia-oxidizing archaea are outcompeted in environments with higher levels of ammonium (Erguder

et al., 2009), so the greater abundance of Nitrosomopumilaceae likely reflects the oligotrophic environment of the offshore FGBNMS reefs during no storm conditions.

This study is also the first to describe bacterial communities associated with *A. clathrodes* using next-generation sequencing technology. Bacterial communities of *A. clathrodes* at the FGBNMS contained Phyla (i.e. Proteobacteria, Firmicutes, Actinobacteria, Chloroflexi, Thaumarchaeota) also present in other *Agelas spp.* from the Florida Keys, Belize, and Central Amazon Shelf (Olson and Gao, 2013; Deignan *et al.*, 2018; Rua *et al.*, 2018; Gantt *et al.*, 2019). However, higher abundances of Archaea, especially Euryarchaeota and Crenarchaeota, and Firmicutes were found in other *Agelas* spp. (Deignan *et al.*, 2018; Rua *et al.*, 2018). Diseased sponges (both *A. clathrodes* and *X. mut*a) sampled after the 2016 LME were dominated by Alphaproteobacteria, especially Rhodobacteraceae. Alphaproteobacteria were also enriched in sponges affected by *Agelas* Wasting Syndrome (Deignan *et al.*, 2018), suggesting that this group of bacteria could play a role in pathogenesis and/or serve as a biomarker of disease risk for FGBNMS sponge communities.

<u>Mitigating the Impacts of Future Storms on Offshore Reefs</u>

This study demonstrates that transport of floodwaters following extreme storms can contribute to shifts in the microbiomes of benthic reef organisms 185 km offshore, promoting bacterial communities that are more variable. Detection of bacteria typically associated with wastewater within these sponge samples illustrates that marine-terrestrial interactions, and thus, the potential impacts of human land and waste management practices, extend far beyond the shoreline. The GoM is regularly impacted by hurricanes, and thus marine communities in the region have evolved in a disturbance regime that includes bursts of storm-generated terrestrial runoff. However, the ongoing expansion of impermeable surfaces (e.g., concrete, pavement) in Texas and other coastal areas, as well as changing extreme storm patterns (e.g., slower moving hurricanes with greater precipitation) are increasing the frequency and intensity of floodwater influx into marine environments.

This study of the potential impacts of the 2016 and 2017 floods was catalyzed because an LME affected the East Bank following the Tax Day Flood. We hypothesize that flood waters associated with other recent extreme storm events (e.g., 2015 Memorial Day Flood, flooding generated by Hurricane Imelda in September 2019) in the region likely also caused sub-lethal stress at FGBNMS. However, targeted sampling of FGBNMS did not occur following these storms. Flood waters reaching the offshore ecosystems of the FGBNMS have other implications beyond disruptions of benthic host-associated microbial communities. For example, excess nutrients, especially nitrogen, promote growth of macroalgae (Lapointe, 1997). Long term monitoring of benthic cover at FGBNMS has documented an increase in macroalgae, without a corresponding loss in corals, over the last two decades (Johnston *et al.*, 2016) and suggests nutrient input may have been affecting these offshore reefs since 1999. Growth of macroalgae on coral reefs is generally detrimental as macroalgae outcompete corals for light and space and promote coral disease (McCook *et al.*, 2001; Barott and Rohwer, 2012; Haas *et al.*, 2016). If unchecked, continued 'pulses' of nutrients from storm-derived flooding events may underlie or exacerbate increased coral-algal interactions and trigger declines at this 'reef of hope', which currently boasts coral cover greater than 50%.

This study demonstrates how water quality changes following severe storms can influence microbiomes of offshore benthic organisms, potentially impacting their physiology and ecosystem functions such as water filtration. Our findings clearly demonstrate the urgent need for: 1) continued mitigation of stormwater runoff and climate change impacts; and 2) establishment of microbial and water quality time series for near- and offshore reef using standardized protocols. This latter program will generate baseline data on the microbiomes of key benthic reef taxa under normal conditions, providing critical context (Glasl, Bourne, *et al.*, 2018) in which to detect and mitigate floodwater-derived stress on reefs.

EXPERIMENTAL PROCEDURES

<u>Pelagic water properties during sample collection periods</u>

The Flower Garden Banks National Marine Sanctuary (FGBNMS, northwest Gulf of Mexico) is currently comprised of three banks: East Bank (EB), Stetson Bank and West Bank (WB) (Figure 1a). To characterize local surface salinity (sensor depth of 2 m) in parts per thousand (ppt) and temperature (°C) representative for the FGBNMS before, during, and after each sampling period, water property data collected each half hour for April - October for the years 2013 - 2018 were downloaded from the Texas Automated Buoy System Real Time Ocean Observations, Buoy V archives (http://tabs.gerg.tamu.edu). Buoy V (27° 53.7960'N, 93° 35.8380'W) is located approximately 3 km from EB and 25 km from WB. Data were filtered to remove all timepoints containing no data and to exclude outlier data (i.e., measurements where salinity abruptly changed to <1 ppt or >70 ppt from 35 ppt). Data were not available (due to instrumentation failure) between the dates of 6 June 2017 through 30 August 2017. The remaining data were then plotted using the ggplot2 package version 3.2.1 using code from https://github.com/rachelwright8/TagSeq_FGB_HurricaneHarvey. Data were summarized to determine means (black line) and daily ranges (grey shading) for April to October over the six-year period. Daily means for each sampling date were also calculated and plotted on top of the six-year summary data. (Figure 1d). For sampling campaigns that spanned more than one day, only the first day of sampling was plotted (the maximum length of a sampling campaign was 5 days). To assess the lags between continental flooding (associated with an extreme storm) and changes in water quality at the FGBNMS, daily salinity mean and range data for April to October of individual years 2015, 2016, 2017 and 2018 (red lines and shading, Supplemental Figure S1a-d) were overlaid on surface salinity averages for the six-year summary data.

Sponge Sample Collections

Samples were collected at five timepoints spanning 2016-2018 from two locations within the FGBNMS; East Bank (27° 52'54.84”, 93° 37'41.84") and West Bank (27°54'28.8", 93°36'0.72", Figure 1a, Table 1, Supplemental Table S1). Samples from July 2016 were samples of dead invertebrate tissue (DIT) collected opportunistically on the day that the EB LME was discovered; sampling capabilities at this time point were extremely limited in terms of available sterile collection materials and dive time, relative to

subsequent sample time points. Thus, the July 2016 DIT samples are a composite representation of the organic mat that had formed on the tops of dying sponges, corals, and other organisms including brittle stars and urchins (Supplemental Table S5). At all other sampling timepoints, fragments were collected from individual *A. clathrodes* and *X. muta* sponges (Figure 1b,c). The same individual sponges were not repeatedly sampled across timepoints due to time constraints in available ship and dive time. In August 2016, samples were collected from 'diseased sponges' that were exhibiting progressive tissue loss, as well as from visually healthy sponges. For all other timepoints, samples were collected from visually healthy sponges as diseased sponges were not observed. In total, 109 samples, collected from depths ranging 18 - 27 m, are analyzed in this study (Table 1, individual sample metadata provided in Supplemental Table S5). Samples were clipped from colonies using health status and species-specific cutting shears that were wiped clean between samples. Each sample was placed in an individual sterile bag for transport to the surface. Once topside, each sample was immediately transferred to liquid nitrogen and stored at -20°C until further processing.

Bacterial Community Analysis

DNA was extracted from 250 mg of sponge sample using the Nucleospin Soil DNA extraction kit (Takara Bio); whereas DNA from DIT samples was extracted using the DNeasy PowerSoil DNA extraction kit (QIAGEN). Clustering of samples from ill health states (i.e. DIT samples, some of which were collected from *X. muta*, and diseased sponge samples) suggests that there was no significant bias from extraction method. Extracted DNA was submitted for high-throughput sequencing of the V4 hypervariable region of the 16S gene using the Illumina Mi-Seq (Paired-end 2x 300 read) platform. High quality sequences were analyzed through the QIIME 1.8 software package to generate Operational taxonomic units (OTUs) clustered at 97% identity (Caporaso *et al.*, 2010). Rarefied bacterial OTU tables were used to calculate alpha diversity metrics and to conduct beta diversity analyses using weighted UniFrac distance matrices. Taxonomy was assigned using the RDP v. 2.12 reference database (Wang *et al.*, 2007). Bacterial Families were also categorized by oxygen requirement using descriptions available in Bergey's Manual of

Bacteriology (Whitman, 2015). Bacterial Families with Genera that have multiple oxygen requirements were classified as "Various". The raw sequence data files were submitted to the NCBI Sequence Read Archive under accession number SRP248232. Additional details for sequence processing and analysis are provided in Supplemental Methods.

Quantitative PCR for human pathogens associated with Hurricane Harvey-derived floodwaters

Species-specific functional genes were chosen as biomarkers to detect and quantify fecal indicator bacteria (*Escherichia coli* and *Enterococcus spp.*), putative pathogenic bacteria in the Family Enterobacteriaceae (*Klebsiella pneumoniae*, *Serratia marcescens*, and *Salmonella enterica*), and other putative pathogenic bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*, Supplemental Table S6). These bacterial species were targeted because OTUs of the same Families were identified in high-throughput sequencing of terrestrial floodwater and sediment samples collected immediately following Hurricane Harvey (Yu *et al.*, 2018). Target gene amplicons were used to establish the standard curve between the threshold cycle (Ct) value and log10 (gene copies) for each pathogenic bacterium individually. To assess gene copy number in a given sample, C_T values of samples were compared to a prepared standard curve that was included in triplicate with each qPCR run (further details are provided in Supplemental Methods). Calculated copy number was normalized to g of wet sponge tissue. The limit of quantification ranged from 20 - 100 gene copies per g tissue.

Statistics

PRIMER-E was used to calculate alpha diversity metrics (Observed OTUs, Shannon Diversity). The weighted UniFrac distance matrix was used to calculate beta-diversity to assess dispersion in bacterial communities among samples at each site within each sponge species and to construct Principle Coordinates Analysis (PCoA) plots to visualize differences in bacterial community structure between sites. PCoA was conducted for all samples (both sponge species), as well as for samples of each species individually. Pairwise Analysis of Similarities (ANOSIM) was used to test for significant differences in bacterial

communities between sites using PRIMER-E v7 (Primer-E Ltd). One-way ANOVA was used to compare results such as alpha diversity metrics, beta-diversity metrics, and quantified bacterial abundances among collection dates (within each sponge species). Due to unequal variances in other data sets, (even after transformation), the non-parametric Kruskal-Wallis (with Mann-Whitney post-hoc comparisons and bonferroni correction) was used to compare relative abundances of major microbial taxa among collection dates (within each sponge species). All data are represented as mean (± SEM), unless otherwise stated.

Acknowledgements

This work was funded by NSF awards OCE-1800914 to AC, OCE-1800905 to LSV, OCE-1800904 to SD, OCE-1800913 to KEFS and JBS, as well as a Rice University Faculty Initiative award to AC and LS, and Rice University start-up funds to AC. AS was supported by a Rice Academy Postdoctoral Fellowship and Rice University start-up funds (to AC). We thank Marissa Nuttall, John Embesi and James MacMillan (NOAA), Jake Emmert and Kaitlin Buhler (Moody Gardens), and Ryan Eckert (Florida Atlantic University) for field and logistical support. We are grateful to Emma Hickerson for facilitating collection permits. We also acknowledge the following members of the science party during sampling cruises: Carsten Grupstra, Jennifer Drummond, Kelsey Sanders, and Anna Knochel (Rice University), Shawn Doyle (TAMU). We also acknowledge Rachel Wright for the code used to retrieve and plot TABS Buoy V data. 2016 samples were collected under FGBNMS-2014-001; 2017 and 2018 samples were collected under FGBNMS-2017-012 to AC. July 2016, August 2016 and September 2017 samples were collected on the R/V Manta, whereas October 2017 and October 2018 samples were collected aboard the R/V Point Sur. We express our sincere gratitude to the crews of these vessels, as well as members of the NOAA FGBNMS office and Moody Gardens Aquarium. All authors declare no conflict of interest.

REFERENCES

Altieri, A.H., Harrison, S.B., Seemann, J., Collin, R., Diaz, R.J., and Knowlton, N. (2017) Tropical dead zones and mass mortalities on coral reefs. *Proc Natl Acad Sci U S A* **114**: 3660–3665.

Angermeier, H., Kamke, J., Abdelmohsen, U.R., Krohne, G., Pawlik, J.R., Lindquist, N.L., and Hentschel, U. (2011) The pathology of sponge orange band disease affecting the Caribbean barrel sponge *Xestospongia muta*. *FEMS Microbiol Ecol* **75**: 218–230.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., et al. (2011) Enterotypes of the human gut microbiome. *Nature* **473**: 174–180.

Barott, K.L., Rodriguez-Mueller, B., Youle, M., Marhaver, K.L., Vermeij, M.J.A., Smith, J.E., and Rohwer, F.L. (2012) Microbial to reef scale interactions between the reef-building coral *Montastraea annularis* and benthic algae. *Proc R Soc B Biol Sci* **279**: 1655–1664.

Barott, K.L. and Rohwer, F.L. (2012) Unseen players shape benthic competition on coral reefs. *Trends Microbiol* **20**: 621–628.

Bell, J.J., Davy, S.K., Jones, T., Taylor, M.W., and Webster, N.S. (2013) Could some coral reefs become sponge reefs as our climate changes? *Glob Chang Biol* **19**: 2613–2624.

Beurmann, S., Ushijima, B., Videau, P., Svoboda, C.M., Chatterjee, A., Aeby, G.S., and Callahan, S.M. (2018) Dynamics of acute montipora white syndrome: Bacterial communities of healthy and diseased *M. capitata* colonies during and after a disease outbreak. *Microbiol (United Kingdom)* **164**: 1240–1253.

Blake, E.S. and Zelinsky, D.A. (2018) National Hurricane Center Tropical Cyclone Report Hurricane Harvey.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. (2010) QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nat Publ Gr* **7**: 335–336.

Castro H F, Williams N H, and Ogram A (2000) Phylogeny of sulfate-reducing bacteria. *FEMS Microbiol Ecol* **31**: 1–9.

Chaves-Fonnegra, A., Zea, S., and Gómez, M.L. (2007) Abundance of the excavating sponge *Cliona delitrix* in relation to sewage discharge at San Andrés Island, SW Caribbean, Colombia. *Bol Investig Mar y Costeras* **36**: 63–78.

Chen, S., Lu, Y.H., Dash, P., Das, P., Li, J., Capps, K., et al. (2019) Hurricane pulses: Small watershed exports of dissolved nutrients and organic matter during large storms in the Southeastern USA. *Sci Total Environ* **689**: 232–244.

Cleary, D.F.R., Swierts, T., Coelho, F.J.R.C., Polónia, A.R.M., Huang, Y.M., Ferreira, M.R.S., et al. (2019) The sponge microbiome within the greater coral reef microbial metacommunity. *Nat Commun* **10**: 1–12.

Congdon, V.M., Bonsell, C., Cuddy, M.R., and Dunton, K.H. (2019) In the wake of a major hurricane: Differential effects on early vs. late successional seagrass species. *Limnol Oceanogr Lett* **4**: 155–163.

Deignan, L.K., Pawlik, J.R., and Erwin, P.M. (2018) *Agelas* Wasting Syndrome Alters Prokaryotic Symbiont Communities of the Caribbean Brown Tube Sponge, *Agelas tubulata*. *Microb Ecol* **76**: 459–466.

Emanuel, K. (2017) Assessing the present and future probability of Hurricane Harvey's rainfall. *Proc Natl Acad Sci U S A* **114**: 12681–12684.

Erguder, T.H., Boon, N., Wittebolle, L., Marzorati, M., and Verstraete, W. (2009) Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS Microbiol Rev* **33**: 855–869.

Fabricius, K.E. (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Mar Pollut Bull* **50**: 125–146.

Fiore, C.L., Labrie, M., Jarett, J.K., and Lesser, M.P. (2015) Transcriptional activity of the giant barrel sponge, *Xestospongia muta* Holobiont: Molecular evidence for metabolic interchange. *Front Microbiol* **6**:.

Gantt, S.E., McMurray, S.E., Stubler, A.D., Finelli, C.M., Pawlik, J.R., and Erwin, P.M. (2019) Testing the relationship between microbiome composition and flux of carbon and nutrients in Caribbean

coral reef sponges. *Microbiome* **7**: 1–13.

Garrity, G.M., Bell, J.A., and Lilburn, T. (2015) Thiotrichaceae fam. nov. In *Bergey's Manual of Systematics of Archaea and Bacteria*. Whitman, W.B., Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., et al. (eds). John Wiley & Sons, Inc.

Glasl, B., Bourne, D.G., Frade, P.R., and Webster, N.S. (2018) Establishing microbial baselines to identify indicators of coral reef health. *Microbiol Aust* **39**: 42–46.

Glasl, B., Smith, C.E., Bourne, D.G., and Webster, N.S. (2018) Exploring the diversity-stability paradigm using sponge microbial communities. *Sci Rep* **8**: 1–9.

Haas, A.F., Fairoz, M.F.M., Kelly, L.W., Nelson, C.E., Dinsdale, E.A., Edwards, R.A., et al. (2016) Global microbialization of coral reefs. *Nat Microbiol* **1**: 1–7.

Le Hénaff, M., Muller-Karger, F.E., Kourafalou, V.H., Otis, D., Johnson, K.A., McEachron, L., and Kang, H.S. (2019) Coral mortality event in the Flower Garden Banks of the Gulf of Mexico in July 2016: Local hypoxia due to cross-shelf transport of coastal flood waters? *Cont Shelf Res* **190**: 103988.

Hughes, D.J., Alderdice, R., Cooney, C., Kühl, M., Pernice, M., Voolstra, C.R., and Suggett, D.J. (2020) Coral reef survival under accelerating ocean deoxygenation. *Nat Clim Chang* **10**: 1–12.

Humphrey, C., Iverson, G., Skibiel, C., Sanderford, C., and Blackmon, J. (2019) Geochemistry of flood waters from the tar river, North Carolina associated with Hurricane Matthew. *Resources* **8**: 1–12.

Humphrey, C., Weber, M., Lott, C., Cooper, T., and Fabricius, K. (2008) Effects of suspended sediments, dissolved inorganic nutrients and salinity on fertilisation and embryo development in the coral *Acropora millepora* (Ehrenberg, 1834). *Coral Reefs* **27**: 837–850.

Johnston, M.A., Embesi, J.A., Eckert, R.J., Nuttall, M.F., Hickerson, E.L., and Schmahl, G.P. (2016) Persistence of coral assemblages at East and West Flower Garden Banks, Gulf of Mexico. *Coral Reefs* **35**: 821–826.

Johnston, M.A., Nuttall, M.F., Eckert, R.J., Blakeway, R.D., Sterne, T.K., Hickerson, E.L., et al. (2019) Localized coral reef mortality event at East Flower Garden Bank, Gulf of Mexico. *Bull Mar Sci* **95**:

239–250.

Kealoha, A.K., Doyle, S.M., Shamberger, K.E.F., Sylvan, J.B., Hetland, R.D., and DiMarco, S.F. (2020) Localized hypoxia may have caused coral reef mortality at the Flower Garden Banks. *Coral Reefs* **39**: 119–132.

Kerswell, A.P. and Jones, R.J. (2003) Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of 'low-salinity bleaching.' *Mar Ecol Prog Ser* **253**: 145–154.

Kiaghadi, A. and Rifai, H.S. (2019) Physical, Chemical, and Microbial Quality of Floodwaters in Houston Following Hurricane Harvey. *Environ Sci Technol* **53**: 4832–4840.

Knight, P.A. and Fell, P.E. (1987) Low salinity induces reversible tissue regression in the estuarine sponge *Microciona prolifera* (Ellis & Solander). *J Exp Mar Bio Ecol* **107**: 253–261.

Knutson, T.R., McBride, J.L., Chan, J., Emanuel, K., Holland, G., Landsea, C., et al. (2010) Tropical cyclones and climate change. *Nat Geosci* **3**: 157–163.

Lapointe, B.E. (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeat Florida. **42**: 1119–1131.

Lapointe, B.E., Langton, R., Bedford, B.J., Potts, A.C., Day, O., and Hu, C. (2010) Land-based nutrient enrichment of the Buccoo Reef Complex and fringing coral reefs of Tobago, West Indies. *Mar Pollut Bull* **60**: 334–343.

Lesser, M.P., Fiore, C., Slattery, M., and Zaneveld, J. (2016) Climate change stressors destabilize the microbiome of the Caribbean barrel sponge, Xestospongia muta. *J Exp Mar Bio Ecol* **475**: 11–18.

Longo, C., Corriero, G., Licciano, M., and Stabili, L. (2010) Bacterial accumulation by the Demospongiae *Hymeniacidon perlevis*: A tool for the bioremediation of polluted seawater. *Mar Pollut Bull* **60**: 1182–1187.

López-Legentil, S., Erwin, P.M., Pawlik, J.R., and Song, B. (2010) Effects of Sponge Bleaching on Ammonia-Oxidizing Archaea: Distribution and Relative Expression of Ammonia Monooxygenase Genes Associated with the Barrel Sponge Xestospongia muta. *Microb Ecol* **60**: 561–571.

Luter, H.M., Gibb, K., and Webster, N.S. (2014) Eutrophication has no short-term effect on the

Cymbastela stipitata holobiont. *Front Microbiol* **5**: 216.

Maldonado, M., Zhang, X., Cao, X., Xue, L., Cao, H., and Zhang, W. (2010) Selective feeding by sponges on pathogenic microbes: A reassessment of potential for abatement of microbial pollution. *Mar Ecol Prog Ser* **403**: 75–89.

McCook, L.J., Jompa, J., and Diaz-Pulido, G. (2001) Competition between corals and algae on coral reefs: A review of evidence and mechanisms. *Coral Reefs* **19**: 400–417.

McMurray, S.E., Blum, J.E., and Pawlik, J.R. (2008) Redwood of the reef: Growth and age of the giant barrel sponge *Xestospongia muta* in the Florida Keys. *Mar Biol* **155**: 159–171.

Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G.L., Cerrano, C., et al. (2017) The sponge microbiome project. *Gigascience* **6**: 1–7.

Montalvo, N.F., Davis, J., Vicente, J., Pittiglio, R., Ravel, J., and Hill, R.T. (2014) Integration of culture-based and molecular analysis of a complex sponge-associated bacterial community. *PLoS One* **9**: 1–8.

Morganti, T.M., Ribes, M., Yahel, G., and Coma, R. (2019) Size Is the Major Determinant of Pumping Rates in Marine Sponges. *Front Physiol* **10**:.

Morrow, K.M., Fiore, C.L., and Lesser, M.P. (2016) Environmental drivers of microbial community shifts in the giant barrel sponge, *Xestospongia muta*, over a shallow to mesophotic depth gradient. *Environ Microbiol* **18**: 2025–2038.

Mußmann, M., Schulz, H.N., Strotmann, B., Kjær, T., Nielsen, L.P., Rosselló-Mora, R.A., et al. (2003) Phylogeny and distribution of nitrate-storing *Beggiatoa* spp. in coastal marine sediments. *Environ Microbiol* **5**: 523–533.

Nelson, H.R. and Altieri, A.H. (2019) Oxygen: the universal currency on coral reefs. *Coral Reefs* **38**: 177–198.

Oakley, J.W. and Guillen, G.J. (2019) Impact of Hurricane Harvey on Galveston Bay Saltmarsh Nekton Communities. *Estuaries and Coasts*.

Olson, J.B. and Gao, X. (2013) Characterizing the bacterial associates of three Caribbean sponges along a

gradient from shallow to mesophotic depths. *FEMS Microbiol Ecol* **85**: 74–84.

Ostrander, C.E., McManus, M.A., DeCarlo, E.H., and Mackenzie, F.T. (2008) Temporal and spatial variability of freshwater plumes in a semienclosed estuarine-bay system. *Estuaries and Coasts* **31**: 192–203.

Parra-velandia, F.J., Zea, S., and Soest, R.W.M.V.S. (2014) Reef sponges of the genus *Agelas* (Porifera: Demospongiae) from the Greater Caribbean. *Zootaxa* **3794**: 301–343.

Pawlik, J.R. and McMurray, S.E. (2020) The Emerging Ecological and Biogeochemical Importance of Sponges on Coral Reefs. *Ann Rev Mar Sci* **12**: 315–337.

Pelletier, E., Kreimeyer, A., Bocs, S., Rouy, Z., Gyapay, G., Chouari, R., et al. (2008) "Candidatus *Cloacamonas acidaminovorans*": Genome sequence reconstruction provides a first glimpse of a new bacterial division. *J Bacteriol* **190**: 2572–2579.

Pita, L., Rix, L., Slaby, B.M., Franke, A., and Hentschel, U. (2018) The sponge holobiont in a changing ocean: from microbes to ecosystems. *Microbiome* **6**: 46.

Postgate, J.R. and Campbell, L.L. (1966) Classification of *Desulfovibrio species*, the nonsporulating sulfate-reducing bacteria. *Bacteriol Rev* **30**: 732–738.

Rabalais, N.N., Harper, D.E., and Turner, R.E. (2001) Responses of nekton and demersal and benthic fauna to decreasing oxygen concentrations. In *Coastal Hypoxia: Consequences for Living Resources and Ecosystem Coastal and Estuarine Studies*. pp. 115–128.

Roffer, M.A., Gawlikowski, G., and Upton, M. (2018) Monitoring of the Hurricane Harvey Plume in the Gulf of Mexico, West Melbourne, FL 32904.

Rosenberg, R. and Diaz, R.J. (1993) Sulfur bacteria (*Beggiatoa* spp.) mats indicate hypoxic conditions in the Inner Stockholm Archipelago. *Ambio* **22**: 32–36.

Rua, C.P.J., de Oliveira, L.S., Froes, A., Tschoeke, D.A., Soares, A.C., Leomil, L., et al. (2018) Microbial and Functional Biodiversity Patterns in Sponges that Accumulate Bromopyrrole Alkaloids Suggest Horizontal Gene Transfer of Halogenase Genes. *Microb Ecol* **76**: 825–838.

Sato, Y., Civiello, M., Bell, S.C., Willis, B.L., and Bourne, D.G. (2016) Integrated approach to

understanding the onset and pathogenesis of black band disease in corals. *Environ Microbiol* **18**: 752–765.

Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., et al. (2012) Assessing the complex sponge microbiota: Core, variable and species-specific bacterial communities in marine sponges. *ISME J* **6**: 564–576.

Simister, R., Taylor, M.W., Tsai, P., and Webster, N. (2012) Sponge-Microbe Associations Survive High Nutrients and Temperatures. *PLoS One* **7**: e52220.

Slaby, B.M., Franke, A., Rix, L., Pita, L., Bayer, K., Jahn, M.T., and Hentschel, U. (2019) Marine Sponge Holobionts in Health and Disease. In *Symbiotic Microbiomes of Coral Reefs Sponges and Corals*. Springer Netherlands, pp. 81–104.

Sutherland, K.P., Porter, J.W., Turner, J.W., Thomas, B.J., Looney, E.E., Luna, T.P., et al. (2010) Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, *Acropora palmata*. *Environ Microbiol* **12**: 1122–1131.

Sutherland, K.P., Shaban, S., Joyner, J.L., Porter, J.W., and Lipp, E.K. (2011) Human pathogen shown to cause disease in the threatened eklhorn coral *Acropora palmata*. *PLoS One* **6**:.

Szmant, A.M. (2002) Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? *Estuaries* **25**: 743–766.

Villegas-Plazas, M., Wos-Oxley, M.L., Sanchez, J.A., Pieper, D.H., Thomas, O.P., and Junca, H. (2019) Variations in Microbial Diversity and Metabolite Profiles of the Tropical Marine Sponge *Xestospongia muta* with Season and Depth. *Microb Ecol* **78**: 243–256.

Wallace, C.C., Yund, P.O., Ford, T.E., Matassa, K.A., and Bass, A.L. (2013) Increase in antimicrobial resistance in bacteria isolated from stranded marine mammals of the Northwest Atlantic. *Ecohealth* **10**: 201–210.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**: 5261–5267.

Waters, J.L. and Ley, R.E. (2019) The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. *BMC Biol* **17**: 1–11.

Wear, S.L. and Thurber, R.V. (2015) Sewage pollution: mitigation is key for coral reef stewardship. *Ann N Y Acad Sci* **1355**: 15–30.

Whitman, W.B. (2015) Bergey's manual of systematics of archaea and bacteria, DeVos, P., Chun, J., Dedysh, S., Hedlund, B., Kämpfer, P., Rainey, F., and Trujillo, M. (eds) Hoboken, NJ: Wiley.

Wright, R.M., Correa, A.M.S., Quigley, L.A., Santiago-Vázquez, L.Z., Shamberger, K.E.F., and Davies, S.W. (2019) Gene Expression of Endangered Coral (*Orbicella* spp.) in Flower Garden Banks National Marine Sanctuary After Hurricane Harvey. *Front Mar Sci* **6**: 672.

Yu, P., Zaleski, A., Li, Q., He, Y., Mapili, K., Pruden, A., et al. (2018) Elevated Levels of Pathogenic Indicator Bacteria and Antibiotic Resistance Genes after Hurricane Harvey's Flooding in Houston. *Environ Sci Technol Lett* **5**: 481–486.

Zaneveld, J.R., McMinds, R., and Thurber, R.V. (2017) Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol* **2**: 17121.

Zouch, H., Karray, F., Armougom, F., Chifflet, S., Hirschler-Réa, A., Kharrat, H., et al. (2017) Microbial diversity in sulfate-reducing marine sediment enrichment cultures associated with anaerobic biotransformation of coastal stockpiled phosphogypsum (Sfax, Tunisia). *Front Microbiol* **8**: 1–11.

TABLEs AND FIGURES

Table 1. Summary of sample collections from two reef sponge species at the East Bank (EB) and West Bank (WB) of the Flower Garden Banks National Marine Sanctuary (northwest Gulf of Mexico) and amplicon sequencing results of the V4 region of the 16S rRNA gene from sponge-associated bacterial communities. Richness (Observed OTUs) and diversity (Shannon H') were calculated from rarefied OTU tables. Data are presented as mean ± (sem).

					Sequence Analysis Summary		
Species	Date	Site	Health Status	N	High Quality Sequences[#]	Observed OTUs[^]	Shannon H'
Various species	Jul. 2016	EB	Dead*	8	48,706 (5,970)	470 (46)	3.286 (0.234)
Agelas clathrodes	Aug. 2016	EB	Diseased	4	108,475 (8,413)	1,338 (79)	3.600 (0.613)
		EB	Healthy	8	98,916 (4,999)	840 (46)	3.670 (0.087)
		WB	Healthy	5	73,449 (9,982)	801 (60)	3.355 (0.277)
	Oct. 2017	EB	Healthy	9	29,487 (2,797)	500 (52)	2.822 (0.258)
		WB	Healthy	8	28,893 (1,194)	541 (31)	3.345 (0.288)
	Oct. 2018	EB	Healthy	10	29,728 (1,111)	910 (20)	4.072 (0.056)
		WB	Healthy	10	29,721 (1,039)	805 (16)	3.901 (0.063)
Xestospongia muta	Aug. 2016	EB	Diseased	5	74,783 (18,104)	987 (77)	4.073 (0.111)
		EB	Healthy	2	103,021 (2,718)	1,189 (105)	4.527 (0.332)
		WB	Healthy	3	107,784 (6,555)	999 (62)	3.806 (0.530)
	Sept. 2017	EB	Healthy	9	36,437 (4,643)	790 (68)	4.386 (0.195)
	Oct. 2017	EB	Healthy	2	28,125 (1,076)	1,219 (58)	4.977 (0.044)
		WB	Healthy	3	30,113 (1,305)	916 (170)	4.136 (0.431)
	Oct. 2018	EB	Healthy	10	31, 029 (3,026)	1,142 (36)	4.552 (0.092)
		WB	Healthy	10	30,808 (1,944)	1,113 (70)	4.449 (0.117)

*samples collection from a variety of dead invertebrate tissue

[#]quality filtering included removal of low quality, short, Mitochondrial, Chloroplast, and Unassigned reads

[^]Operational Taxonomic Units (OTUs) after rarefying to equal depth (20,000 reads)

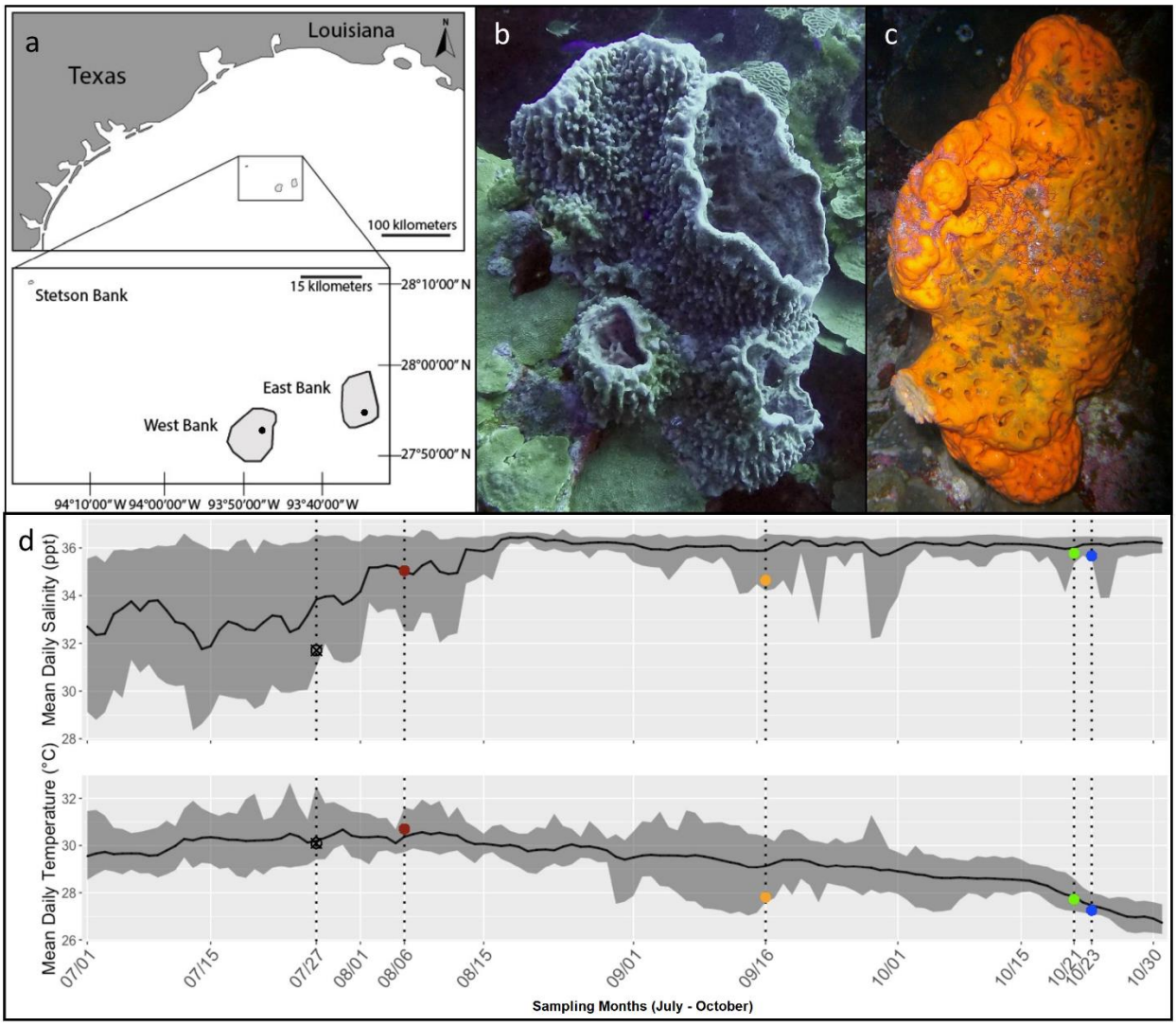

Figure 1. **Summary of study site, host taxa and local abiotic conditions associated with this study.** A) Map of Flower Garden Banks National Marine Sanctuary (northwest Gulf of Mexico) with sites of sponge collection indicated as black dots; B) Representative *Xestospongia muta* sponge; C) Representative *Agelas clathrodes* sponge; D) Surface Salinity (ppt, top) and temperature (°C, bottom) at buoy V (3 km from sampling sites) spanning the months July through October in which sampling occurred for this study. Black lines represent daily means from 2013–2018 and grey shaded areas encompass minimum and maximum values from 2013–2018. Dotted lines and symbols represent mean daily values on the date of each sample collection: black circle = 27 July 2016, dark red circle = 6 August 2016, orange circle = 16 September 2017, green circle = 21 October 2017, and blue circle = 23 October 2018.

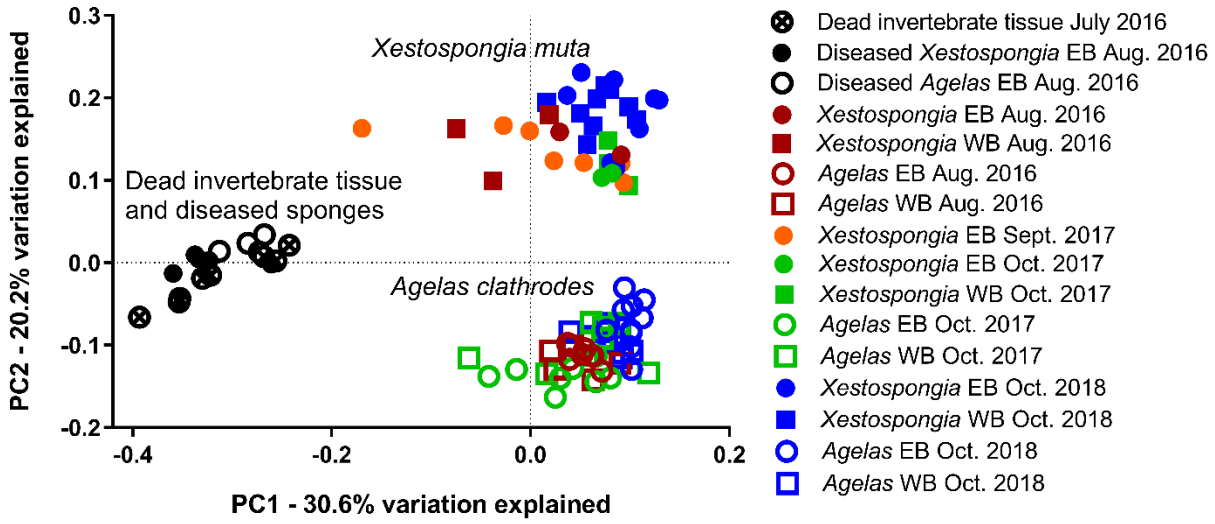

Figure 2. Principle Coordinate Analysis of the weighted UniFrac distance matrix for bacterial communities analyzed in this study. Empty symbols = *Agelas clathrodes* samples; filled symbols = *Xestospongia muta* samples. Black symbols = samples from dead invertebrate tissue (DIT) and diseased sponges during (July 2016) and immediately after (August 2016) the Localized Mortality Event (LME); Red symbols = visually healthy sponge samples collected immediately after the 2016 LME. Orange symbols = samples collected immediately following Hurricane Harvey (Sept. 2017) from visually healthy sponges; Green symbols = samples collected one month following Hurricane Harvey (Oct. 2017) from visually healthy sponges. Blue symbols = samples collected in a no-flood (baseline) year from visually healthy sponges (Oct. 2018). Circles = samples collected from East Bank (EB); squares = samples collected from West Bank (WB) of the Flower Garden Banks National Marine Sanctuary (northern Gulf of Mexico).

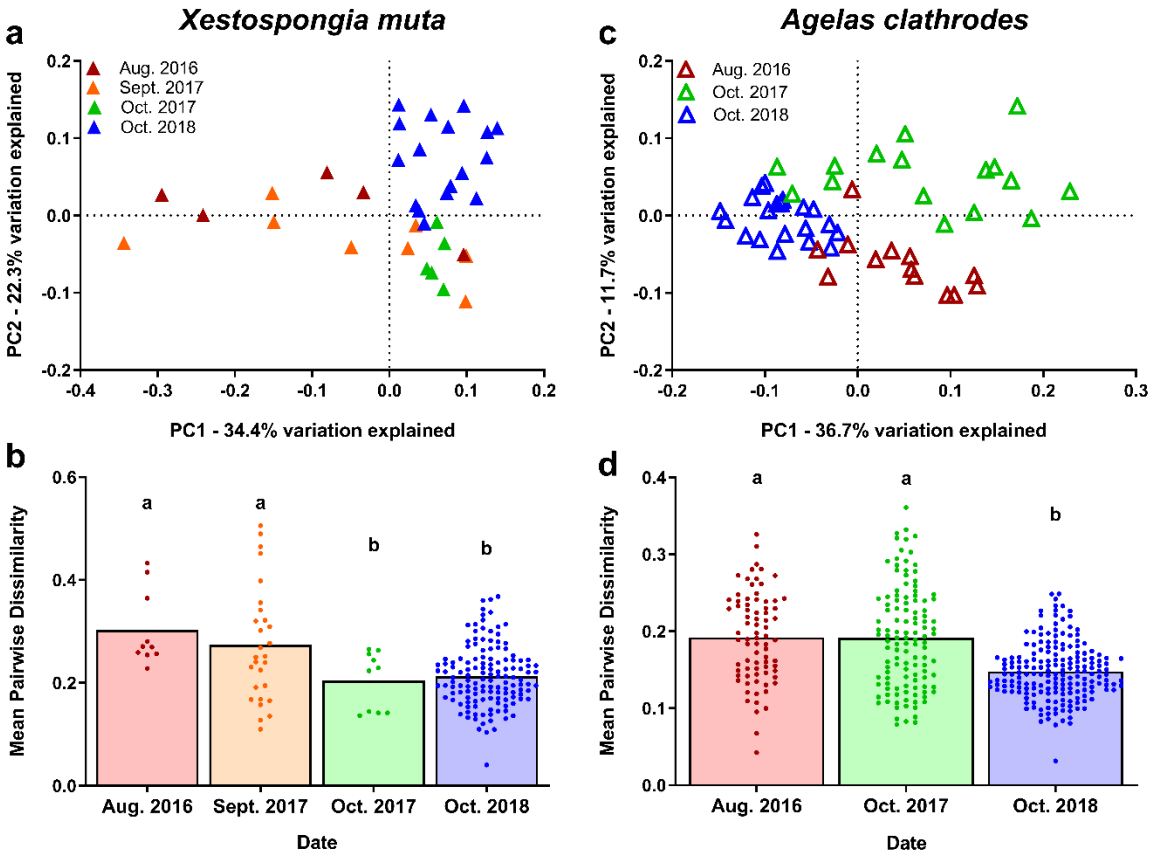

Figure 3. Sponge-associated bacterial communities differed in composition and variability following extreme storm-derived floods. Principle Coordinate Analysis of the weighted UniFrac distance matrices for visually healthy a) *Xestospongia muta* and c) *Agelas clathrodes* bacterial communities. Mean (with individual value dots) pairwise dissimilarity values for b) *X. muta* and d) *A. clathrodes*. Red = August 2016. Orange = September 2017; Green = October 2017. Blue = October 2018 associated with no flooding stress. Bars represent mean (± sem). Within a species, bars that do not share a letter are significantly different based on one-way ANOVA with Tukey's comparisons ($p < 0.05$).

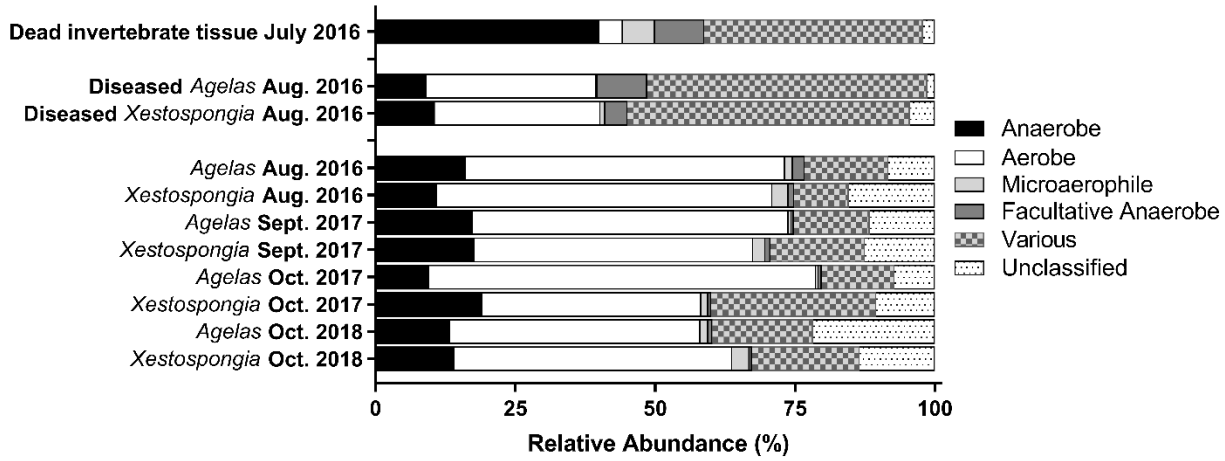

Figure 4. Percent abundance of bacterial sequences classified by oxygen requirement, for dead invertebrate tissue (DIT) samples (July 2016), diseased sponges (August 2016), and visually healthy sponges (2016, 2017, 2018). Flood events occurred in the region in 2016 and 2017, but not in 2018. Bacterial taxonomy was assigned using the RDP v2.12 database, and oxygen requirements of each Family were classified as using Bergey's Manual of Systematics of Archaea and Bacteria (Whitman et al., 2015). Families with more than one type of oxygen requirement were categorized as ‘Various’.

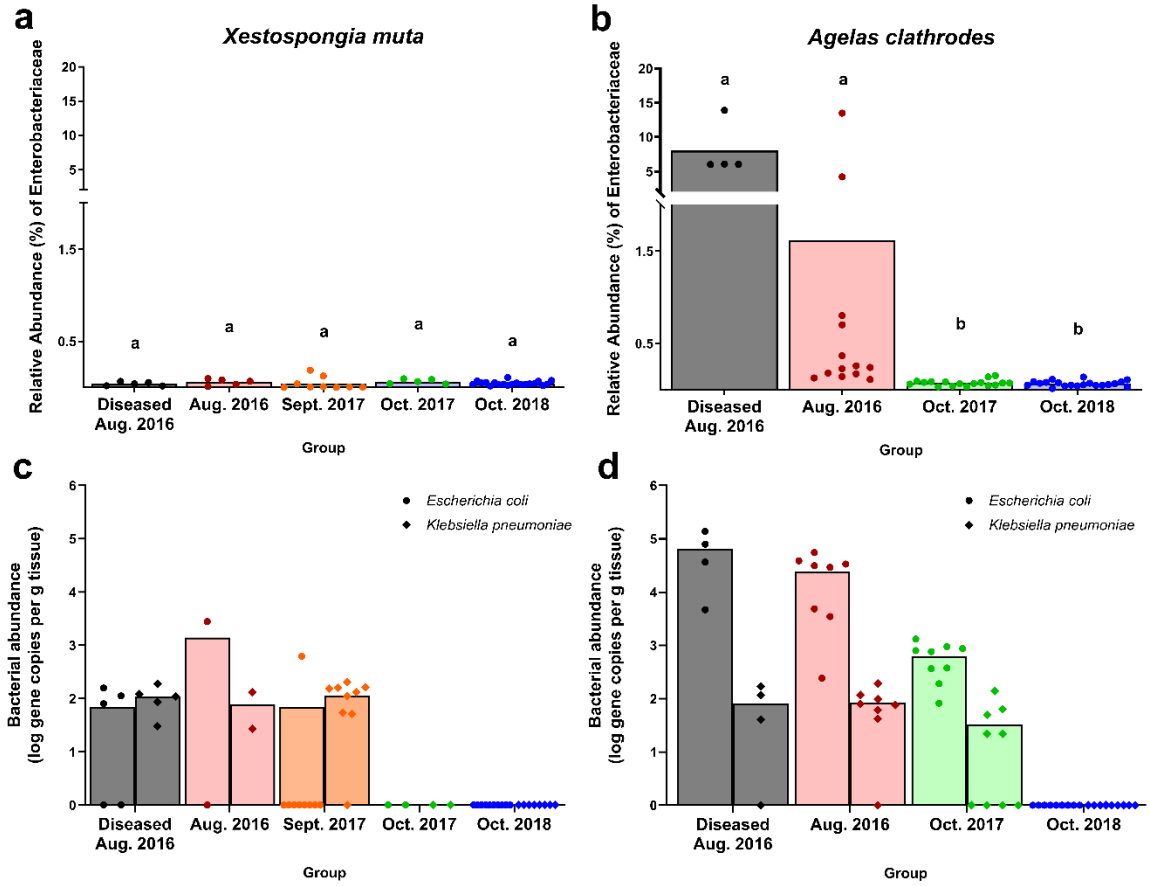

Figure 5. Relative abundance of sequences in the Family Enterobacteriaceae (a, b) and to specific human pathogens (*Escherichia coli;* c, and *Klebsiella pneumoniae)*; d), across sites and years. Data in (a,b) are based on Illumina MiSeq amplicon sequencing of the V4 region of the bacterial 16S rRNA gene, and are presented as mean ± sem. Data in (c,d) are quantitative PCR amplification of the bacterial *ybbW* and *phoE* genes for *E. coli* and *K. pneumoniae*, respectively. Black bars = affected sponges in 2016; red bars = healthy sponges in 2017; green bars = healthy sponges in 2017; blue bars = healthy sponges in 2018 (no flood, baseline year). Groups that share a letter (in a, b) are not significantly different based on Mann-Whitney pairwise comparisons across groups within a species. ND = not detected.