Biodiversity of Macrofauna Associated with Sponges across Ecological Gradients in the Central Red Sea

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ABSTRACT

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Between 33 and 91 percent of marine species are currently undescribed, with the majority occurring in tropical and offshore environments. Sponges act as important microhabitats and promote biodiversity by harboring a wide variety of macrofauna and microbiota, but little is known about the relationships between the sponges and their symbionts. This study uses DNA barcoding to examine the macrofaunal communities associated with sponges of the central Saudi Arabian Red Sea, a drastically understudied ecosystem with high biodiversity and endemism. In total, 185 epifaunal and infaunal operational taxonomic units (OTUs) were distinguished from the 1399 successfully-sequenced macrofauna individuals from 129 sponges representing seven sponge species, one of which (*Stylissa carteri*) was intensively studied. A significant difference was found in the macrofaunal community composition of *Stylissa carteri* along a cross-shelf gradient using relative OTU abundance (Bray-Curtis diversity index). The abundance of *S. carteri* also follows a cross-shelf gradient, increasing with proximity to shore. The difference in macrofaunal communities of several species of sponges at one location was found to be significant as well, using OTU presence (binary Jaccard diversity index). Four of the seven sponge species collected were dominated by a single annelid OTU, each unique to one sponge species. A fifth was dominated by four arthropod OTUs, all species-specific as well. Region-based diversity differences may be attributed to environmental factors

such as reef morphology, water flow, and sedimentation, whereas species-based differences may be caused by sponge morphology, microbial abundances, and chemical defenses. As climate change and ocean acidification continue to modify coral reef ecosystems, understanding the ecology of sponges and their role as microhabitats may become more important. This thesis also includes a supplemental document in the form of a spreadsheet showing the number of macrofauna individuals of each OTU found within each sponge sample.

Keywords: barcoding, diversity index, macrofauna, Porifera, Red Sea, *Stylissa carteri*

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LIST OF ABBREVIATIONS

- BIN: Barcode index number
- BLAST: Basic local alignment search tool
- BOLD: Barcode of life data system
- COI: Cytochrome *c* oxidase subunit 1
- CROP: Clustering 16S rRNA for OTU prediction
- DNA: Deoxyribonucleic acid
- HMA: High microbial abundance
- LMA: Low microbial abundance
- OTU: Operational taxonomic unit
- PC: Principal component
- PCoA: Principal coordinate analysis
- PCR: Polymerase chain reaction
- PERMANOVA: Permutational multivariate analysis of variance
- QIIME: Quantitative insights into microbial ecology
- SCUBA: Self-contained underwater breathing apparatus
- UPGMA: Unweighted pair group method with arithmetic mean

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INTRODUCTION

1.1 Measuring Biodiversity

Tropical coral reefs are among the most diverse ecosystems on the planet. Studying biodiversity in these environments is important not only for gaining knowledge about the variety of organisms present, but also for knowing how the environment shifts and is affected by climate change and anthropogenic stresses. Recent studies show that between 33 and 91 percent of marine species are currently undescribed (Mora et al. 2011, Appeltans et al. 2012). The majority of these are invertebrates from tropical and offshore environments (Appeltans et al. 2012).

Molecular methods, particularly high-throughput DNA sequencing, are revolutionizing our ability to conduct biodiversity surveys (Leray and Knowlton 2015). DNA barcoding is very quick and accurate, as well as more convenient than traditional methods, which require long hours and expert taxonomists using identification by morphology and microscopy (Bourlat et al. 2013). Barcoding excels where morphological studies cannot, giving positive identifications to organisms that are difficult to distinguish or have an impaired physical appearance (e.g., only a partial organism). DNA barcoding also often has the ability to provide taxonomic resolution exceeding that of morphological studies (Hebert et al. 2015). Due to its rapid and rigorous production of information, DNA barcoding provides evidence for the vast number of marine species that have not yet been described or cataloged. Plaisance et al. (2011) and Leray and Knowlton (2015) report under-estimation of macroinvertebrates and fish using sequencing techniques of this kind.

1.2 Sponges as Hosts

Sponges are widely known as simple, sessile, filter-feeding animals. They are often overlooked in biodiversity studies due to the lack of sufficient and reliable field guides as well as difficulty of identification in the laboratory, which requires tedious microscopic work for an absolute match (Diaz and Rutzler 2001, Wulff 2001). Many species are currently undescribed, making them even more troublesome to work with (Wulff 2001). Nevertheless, sponges act as important microhabitats and promote biodiversity through their associated infauna as one of the most diverse components of reef systems (Diaz and Rutzler 2001).

1.2a Symbiotic Relationships

Diaz and Rutzler (2001) describe sponges as an essential component of coral reef environments in the Caribbean, due in part to their symbiotic relationships with microbial communities. Sponges have been recognized to belong to one of two classifications in relation to their microbiota (Vacelet and Donadey 1977). Those with a very high bacterial density that is easily seen with a light microscope have been termed high microbial abundance (HMA) sponges, while those with a low bacterial density that is difficult or impossible to see with a light microscope have been termed low microbial abundance (LMA) sponges (Vacelet and Donadey 1977, Hentschel et al. 2003). LMA sponges have microbial abundances approximately equal to that of seawater with only one or two different types of bacteria, while HMA sponges typically have more different types of bacteria and microbial abundances two to four orders of magnitude greater, occupying more volume than that of the animal cells (Vacelet and Donadey 1977, Hentschel et al. 2003, Gloeckner et al. 2014). Vacelet and Donadey (1977) describe

sponges with a high bacterial density as large, massive, or thick-walled tubular sponges. While LMA sponges have a well-developed aquiferous system and a mesohyl of lower density, HMA sponges have a much denser mesohyl and higher rates of water flow (Vacelet and Donadey 1977, Weisz et al. 2008).

In addition to bacteria, sponges harbor a large variety of macrofauna. Macroinvertebrate communities associated with sponges are commonly dominated by crustaceans and polychaetes, followed by echinoderms and molluscs (Wendt et al. 1985, Voultsiadou-Koukoura et al. 1987, Ribeiro et al 2003, Abdo 2007, Padua et al. 2012). Fish have also been found to live in sponges, with some being obligate sponge-dwellers (Tyler and Bohlke 1972).

The complex structure of sponges provides an interesting microhabitat for symbiotic residents. Pearse (1950) describes large individuals with substantial internal canals as "veritable living hotels." Several studies show a positive correlation between sponge volume and macrofaunal abundance or species richness (Westinga and Hoetjes 1981, Villamizar and Laughlin 1991, Gherardi et al. 2001, Ribeiro et al. 2003, Hultgren and Duffy 2010, Padua et al. 2012). Others emphasize morphological features, particularly the importance of large and distinct internal canals (Klitgaard 1995, Duffy 1996, Koukouras et al. 1996, Hultgren and Duffy 2010, Kersken et al. 2014). In many cases, macrofauna take refuge in sponges for protection from predators or for camouflage, thereby increasing their chances of survival (Bell 2008). Some species spend most of their life cycles inside their hosts (Magnino et al. 1999) and may even use them as breeding grounds (Ribeiro et al. 2003, Abdo 2007).

Another benefit of sponge-macrofauna relationships is food availability. Sponges may provide an indirect food source via water flow through their internal canals for filter feeders such as some polychaetes, barnacles, and brittle stars, which feed on the plankton and small particles carried by the seawater (Wendt et al. 1985, Abdo 2007). Fish may receive both food and oxygen from water currents (Tyler and Bohlke 1972). Some sponges provide a direct food source for residents such as nudibranchs (Proksch 1994), chitons (Klitgaard 1995), and snapping shrimps (Duris et al. 2011), the latter of which have specialized mouths and claws for feeding on their hosts. The regenerative properties of sponges may provide a continual food source for this type of parasitic species (Duris et al. 2011).

Although most symbiotic relationships with sponges seem to be commensal or parasitic, it is possible for the hosts to benefit as well. For example, in what is most likely a mutualistic relationship with *Callyspongia sp.*, brittle stars have been found to clean the surface of their host sponges in exchange for shelter and food (Hendler 1984). However, the nature of most relationships is not fully understood and requires further examination.

1.2b Differing Macrofaunal Communities

Macrofaunal communities associated with a single sponge species may change between different sites or reefs of a general region (Pearse 1950, Westinga and Hoetjes 1981, Abdo 2007). Cinar and Ergen (1998) attribute differences in polychaete communities to various degrees of water movement and total vegetation cover. Water movement is strongly correlated with sedimentation, which may be yet another contributing factor. Epifauna and infauna may also differ between sponge species

(Wendt et al. 1985, Koukouras et al. 1996, Abdo 2007). These differences are commonly attributed to sponge morphology (Pearse 1950, Wendt et al. 1985, Koukouras et al. 1996, Kersken et al. 2014). Some species of macroinvertebrates are sponge species-specific and have only been found in one host species (Bell 2008). Environmental factors and host-symbiont relationships require further attention in order to fully understand differences in macrofaunal communities.

1.2c Global Change

In addition to having symbiotic relationships with microbiota and macrofauna, sponges benefit coral reef ecosystems by contributing to reef framework and filtering seawater (Diaz and Rutzler 2001). They may act as a glue that binds corals to the reef and stabilizes the structure (Wulff 2001). As one of the most prominent reef groups, they also contribute significantly to reef diversity, abundance, and space competition. They may rival the abundance of coral and algae in the Caribbean (Diaz and Rutzler 2001), and may surpass these major groups in biomass as well (Rutzler 1978). At the same time that coral has been declining in the Caribbean throughout the past decade or two, some sponges have been increasing (Bell et al. 2013, Ruzicka et al. 2013), with particular attention focused on *Xestospongia muta* (McMurray et al. 2015). *Xestospongia muta* is not only more abundant than in the past, but the rate of its population growth is increasing as well. Bell et al. (2013) attribute the increase of Caribbean sponges to a low sensitivity to rising temperature and ocean acidification. As other space competitors (hard corals and algae) decline, sponges have the chance to expand. They have even been shown to be more tolerant of lower light conditions, increased sedimentation, and mild organic pollution (Alcolado 1994, Zea 1994). Wulff (1995) found sponges capable

of surviving severe partial mortality and undergoing frequent fragmentation, further adding to their set of survival skills. As global climate change and ocean acidification continue to change modern coral reef ecosystems, understanding the ecology of sponges and their role as hosts may become more important.

1.3 Study System

The Red Sea is a region of high biodiversity and endemism, which was recently found to be even higher than previously thought (e.g., DiBattista et al. 2013). It is a unique body of water with extremely high temperatures (20-32°C) and salinity (37-42‰) (Raitsos et al. 2011). Despite these characteristics, the Red Sea remains a drastically understudied region. Sponges are understudied worldwide, which is only accentuated in the Red Sea. As of 2013, only 23 studies of sponges were conducted in the Red Sea, as opposed to 88 in the Great Barrier Reef System and 170 in the Caribbean (Berumen et al. 2013). From those 23 studies in the Red Sea, only five occurred outside the Gulf of Aqaba, and only two of those were related to symbiotic interactions with infauna (Ilan et al. 1999, Magnino et al. 1999, Berumen et al. 2013).

The main focus species of this study, *Stylissa carteri* (syn. *Axinella carteri*), is an abundant sponge in coastal waters of the Red Sea and exhibits an interesting morphology. Its characteristic folds and ridges form canals and protected areas, showing promise of hosting macrofaunal symbionts. Currently, there are no studies examining the epifaunal and infaunal communities of *S. carteri* in the Red Sea or elsewhere.

1.4 Aim of Study

The aim of this study is to assess the macrofaunal communities of sponges in the central Red Sea, using DNA barcoding techniques. The diversity of epifauna and infauna of *Stylissa carteri* will be examined on a fine scale, across various ecological gradients. Macrofaunal diversities will also be compared between several sponge species at one location. I hypothesize a change in the macrofaunal communities of *S. carteri* across both a cross-shelf gradient and an even finer-scale exposure gradient. Although I expect to find similar taxa associated with all individual sponges of the same species, I expect to see changes in the relative abundances of such macrofaunal groups between regions. Therefore, a dissimilarity index taking into account relative abundance will be focused on to compare region-based diversity. I also hypothesize differences between the macrofaunal communities of the various sponge species collected at a single location. Here, I expect the presence of different taxa in each sponge species to be the most important diversifying factor, so a dissimilarity index taking into account presence-absence only data will be focused on to compare species-based diversity. Together, these results will provide valuable information concerning the role of *S. carteri* and other Red Sea sponges as hosts.

MATERIALS AND METHODS

2.1 Sample Collection

Sponge samples were taken from the exposed (west) and sheltered (east) sides of ten reefs in the central Red Sea, off the coast of Thuwal, Saudi Arabia (Fig. 1, Campbell 2015). The study reefs include three inshore, three midshelf, three offshore, and one

miscellaneous reef (Table 1). For the purpose of this study, the miscellaneous reef (Qita' Al-Girsh) is labeled as such because it is a midshelf reef surrounded by deep water, setting it apart from both the midshelf and offshore groupings. Before samples were collected, surveys were conducted in order to gain perspective on the abundance of the focal species, *Stylissa carteri*. At each of the 20 sites, three replicate 25-meter transects were laid and all *S. carteri* within two meters of either side were counted (i.e., a total of 100 square meters in each replicate transect).

Five *S. carteri* individuals were collected utilizing SCUBA in June 2015 from each site at depths of 7.7 to 25.3 meters. Five individuals of each of six additional sponge species (with the exception of one species of which only four individuals were found) were collected at one of the sites, sheltered Al-Fahal. An effort was made to collect sponges of different known microbial abundance classifications (LMA vs. HMA). The total number of sampled sponges was 129 (Table 2). Each sponge (in most cases the entire individual) was covered with a plastic bag in situ and removed from the substratum using a dive knife. As sponges were collected, their depths were recorded.

Upon return to the lab, the sponges were immediately processed. Each sponge was immersed in filtered seawater to obtain mass by volume displacement before being carefully dissected. The filtered seawater and any water from the plastic bags was examined for fauna to collect anything that may have escaped during transportation and volume measurement. All visible (greater than approximately one millimeter) epifauna and infauna were individually collected, photographed, and stored in 80 percent ethanol at -20 degrees Celsius. Tissue samples were also taken from the sponges for barcoding.

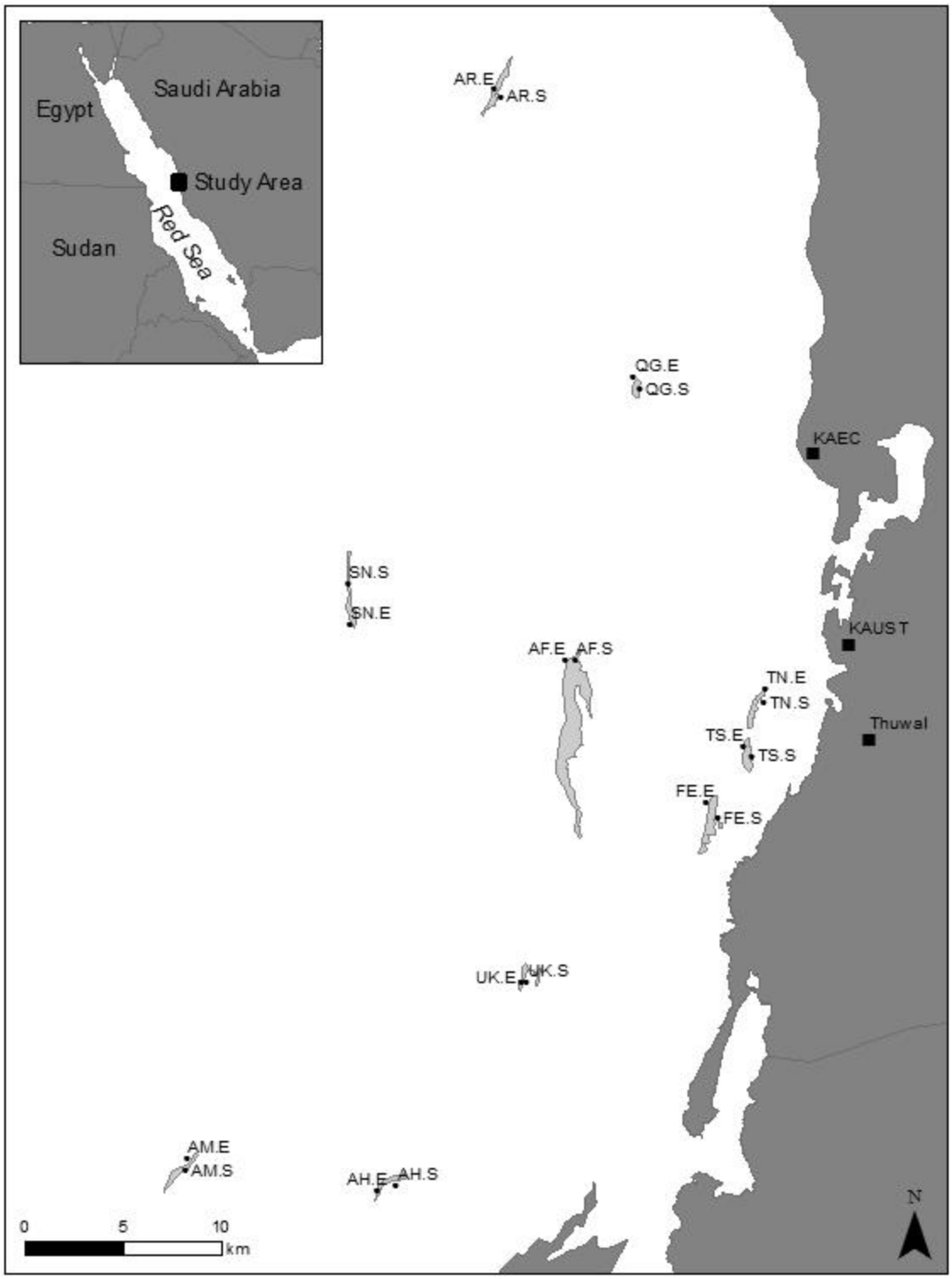

Fig. 1 Sponge collection sites in the central Saudi Arabian Red Sea. The sheltered and exposed sides of each of ten reefs are labeled for a total of 20 sites (Campbell 2015). Site abbreviations follow Table 1.

Table 1 Information on the collection sites, including full reef name, region and exposure, location, and the sponge species collected.

Label	Reef	Region	Exposure	Latitude	Longitude	Sponge Species
AR.E	Abu Romah	Offshore	Exposed	22°34.034'	38°55.541'	*Stylissa carteri*
AR.S	Abu Romah	Offshore	Sheltered	22°33.833'	38°55.717'	*Stylissa carteri*
SN.E	Shib Nazar	Offshore	Exposed	22°19.317'	38°51.252'	*Stylissa carteri*
SN.S	Shib Nazar	Offshore	Sheltered	22°20.434'	38°51.227'	*Stylissa carteri*
AM.E	Abu Madafi	Offshore	Exposed	22°04.594'	38°46.505'	*Stylissa carteri*
AM.S	Abu Madafi	Offshore	Sheltered	22°04.258'	38°46.433'	*Stylissa carteri*
QG.E	Qita' Al-Girsh	Misc.	Exposed	22°26.105'	38°59.638'	*Stylissa carteri*
QG.S	Qita' Al-Girsh	Misc.	Sheltered	22°25.796'	38°59.855'	*Stylissa carteri*
AF.E	Al-Fahal	Midshelf	Exposed	22°18.316'	38°57.648'	*Stylissa carteri*
AF.S	Al-Fahal	Midshelf	Sheltered	22°18.341'	38°57.930'	*Stylissa carteri*
UK.E	Um Al-Kathel	Midshelf	Exposed	22°09.467'	38°56.336'	*Stylissa carteri*
UK.S	Um Al-Kathel	Midshelf	Sheltered	22°09.471'	38°56.470'	*Stylissa carteri*
AH.E	Abu Henshan	Midshelf	Exposed	22°03.723'	38°52.085'	*Stylissa carteri*
AH.S	Abu Henshan	Midshelf	Sheltered	22°03.863'	38°52.636'	*Stylissa carteri*
TN.E	Tahla North	Inshore	Exposed	22°17.545'	39°03.562'	*Stylissa carteri*
TN.S	Tahla North	Inshore	Sheltered	22°17.151'	39°03.530'	*Stylissa carteri*
TS.E	Tahla South	Inshore	Exposed	22°15.927'	39°02.899'	*Stylissa carteri*
TS.S	Tahla South	Inshore	Sheltered	22°15.679'	39°03.154'	*Stylissa carteri*
FE.E	Fsar East	Inshore	Exposed	22°14.390'	39°01.817'	*Stylissa carteri*
FE.S	Fsar East	Inshore	Sheltered	22°13.980'	39°02.181'	*Stylissa carteri*
AF.Hy	Al-Fahal	Midshelf	Sheltered	22°18.341'	38°57.930'	*Hyrtios* sp.
AF.CN	Al-Fahal	Midshelf	Sheltered	22°18.341'	38°57.930'	*Chalinula novo*
AF.Cr	Al-Fahal	Midshelf	Sheltered	22°13.435'	38°58.229'	*Crella* sp.
AF.XT	Al-Fahal	Midshelf	Sheltered	22°13.435'	38°58.229'	*Xestospongia testudinari*
AF.TS	Al-Fahal	Midshelf	Sheltered	22°13.435'	38°58.229'	*Theonella swinhoei*
AF.Ca	Al-Fahal	Midshelf	Sheltered	22°13.435'	38°58.229'	*Callyspongia* sp.

Table 2 Information on the sponge samples, including collection location, region and exposure, date of collection, sponge species, depth of collection, mass by volume displacement, number of associated macrofauna individuals, and density of macrofauna per ten grams of sponge.

Sample	Region	Reef	Exposure	Date	Species	Depth (m)	Mass (g)	Macrofauna	Fauna/10g
S.001	Inshore	Fsar East	Exposed	04.06.15	*Stylissa carteri*	10.1	130	18	1.385
S.002	Inshore	Fsar East	Exposed	04.06.15	*Stylissa carteri*	9.6	85	8	0.941
S.003	Inshore	Fsar East	Exposed	04.06.15	*Stylissa carteri*	10.0	190	30	1.579
S.004	Inshore	Fsar East	Exposed	04.06.15	*Stylissa carteri*	9.8	50	7	1.400
S.005	Inshore	Fsar East	Exposed	04.06.15	*Stylissa carteri*	11.1	260	7	0.269
S.006	Inshore	Fsar East	Sheltered	04.06.15	*Stylissa carteri*	11.3	55	4	0.727
S.007	Inshore	Fsar East	Sheltered	04.06.15	*Stylissa carteri*	11.7	45	5	1.111
S.008	Inshore	Fsar East	Sheltered	04.06.15	*Stylissa carteri*	9.8	75	4	0.533
S.009	Inshore	Fsar East	Sheltered	04.06.15	*Stylissa carteri*	12.0	100	12	1.200
S.010	Inshore	Fsar East	Sheltered	04.06.15	*Stylissa carteri*	9.9	70	5	0.714
S.011	Offshore	Shib Nazar	Sheltered	07.06.15	*Stylissa carteri*	10.2	90	2	0.222
S.012	Offshore	Shib Nazar	Sheltered	07.06.15	*Stylissa carteri*	10.7	45	8	1.778
S.013	Offshore	Shib Nazar	Sheltered	07.06.15	*Stylissa carteri*	12.8	100	13	1.300
S.014	Offshore	Shib Nazar	Sheltered	07.06.15	*Stylissa carteri*	12.3	50	6	1.200
S.015	Offshore	Shib Nazar	Sheltered	07.06.15	*Stylissa carteri*	10.9	90	3	0.333
S.016	Midshelf	Al-Fahal	Sheltered	07.06.15	*Stylissa carteri*	10.2	65	14	2.154
S.017	Midshelf	Al-Fahal	Sheltered	07.06.15	*Stylissa carteri*	7.9	45	7	1.556
S.018	Midshelf	Al-Fahal	Sheltered	07.06.15	*Stylissa carteri*	8.1	50	5	1.000
S.019	Midshelf	Al-Fahal	Sheltered	07.06.15	*Stylissa carteri*	10.3	30	8	2.667
S.020	Midshelf	Al-Fahal	Sheltered	07.06.15	*Stylissa carteri*	9.1	60	3	0.500

Table 2 (cont.)

Sample	Region	Reef	Exposure	Date	Species	Depth (m)	Mass (g)	Macrofauna	Fauna/10g
S.021	Offshore	Shib Nazar	Exposed	08.06.15	*Stylissa carteri*	22.4	20	1	0.500
S.022	Offshore	Shib Nazar	Exposed	08.06.15	*Stylissa carteri*	23.6	35	3	0.857
S.023	Offshore	Shib Nazar	Exposed	08.06.15	*Stylissa carteri*	22.9	110	7	0.636
S.024	Offshore	Shib Nazar	Exposed	08.06.15	*Stylissa carteri*	19.3	60	3	0.500
S.025	Offshore	Shib Nazar	Exposed	08.06.15	*Stylissa carteri*	18.1	50	11	2.200
S.026	Midshelf	Al-Fahal	Exposed	08.06.15	*Stylissa carteri*	10.8	30	10	3.333
S.027	Midshelf	Al-Fahal	Exposed	08.06.15	*Stylissa carteri*	12.2	30	4	1.333
S.028	Midshelf	Al-Fahal	Exposed	08.06.15	*Stylissa carteri*	11.4	105	9	0.857
S.029	Midshelf	Al-Fahal	Exposed	08.06.15	*Stylissa carteri*	12.9	55	7	1.273
S.030	Midshelf	Al-Fahal	Exposed	08.06.15	*Stylissa carteri*	11.6	50	8	1.600
S.031	Offshore	Abu Romah	Exposed	09.06.15	*Stylissa carteri*	16.0	45	0	0.000
S.032	Offshore	Abu Romah	Exposed	09.06.15	*Stylissa carteri*	15.8	20	3	1.500
S.033	Offshore	Abu Romah	Exposed	09.06.15	*Stylissa carteri*	14.9	95	8	0.842
S.034	Offshore	Abu Romah	Exposed	09.06.15	*Stylissa carteri*	14.9	30	7	2.333
S.035	Offshore	Abu Romah	Exposed	09.06.15	*Stylissa carteri*	15.3	15	1	0.667
S.036	Offshore	Abu Romah	Sheltered	09.06.15	*Stylissa carteri*	10.4	135	17	1.259
S.037	Offshore	Abu Romah	Sheltered	09.06.15	*Stylissa carteri*	11.3	125	40	3.200
S.038	Offshore	Abu Romah	Sheltered	09.06.15	*Stylissa carteri*	11.1	95	18	1.895
S.039	Offshore	Abu Romah	Sheltered	09.06.15	*Stylissa carteri*	11.1	100	11	1.100
S.040	Offshore	Abu Romah	Sheltered	09.06.15	*Stylissa carteri*	11.4	80	6	0.750
S.041	Misc.	Qita' Al-Girsh	Exposed	10.06.15	*Stylissa carteri*	10.6	105	4	0.381
S.042	Misc.	Qita' Al-Girsh	Exposed	10.06.15	*Stylissa carteri*	12.0	100	12	1.200
S.043	Misc.	Qita' Al-Girsh	Exposed	10.06.15	*Stylissa carteri*	14.5	155	11	0.710
S.044	Misc.	Qita' Al-Girsh	Exposed	10.06.15	*Stylissa carteri*	13.8	115	6	0.522
S.045	Misc.	Qita' Al-Girsh	Exposed	10.06.15	*Stylissa carteri*	14.6	50	17	3.400
S.046	Misc.	Qita' Al-Girsh	Sheltered	10.06.15	*Stylissa carteri*	10.9	65	12	1.846
S.047	Misc.	Qita' Al-Girsh	Sheltered	10.06.15	*Stylissa carteri*	13.9	75	26	3.467
S.048	Misc.	Qita' Al-Girsh	Sheltered	10.06.15	*Stylissa carteri*	11.0	85	9	1.059
S.049	Misc.	Qita' Al-Girsh	Sheltered	10.06.15	*Stylissa carteri*	9.3	65	7	1.077
S.050	Misc.	Qita' Al-Girsh	Sheltered	10.06.15	*Stylissa carteri*	9.3	100	7	0.700
S.051	Inshore	Tahla North	Exposed	11.06.15	*Stylissa carteri*	13.7	80	21	2.625
S.052	Inshore	Tahla North	Exposed	11.06.15	*Stylissa carteri*	14.6	65	3	0.462
S.053	Inshore	Tahla North	Exposed	11.06.15	*Stylissa carteri*	15.2	165	5	0.303
S.054	Inshore	Tahla North	Exposed	11.06.15	*Stylissa carteri*	13.8	115	14	1.217
S.055	Inshore	Tahla North	Exposed	11.06.15	*Stylissa carteri*	15.8	100	43	4.300
S.056	Inshore	Tahla North	Sheltered	11.06.15	*Stylissa carteri*	9.6	70	4	0.571
S.057	Inshore	Tahla North	Sheltered	11.06.15	*Stylissa carteri*	14.4	55	2	0.364
S.058	Inshore	Tahla North	Sheltered	11.06.15	*Stylissa carteri*	14.1	55	1	0.182
S.059	Inshore	Tahla North	Sheltered	11.06.15	*Stylissa carteri*	10.0	120	12	1.000
S.060	Inshore	Tahla North	Sheltered	11.06.15	*Stylissa carteri*	9.4	75	12	1.600
S.061	Midshelf	Um Al-Kathel	Exposed	15.06.15	*Stylissa carteri*	14.4	70	2	0.286
S.062	Midshelf	Um Al-Kathel	Exposed	15.06.15	*Stylissa carteri*	13.6	90	12	1.333
S.063	Midshelf	Um Al-Kathel	Exposed	15.06.15	*Stylissa carteri*	14.4	115	6	0.522
S.064	Midshelf	Um Al-Kathel	Exposed	15.06.15	*Stylissa carteri*	13.9	65	13	2.000
S.065	Midshelf	Um Al-Kathel	Exposed	15.06.15	*Stylissa carteri*	14.1	45	6	1.333
S.066	Midshelf	Al-Fahal	Sheltered	14.06.15	*Hyrtios* sp.	7.7	145	87	6.000
S.067	Midshelf	Al-Fahal	Sheltered	14.06.15	*Hyrtios* sp.	8.8	100	54	5.400
S.068	Midshelf	Al-Fahal	Sheltered	14.06.15	*Hyrtios* sp.	10.6	110	25	2.273
S.069	Midshelf	Al-Fahal	Sheltered	14.06.15	*Hyrtios* sp.	10.9	160	78	4.875
S.070	Midshelf	Al-Fahal	Sheltered	14.06.15	*Hyrtios* sp.	9.4	215	33	1.535
S.071	Midshelf	Al-Fahal	Sheltered	14.06.15	*Chalinula novo*	8.6	100	501	50.100
S.072	Midshelf	Al-Fahal	Sheltered	14.06.15	*Chalinula novo*	7.9	100	507	50.700
S.073	Midshelf	Al-Fahal	Sheltered	14.06.15	*Chalinula novo*	9.2	35	502	143.429
S.074	Midshelf	Al-Fahal	Sheltered	14.06.15	*Chalinula novo*	9.8	35	501	143.143
S.075	Midshelf	Al-Fahal	Sheltered	14.06.15	*Chalinula novo*	9.5	55	500	90.909
S.076	Midshelf	Um Al-Kathel	Sheltered	15.06.15	*Stylissa carteri*	9.3	60	4	0.667
S.077	Midshelf	Um Al-Kathel	Sheltered	15.06.15	*Stylissa carteri*	9.9	75	2	0.267
S.078	Midshelf	Um Al-Kathel	Sheltered	15.06.15	*Stylissa carteri*	7.7	105	11	1.048
S.079	Midshelf	Um Al-Kathel	Sheltered	15.06.15	*Stylissa carteri*	9.8	105	7	0.667
S.080	Midshelf	Um Al-Kathel	Sheltered	15.06.15	*Stylissa carteri*	7.8	90	2	0.222
S.081	Midshelf	Abu Henshan	Exposed	17.06.15	*Stylissa carteri*	12.2	105	4	0.381
S.082	Midshelf	Abu Henshan	Exposed	17.06.15	*Stylissa carteri*	13.8	80	6	0.750
S.083	Midshelf	Abu Henshan	Exposed	17.06.15	*Stylissa carteri*	13.4	50	1	0.200
S.084	Midshelf	Abu Henshan	Exposed	17.06.15	*Stylissa carteri*	12.2	95	5	0.526
S.085	Midshelf	Abu Henshan	Exposed	17.06.15	*Stylissa carteri*	11.6	205	11	0.537

Table 2 (cont.)

Sample	Region	Reef	Exposure	Date	Species	Depth (m)	Mass (g)	Macrofauna	Fauna/10g
S.086	Midshelf	Abu Henshan	Sheltered	17.06.15	*Stylissa carteri*	13.8	185	11	0.595
S.087	Midshelf	Abu Henshan	Sheltered	17.06.15	*Stylissa carteri*	13.4	150	10	0.667
S.088	Midshelf	Abu Henshan	Sheltered	17.06.15	*Stylissa carteri*	14.0	165	17	1.030
S.089	Midshelf	Abu Henshan	Sheltered	17.06.15	*Stylissa carteri*	12.9	120	8	0.667
S.090	Midshelf	Abu Henshan	Sheltered	17.06.15	*Stylissa carteri*	12.1	105	6	0.571
S.091	Midshelf	Al-Fahal	Sheltered	18.06.15	*Crella* sp.	11.3	50	78	15.600
S.092	Midshelf	Al-Fahal	Sheltered	18.06.15	*Crella* sp.	11.0	50	44	8.800
S.093	Midshelf	Al-Fahal	Sheltered	18.06.15	*Crella* sp.	9.8	95	67	7.053
S.094	Midshelf	Al-Fahal	Sheltered	18.06.15	*Crella* sp.	12.1	105	44	4.190
S.095	Midshelf	Al-Fahal	Sheltered	18.06.15	*Crella* sp.	11.3	45	23	5.111
S.096	Midshelf	Al-Fahal	Sheltered	18.06.15	*Xestospongia testudinaria*	12.8	120	500	41.667
S.097	Midshelf	Al-Fahal	Sheltered	18.06.15	*Xestospongia testudinaria*	14.5	330	500	15.152
S.098	Midshelf	Al-Fahal	Sheltered	18.06.15	*Xestospongia testudinaria*	14.6	205	500	24.390
S.099	Midshelf	Al-Fahal	Sheltered	18.06.15	*Xestospongia testudinaria*	13.9	225	500	22.222
S.100	Midshelf	Al-Fahal	Sheltered	23.06.15	*Xestospongia testudinaria*	25.3	205	500	24.390
S.101	Midshelf	Al-Fahal	Sheltered	18.06.15	*Theonella swinhoei*	18.9	75	23	3.067
S.102	Midshelf	Al-Fahal	Sheltered	18.06.15	*Theonella swinhoei*	18.9	170	203	11.941
S.103	Midshelf	Al-Fahal	Sheltered	18.06.15	*Theonella swinhoei*	22.8	70	20	2.857
S.104	Midshelf	Al-Fahal	Sheltered	23.06.15	*Theonella swinhoei*	21.5	120	205	17.083
S.105	Midshelf	Al-Fahal	Sheltered	23.06.15	*Theonella swinhoei*	19.6	280	217	7.750
S.106	Inshore	Tahla South	Exposed	21.06.15	*Stylissa carteri*	13.6	120	10	0.833
S.107	Inshore	Tahla South	Exposed	21.06.15	*Stylissa carteri*	10.5	70	13	1.857
S.108	Inshore	Tahla South	Exposed	21.06.15	*Stylissa carteri*	14.8	100	11	1.100
S.109	Inshore	Tahla South	Exposed	21.06.15	*Stylissa carteri*	13.0	65	13	2.000
S.110	Inshore	Tahla South	Exposed	21.06.15	*Stylissa carteri*	11.5	60	2	0.333
S.111	Inshore	Tahla South	Sheltered	21.06.15	*Stylissa carteri*	13.3	70	10	1.429
S.112	Inshore	Tahla South	Sheltered	21.06.15	*Stylissa carteri*	9.9	60	8	1.333
S.113	Inshore	Tahla South	Sheltered	21.06.15	*Stylissa carteri*	11.9	205	22	1.073
S.114	Inshore	Tahla South	Sheltered	21.06.15	*Stylissa carteri*	10.2	55	7	1.273
S.115	Inshore	Tahla South	Sheltered	21.06.15	*Stylissa carteri*	12.2	195	16	0.821
S.116	Offshore	Abu Madafi	Exposed	22.06.15	*Stylissa carteri*	11.0	55	3	0.545
S.117	Offshore	Abu Madafi	Exposed	22.06.15	*Stylissa carteri*	19.4	50	7	1.400
S.118	Offshore	Abu Madafi	Exposed	22.06.15	*Stylissa carteri*	12.2	10	12	12.000
S.119	Offshore	Abu Madafi	Exposed	22.06.15	*Stylissa carteri*	20.0	55	37	6.727
S.120	Offshore	Abu Madafi	Exposed	22.06.15	*Stylissa carteri*	13.9	45	4	0.889
S.121	Offshore	Abu Madafi	Sheltered	22.06.15	*Stylissa carteri*	11.9	135	4	0.296
S.122	Offshore	Abu Madafi	Sheltered	22.06.15	*Stylissa carteri*	14.2	125	54	4.320
S.123	Offshore	Abu Madafi	Sheltered	22.06.15	*Stylissa carteri*	13.9	95	8	0.842
S.124	Offshore	Abu Madafi	Sheltered	22.06.15	*Stylissa carteri*	16.3	50	10	2.000
S.125	Offshore	Abu Madafi	Sheltered	22.06.15	*Stylissa carteri*	13.6	120	5	0.417
S.126	Midshelf	Al-Fahal	Sheltered	23.06.15	*Callyspongia* sp.	14.1	190	30	1.579
S.127	Midshelf	Al-Fahal	Sheltered	23.06.15	*Callyspongia* sp.	22.3	95	27	2.842
S.128	Midshelf	Al-Fahal	Sheltered	23.06.15	*Callyspongia* sp.	14.1	215	28	1.302
S.129	Midshelf	Al-Fahal	Sheltered	23.06.15	*Callyspongia* sp.	13.2	35	11	3.143

2.2 DNA Barcoding

Macrofauna specimens were taken to the Smithsonian National Museum of Natural History in Washington, D.C. for processing. There, tissue was sub-sampled from each specimen and placed in 96-well plates. In cases where the individual was too small, the entire specimen was used. DNA was extracted by proteinase K digestion and phenol

extraction on an AutoGenprep 965, manufactured by Autogen. PCR amplification and Sanger sequencing used standard protocols and previously published primers (jgLCO1490 and jgHCO2198) to sequence part of the mitochondrial COI gene in both directions (Geller et al. 2013, Leray and Knowlton 2015). Additional primer pairs dgLCO1490/dgHCO2198 and mlCOIintF/jgHCO were used on samples that did not amplify with the initial primers (Meyer 2003, Leray et al. 2013).

2.3 Sequence Analysis

Raw sequences were uploaded to Geneious 8.1.6 for forward and reverse assembly, minor editing, and alignment. CROP v1.33, a Bayesian clustering program (Hao et al. 2011), was used to cluster the sequences into OTUs with a dissimilarity threshold between six and eight percent. This flexible cutoff has been shown to provide the best results for marine invertebrates (Leray et al. 2013). The sequence outputs from Geneious were also input to BOLD, where they were divided into BINs for further comparison of sequence relationships (Ratnasingham and Hebert 2007). Mothur v.1.36.1 (Schloss et al. 2009) was used to determine the number of OTUs at different levels of dissimilarity cutoff (by the farthest neighbor method), which were then used to construct a step function graph (Fig. 2). The three clustering tools (CROP, BIN, mothur) were tested in parallel for sample consistency and they gave similar OTU divisions, with 190, 198, and 184 OTUs/BINs, respectively. CROP's flexible threshold has been proven efficient by Leray et al. (2013) and lies within the limits of the plateau of the step function graph. Therefore, the CROP output, which includes a representative sequence for each OTU, was used to make an OTU table in mothur, which was then used for statistical analysis. The CROP output of representative sequences was also uploaded to

GenBank for taxonomic identification via BLAST searches. A species level match was accepted with a reference similarity greater than 97 percent. Photo identification by taxonomic experts was used to confirm results from the sequence database and assign a higher taxonomic level to sequences without a direct match.

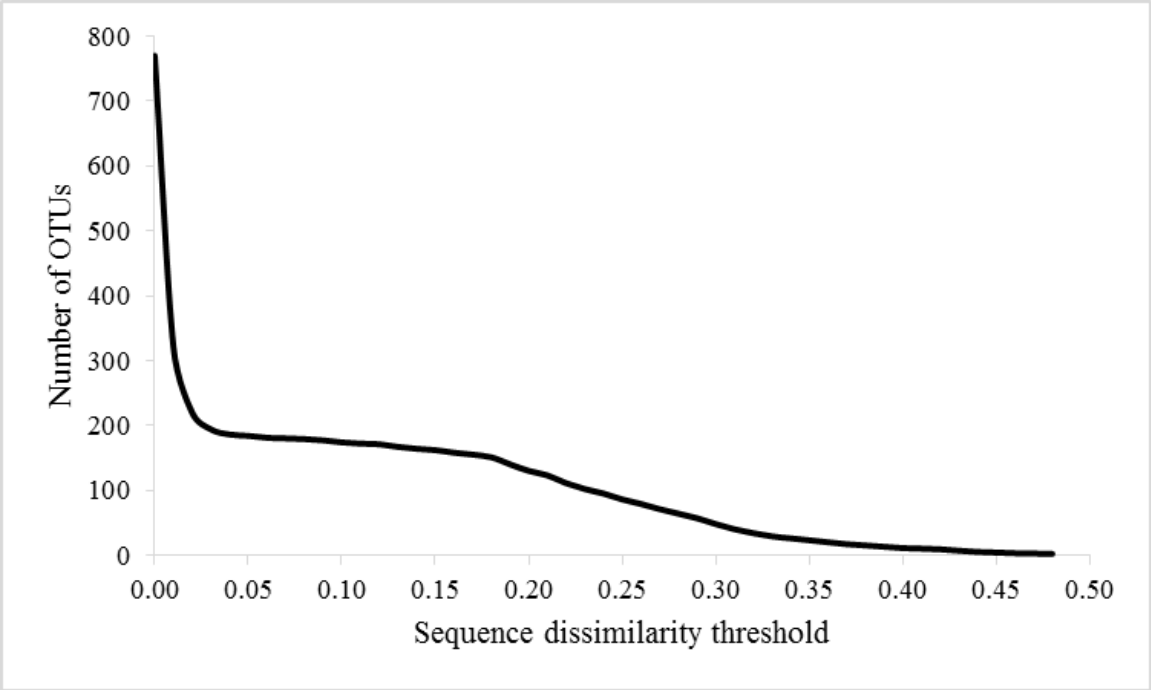

Fig. 2 Step function graph showing the number of distinguished OTUs vs. the threshold for sequence dissimilarity. Data were produced using mothur.

2.4 Statistical Analysis

The OTU table output from mothur was modified to create a second dataset in which the five replicate samples were pooled to create a single sample for each collection site because many sponge samples contained very few macrofauna individuals. Both datasets were used to calculate individual-based rarefaction curves and nonparametric species richness estimators in EstimateS 9.1.0 (Colwell 2005). *Stylissa carteri* samples from all 20 collection sites and all samples from the sheltered side of Al-Fahal were

separated into two tables for input to MacQIIME 1.9.1 (Caporaso et al. 2010). Phylum-level taxonomy tables were constructed with both relative and absolute abundance. Jaccard (presence-absence) and Bray-Curtis (relative abundance) metrics were used to examine differences in beta diversity, which was visualized using PCoA and UPGMA-based hierarchical clustering trees with jackknife support. Differences in macrofaunal community composition between sites, regions, and sponge species were examined using PERMANOVA (Anderson 2001).

Although relative abundance and presence-absence data were both used for each dataset, the Bray-Curtis similarity index was primarily used to compare the beta diversity of *S. carteri* macrofaunal communities between regions, while the Jaccard index was primarily used for comparison between sponge species at Al-Fahal. The abundance-influenced method of Bray-Curtis was chosen for region-based studies to examine the hypothesized differences in abundance of the various epifauna and infauna of *S. carteri* across ecological gradients. The binary method of Jaccard was chosen for species-based studies to examine the hypothesized differences in macrofaunal community composition and species richness among several species of sponges at a single collection site.

RESULTS

3.1 Sample Summary

The abundance surveys of *Stylissa carteri* revealed a trend following the offshore-inshore environmental gradient (Fig. 3). Abundance increases from offshore to inshore, with the exception of the sheltered side of Shib Nazar, which has an unusually high

number of *S. carteri* individuals. In addition, all but two reefs (Qita' Al-Girsh and Fsar East) show a higher abundance of *S. carteri* on the sheltered side as opposed to the exposed side of the reef.

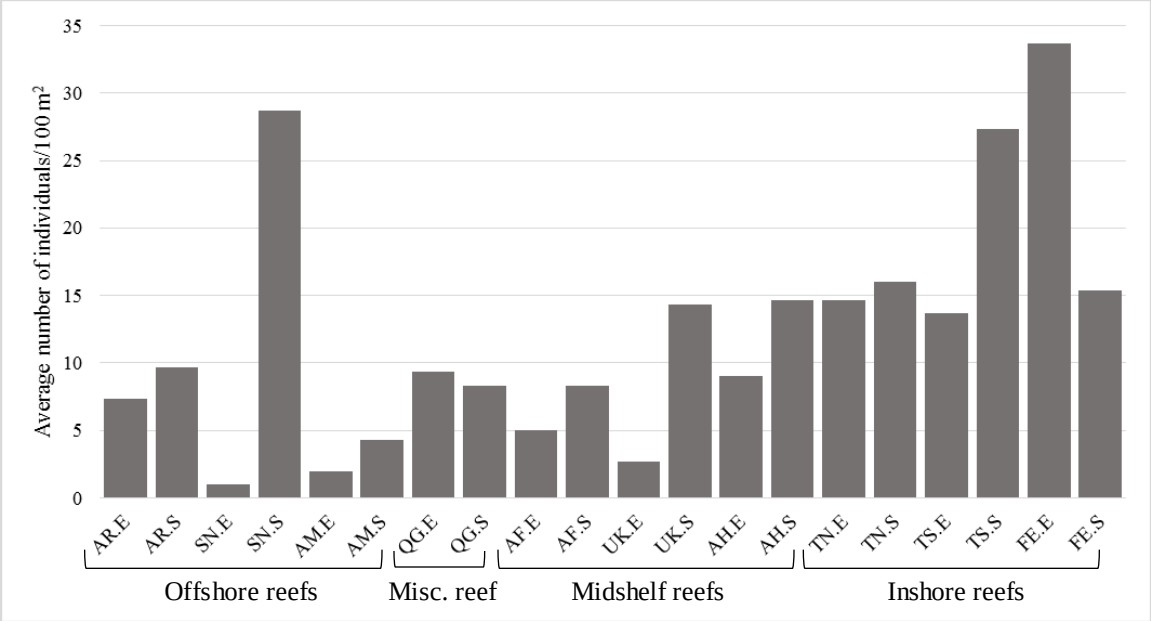

Fig. 3 Average abundance of *Stylissa carteri* at each collection site. Values are the averages of three replicate transects of 100 m^2 (25 X 4 m) each. Order of sites follows the cross-shelf gradient, from offshore to inshore. Site abbreviations follow Table 1.

In total, 1469 epifauna and infauna individuals from 127 sponges were collected and sequenced. Barnacles and a species of mollusc from the family Haminoeidae were found in *S. carteri*, but were not sampled due to difficulty of extraction from the sponges. In cases where annelids found in the additional sponge species were too numerous to be counted and appeared to be identical, their abundances were estimated. When numerous individuals of apparently identical annelids were found, representatives were taken for photographing and sequencing. Tissue was sub-sampled from 16 of the 129 sponge specimens (four *S. carteri* and two of each additional species) for confirmation of the photo identifications.

Sequencing was successful for 1399 macrofauna samples and 12 sponge samples, comprising five of the seven sponge species. Both the overall and macrofaunal success rates were greater than 95 percent.

3.2 Summary Statistics

CROP divided the sequences obtained into 189 OTUs, five of which consisted of the host sponges (Table 3). Sequencing of the annelids found in *Chalinula novo* was unsuccessful, so the annelids' estimated abundances were reported as a separate unit for analyses, giving a total of 185 macrofaunal OTUs found in the sponges. Arthropoda was the most diverse group of sponge associates (83 OTUs), followed by Annelida (79 OTUs), Chordata (7 OTUs), Echinodermata (6 OTUs), Mollusca (5 OTUs), Sipuncula (2 OTUs), Cnidaria (1 OTU), Nemertea (1 OTU), and Platyhelminthes (1 OTU). GenBank listed a level of similarity greater than 97 percent for only 16 of the OTUs, providing a species-level match for six Arthropoda, three Chordata, two Echinodermata, three of the host Porifera that were barcoded successfully, and two Sipuncula.

Table 3 Summary of the OTUs distinguished by CROP with a dissimilarity threshold between six and eight percent. OTUs are listed in alphabetical order by phylum. Taxon was determined using GenBank, with match confidence and the representative sequences listed.

OTU	Sequence	GenBank %	Phylum	OTU	Sequence	GenBank %	Phylum
2	C_0725	72	Annelida	30	C_0522	74	Annelida
3	C_0117	81	Annelida	32	C_0136	71	Annelida
4	C_0397	77	Annelida	33	C_1107	83	Annelida
12	C_1207	82	Annelida	34	C_1381	79	Annelida
14	C_0663	79	Annelida	36	C_0102	82	Annelida
16	C_0243	77	Annelida	37	C_1355	79	Annelida
20	C_0156	82	Annelida	39	C_1045	74	Annelida
22	C_1183	84	Annelida	46	C_0588	81	Annelida
23	C_1088	78	Annelida	47	C_1185	75	Annelida
24	C_0757	84	Annelida	50	C_1113	74	Annelida
27	C_1022	76	Annelida	52	C_0727	72	Annelida

Table 3 (cont.)

OTU	Sequence	GenBank %	Phylum	OTU	Sequence	GenBank %	Phylum
53	C_0343	72	Annelida	168	C_0632	82	Annelida
57	C_0165	74	Annelida	169	C_0306	81	Annelida
58	C_0633	73	Annelida	173	C_0631	76	Annelida
60	C_0458	72	Annelida	174	C_0345	77	Annelida
64	C_0493	71	Annelida	176	C_0980	80	Annelida
70	C_1142	74	Annelida	186	C_1275	76	Annelida
72	C_0386	71	Annelida	5	C_1160	83	Arthropoda
73	C_1321	78	Annelida	6	C_0917	79	Arthropoda
76	C_1081	83	Annelida	9	C_0212	77	Arthropoda
77	C_0353	83	Annelida	10	C_0348	86	Arthropoda
78	C_1074	90	Annelida	11	C_0749	92	Arthropoda
79	C_1110	84	Annelida	15	C_0628	83	Arthropoda
82	C_0194	93	Annelida	17	C_1212	77	Arthropoda
84	C_1383	80	Annelida	18	C_1136	78	Arthropoda
85	C_0915	82	Annelida	19	C_0106	83	Arthropoda
86	C_0658	78	Annelida	21	C_1462	81	Arthropoda
88	C_0201	80	Annelida	26	C_0800	72	Arthropoda
89	C_0324	76	Annelida	29	C_0724	91	Arthropoda
90	C_0095	83	Annelida	31	C_1443	83	Arthropoda
94	C_0473	89	Annelida	35	C_0572	80	Arthropoda
97	C_0657	81	Annelida	38	C_0067	86	Arthropoda
101	C_0026	80	Annelida	40	C_0206	78	Arthropoda
104	C_0355	81	Annelida	41	C_0740	81	Arthropoda
105	C_0149	76	Annelida	42	C_0920	78	Arthropoda
107	C_0303	71	Annelida	43	C_1474	83	Arthropoda
108	C_0712	78	Annelida	44	C_1253	83	Arthropoda
109	C_0537	81	Annelida	45	C_0690	99	Arthropoda
111	C_1214	73	Annelida	48	C_1255	92	Arthropoda
112	C_1059	79	Annelida	49	C_0064	90	Arthropoda
114	C_1166	73	Annelida	51	C_0652	91	Arthropoda
116	C_0356	71	Annelida	54	C_1103	80	Arthropoda
118	C_1139	76	Annelida	55	C_0309	81	Arthropoda
120	C_0116	85	Annelida	56	C_0799	79	Arthropoda
121	C_0304	84	Annelida	59	C_0103	93	Arthropoda
122	C_0913	77	Annelida	61	C_0754	83	Arthropoda
126	C_1411	76	Annelida	62	C_0020	83	Arthropoda
127	C_1085	77	Annelida	63	C_1423	83	Arthropoda
128	C_0354	78	Annelida	65	C_1248	92	Arthropoda
129	C_1375	81	Annelida	66	C_1454	99	Arthropoda
131	C_1276	77	Annelida	67	C_1171	99	Arthropoda
137	none		Annelida	68	C_1266	86	Arthropoda
138	C_1156	81	Annelida	69	C_0611	89	Arthropoda
139	C_1157	76	Annelida	71	C_0112	77	Arthropoda
144	C_0335	83	Annelida	74	C_1335	80	Arthropoda
145	C_0180	82	Annelida	80	C_0219	77	Arthropoda
147	C_0297	73	Annelida	91	C_1238	81	Arthropoda
149	C_0189	82	Annelida	92	C_1169	90	Arthropoda
150	C_1089	82	Annelida	93	C_1158	81	Arthropoda
152	C_0193	82	Annelida	95	C_1174	85	Arthropoda
157	C_0434	80	Annelida	96	C_0175	76	Arthropoda
162	C_0070	82	Annelida	98	C_1371	76	Arthropoda

Table 3 (cont.)

OTU	Sequence	GenBank %	Phylum	OTU	Sequence	GenBank %	Phylum
100	C_1479	99	Arthropoda	182	C_1476	78	Arthropoda
103	C_1124	73	Arthropoda	184	C_1413	87	Arthropoda
106	C_0029	77	Arthropoda	185	C_0177	81	Arthropoda
110	C_0237	73	Arthropoda	188	C_0430	86	Arthropoda
113	C_1149	79	Arthropoda	190	C_1055	80	Arthropoda
123	C_0861	76	Arthropoda	1	C_0019	92	Chordata
124	C_1350	77	Arthropoda	25	C_1167	100	Chordata
125	C_0700	77	Arthropoda	83	C_1100	92	Chordata
130	C_1365	98	Arthropoda	87	C_0369	97	Chordata
135	C_0059	79	Arthropoda	117	C_1168	83	Chordata
140	C_1364	83	Arthropoda	143	C_0269	99	Chordata
141	C_1134	75	Arthropoda	183	C_0482	99	Chordata
142	C_1101	83	Arthropoda	119	C_0241	88	Cnidaria
146	C_1215	84	Arthropoda	7	C_0052	82	Echinodermata
148	C_0066	92	Arthropoda	28	C_1303	91	Echinodermata
151	C_0692	81	Arthropoda	81	C_1080	95	Echinodermata
153	C_0337	97	Arthropoda	99	C_1182	99	Echinodermata
154	C_0375	91	Arthropoda	133	C_0706	79	Echinodermata
155	C_1249	84	Arthropoda	136	C_1351	98	Echinodermata
156	C_0240	83	Arthropoda	13	C_0624	70	Mollusca
158	C_1260	86	Arthropoda	132	C_0361	84	Mollusca
159	C_0591	86	Arthropoda	170	C_0404	84	Mollusca
160	C_1150	87	Arthropoda	171	C_1105	86	Mollusca
161	C_1254	83	Arthropoda	180	C_0475	85	Mollusca
163	C_1261	86	Arthropoda	75	C_1361	72	Nemertea
164	C_0239	91	Arthropoda	134	C_0221	77	Platyhelminthes
166	C_0660	98	Arthropoda	8	C_0028	100	Porifera
167	C_0589	84	Arthropoda	102	S_127	94	Porifera
172	C_1267	86	Arthropoda	165	S_096	100	Porifera
175	C_0791	85	Arthropoda	187	S_102	99	Porifera
177	C_0788	81	Arthropoda	189	S_092	87	Porifera
178	C_1216	89	Arthropoda	115	C_0882	100	Sipuncula
179	C_0760	90	Arthropoda	181	C_0198	99	Sipuncula

3.3 Region-Based Patterns

In total, 146 macrofaunal OTUs from 937 sequences were found in *S. carteri* across all collection sites. The average number of epifauna and infauna individuals found associated with *S. carteri* from a single site was 46.85, with a minimum value of 19 from the exposed side of Abu Romah and a maximum value of 87 from the sheltered side of the same reef. The average number of OTUs was 17.95, with a minimum of five from

the sheltered side of Fsar East and a maximum of 28 from the sheltered side of Abu Romah. Rarefaction curves did not reach an asymptote for any region, indicating that not all present species were found (Fig. 4).

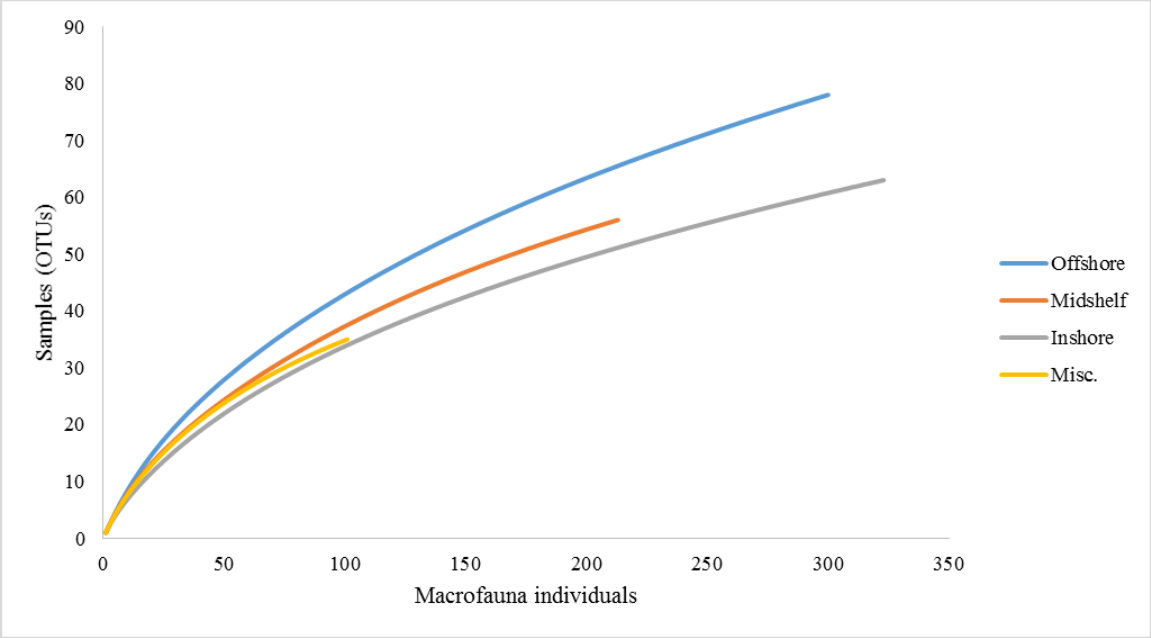

Fig. 4 Individual-based rarefaction curves representing the sampling effort at all regions. Data were produced using EstimateS and the number of OTUs present was plotted vs. estimated diversity.

PERMANOVA analysis using the Bray-Curtis similarity index computed a significant difference between macrofaunal communities of *S. carteri* of different regions (offshore, midshelf, inshore, misc., p=0.021), but not of different sides of the reefs (exposed, sheltered, p=0.155). The same analysis using the Jaccard index computed a significant difference for both ecological gradients (cross-shelf, p=0.020; exposure, p=0.016). Focusing on the Bray-Curtis similarity index, the two-dimensional PCoA plot of PC1 vs. PC3 shows a region-based separation, particularly of inshore and misc. samples versus offshore and midshelf samples (Fig. 5). Bray-Curtis pairwise

comparisons (Table 4) show a similarity value lower than 0.1 for 28 pairs of collection sites, including ten inshore/offshore, six midshelf/offshore, and six inshore/midshelf. Binary Jaccard pairwise comparisons are also shown in Table 4.

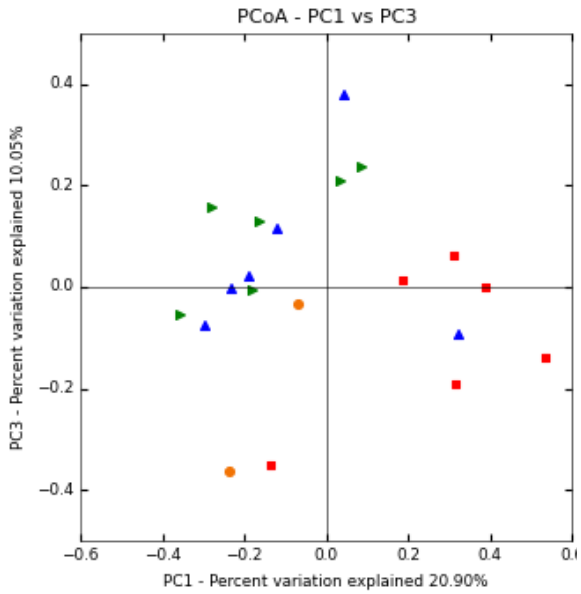

Fig. 5 Two-dimensional PCoA plot of the pooled *Stylissa carteri* samples, depicting similarity in community composition based on OTU relative abundance using the Bray-Curtis diversity index. Data were produced using QIIME. (Red squares=inshore reefs, orange circles=misc. reef (Qita' Al-Girsh), blue triangles=midshelf reefs, green triangles=offshore reefs).

Table 4 Pairwise comparisons of the collection sites. The bottom left portion is based on relative abundance of OTUs (Bray-Curtis diversity index) and the top right portion is based on OTU presence (binary Jaccard diversity index) in QIIME. The numbers shown represent the fraction of similarity between each pair. Pairs with a similarity value equal to or greater than 0.5 are lightly shaded and those with a similarity value equal to or less than 0.1 are more prominently shaded. Site abbreviations follow Table 1.

	AR.E	AR.S	SN.E	SN.S	AM.E	AM.S	QG.E	QG.S	AF.E	AF.S	UK.E	UK.S	AH.E	AH.S	TN.E	TN.S	TS.E	TS.S	FE.E	FE.S
AR.E	-	0.166667	0.166667	0.148148	0.151515	0.148148	0.193548	0.142857	0.193548	0.076923	0.208333	0.125	0.4	0.26087	0.2	0.217391	0.066667	0.083333	0.137931	0.117647
AR.S	0.188679	-	0.139535	0.184211	0.106383	0.153846	0.108696	0.210526	0.085106	0.2	0.102564	0.205882	0.166667	0.228571	0.219512	0.235294	0.069767	0.152174	0.27027	0.064516
SN.E	0.272727	0.142857	-	0.1875	0.125	0.151515	0.222222	0.114286	0.128205	0.166667	0.058824	0.133333	0.166667	0.090909	0.102564	0.166667	0.083333	0.069767	0.081081	0
SN.S	0.291667	0.275862	0.296296	-	0.078947	0.214286	0.142857	0.206897	0.25	0.192308	0.066667	0.25	0.192308	0.142857	0.147059	0.148148	0.060606	0.105263	0.16129	0.047619
AM.E	0.202532	0.136054	0.141176	0.089888	-	0.108108	0.119048	0.105263	0.068182	0.055556	0.083333	0.057143	0.151515	0.181818	0.121951	0.151515	0.05	0.088889	0.075	0.074074
AM.S	0.141414	0.203593	0.095238	0.146789	0.157143	-	0.111111	0.25	0.142857	0.24	0.066667	0.111111	0.148148	0.142857	0.147059	0.192308	0.09375	0.105263	0.2	0.1
QG.E	0.28125	0.166667	0.314286	0.27027	0.190476	0.112	-	0.171429	0.210526	0.088235	0.1875	0.16129	0.233333	0.1875	0.153846	0.193548	0.051282	0.090909	0.105263	0.12
QG.S	0.186667	0.237762	0.148148	0.282353	0.103448	0.161765	0.19802	-	0.138889	0.142857	0.1	0.107143	0.185185	0.1	0.212121	0.185185	0.125	0.102564	0.15625	0.095238
AF.E	0.290909	0.097561	0.229508	0.307692	0.083333	0.103448	0.271605	0.152174	-	0.15625	0.1875	0.125	0.233333	0.151515	0.216216	0.15625	0.078947	0.090909	0.135135	0.037037
AF.S	0.185185	0.344262	0.233333	0.375	0.063158	0.156522	0.2	0.241758	0.197183	-	0.074074	0.173913	0.12	0.16	0.16129	0.217391	0.066667	0.147059	0.137931	0
UK.E	0.280702	0.128	0.063492	0.119403	0.163265	0.118644	0.26506	0.12766	0.189189	0.109589	-	0.217391	0.26087	0.25	0.121212	0.26087	0.1	0.111111	0.096774	0.176471
UK.S	0.181818	0.321429	0.2	0.259259	0.070588	0.07619	0.171429	0.123457	0.163934	0.266667	0.15873	-	0.173913	0.217391	0.166667	0.227273	0.107143	0.1875	0.142857	0.125
AH.E	0.478261	0.263158	0.269231	0.392857	0.16092	0.11215	0.361111	0.240964	0.31746	0.354839	0.276923	0.192308	-	0.26087	0.2	0.272727	0.103448	0.147059	0.137931	0.117647
AH.S	0.225352	0.258993	0.103896	0.123457	0.196429	0.333333	0.164948	0.074074	0.159091	0.114943	0.177778	0.181818	0.227848	-	0.233333	0.45	0.137931	0.176471	0.172414	0.176471
TN.E	0.190476	0.323699	0.108108	0.208696	0.150685	0.13253	0.259542	0.521127	0.196721	0.247934	0.209677	0.126126	0.283186	0.15942	-	0.16129	0.081081	0.119048	0.322581	0.038462
TN.S	0.24	0.220339	0.178571	0.133333	0.241758	0.216216	0.289474	0.137931	0.179104	0.181818	0.521739	0.25	0.275862	0.313253	0.205128	-	0.230769	0.258065	0.222222	0.1875
TS.E	0.088235	0.073529	0.081081	0.051282	0.146789	0.093023	0.148936	0.095238	0.094118	0.166667	0.183908	0.108108	0.105263	0.09901	0.118519	0.3	-	0.131579	0.088235	0.095238
TS.S	0.1	0.189189	0.069767	0.088889	0.181818	0.156028	0.169811	0.08547	0.103093	0.166667	0.363636	0.209302	0.136364	0.247788	0.14966	0.478261	0.218182	-	0.189189	0.153846
FE.E	0.137931	0.193548	0.107527	0.14433	0.109375	0.243243	0.19469	0.145161	0.153846	0.116505	0.320755	0.107527	0.147368	0.116667	0.220779	0.363636	0.136752	0.387597	-	0.090909
FE.S	0.12766	0.034783	0	0.035088	0.136364	0.092593	0.219178	0.047619	0.0625	0	0.515152	0.075472	0.109091	0.1	0.105263	0.474576	0.181818	0.494382	0.520833	-

Visualization of the taxa (labeled by phyla) present at each collection site shows no trends in absolute abundance (Fig. 6). However, the visualization of relative abundance is more interesting (Fig. 7). Echinodermata, particularly brittle stars, constitute a larger proportion of sponge-associated communities inshore. Similarly, Chordata are found at five of the six inshore sites and only two midshelf sites and one site of each remaining region. Mollusca are found only at the miscellaneous reef (Qita' Al-Girsh) and the exposed sides of the three inshore reefs. The only Cnidaria species is from the offshore region, along with the two sea urchins found in this study.

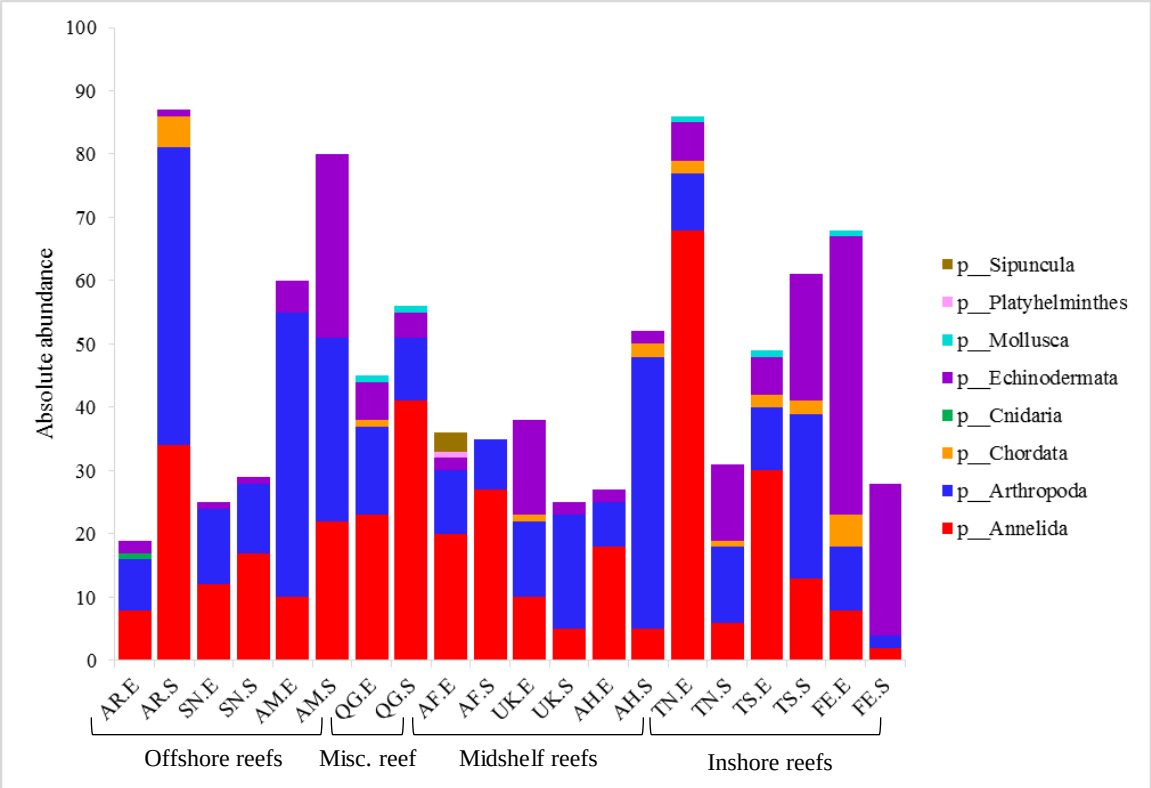

Fig. 6 Absolute abundance of macrofauna individuals found in pooled *Stylissa carteri* samples, categorized by phyla. Collection sites are organized in order of the cross-shelf gradient, from offshore to inshore. Site abbreviations follow Table 1.

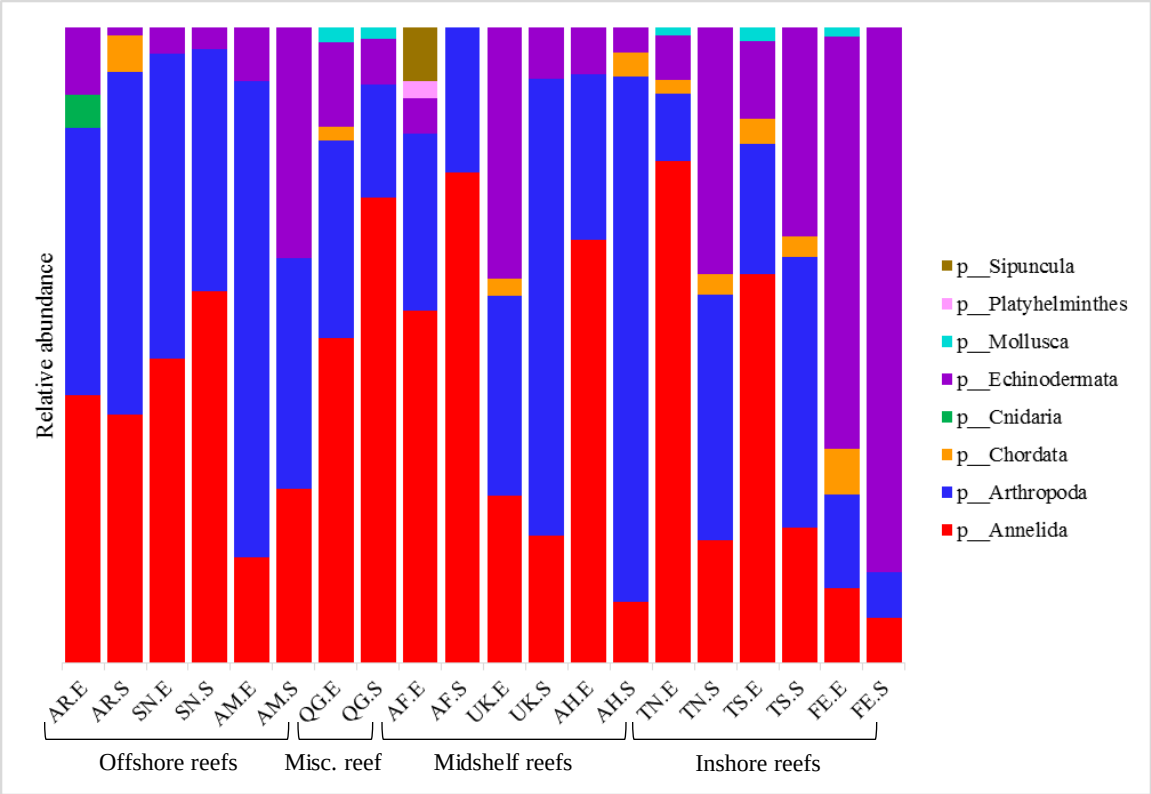

Fig. 7 Relative abundance of macrofauna found in pooled *Stylissa carteri* samples, categorized by phyla. Collection sites are organized in order of the cross-shelf gradient, from offshore to inshore. Site abbreviations follow Table 1.

3.4 Species-Based Patterns

PERMANOVA analysis using both the Bray-Curtis similarity index and the Jaccard index computed a significant difference between macrofaunal communities of the seven sponge species collected from the sheltered side of Al-Fahal (p=0.001). Focusing on the Jaccard index, two-dimensional PCoA plots (Fig. 8a-c) show a clear distinction of *Xestospongia testudinaria*, *Hyrtios* sp., and *Crella* sp. samples. *Theonella swinhoei* and *S. carteri*, as well as *Callyspongia* sp. and *Chalinula novo* appear to be grouped closer together. Binary Jaccard pairwise comparisons show a similarity value lower than 0.1 for all but one pair of species: *Callyspongia* sp./*Chalinula novo*, with a value of 0.3125

(Table 5). The UPGMA-based clustering tree further supports these trends with the four or five sponges of each species grouped together (Fig. 9). *Callyspongia* sp. and *Chalinula novo* differentiate into separate clusters from the same branch. Bray-Curtis pairwise comparisons are also shown in Table 5.

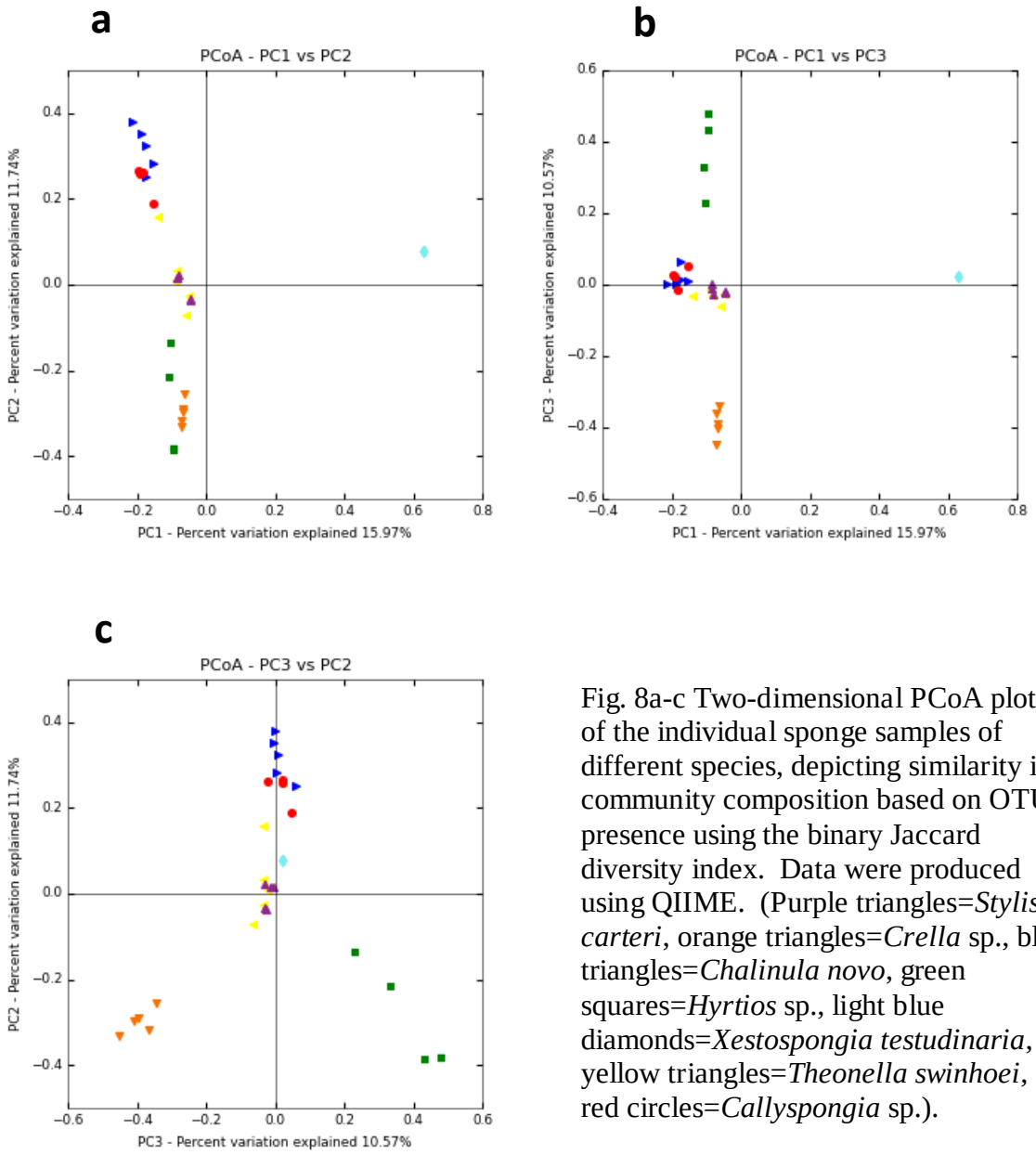

Fig. 8a-c Two-dimensional PCoA plots of the individual sponge samples of different species, depicting similarity in community composition based on OTU presence using the binary Jaccard diversity index. Data were produced using QIIME. (Purple triangles=*Stylissa carteri*, orange triangles=*Crella* sp., blue triangles=*Chalinula novo*, green squares=*Hyrtios* sp., light blue diamonds=*Xestospongia testudinaria*, yellow triangles=*Theonella swinhoei*, red circles=*Callyspongia* sp.).

Table 5 Pairwise comparisons of the sponge species on the sheltered side of Al-Fahal. The bottom left portion is based on relative abundance of OTUs (Bray-Curtis diversity index) and the top right portion is based on OTU presence (binary Jaccard diversity index) in QIIME. The numbers shown represent the fraction of similarity between each pair. The only pair with a similarity value greater than 0.1 (0.3125) is lightly shaded. Sponges are labeled by genus.

	Stylissa	*Hyrtios*	*Chalinula*	*Crella*	*Xestospongia*	*Theonella*	*Callyspongia*
Stylissa	-	0.0869565	0.0526316	0	0	0.05	0.0740741
Hyrtios	0.0130293	-	0.0625	0.0416667	0	0.0540541	0.04
Chalinula	0.0007859	0.0007189	-	0	0	0.0625	0.3125
Crella	0	0.0040733	0	-	0	0.05	0
Xestospongia	0	0	0	0	-	0	0
Theonella	0.0056899	0.0085106	0.0012587	0.0045096	0	-	0.075
Callyspongia	0.0610687	0.0163043	0.0076746	0	0	0.0183246	-

Visualization of the taxa (labeled by phyla) present in each sponge species shows clear distinguishing features (Fig. 10). The macrofaunal communities of *X. testudinaria* include only Annelida. *Chalinula novo* and *T. swinhoei* are also dominated by Annelida specimens, which make up 98.8-100 percent and 85.7-98.5 percent of their communities, respectively. *Stylissa carteri* and *Hyrtios* sp. are more variable with 71.4-100 percent and 53.4-94.3 percent Annelida, respectively. *Crella* sp. is dominated by Arthropoda specimens, which constitute 92.4-100 percent of its macrofaunal communities. *Callypongia* sp. contains a much lower abundance of Arthropoda, but they are still responsible for 75.0-100 percent of the communities. Rarefaction plots indicate that *X. testudinaria* and *Chalinula novo* are very well sampled. However, the curves representing the other sponge species never level off, indicating there are more macrofaunal species to be found (Fig. 11).

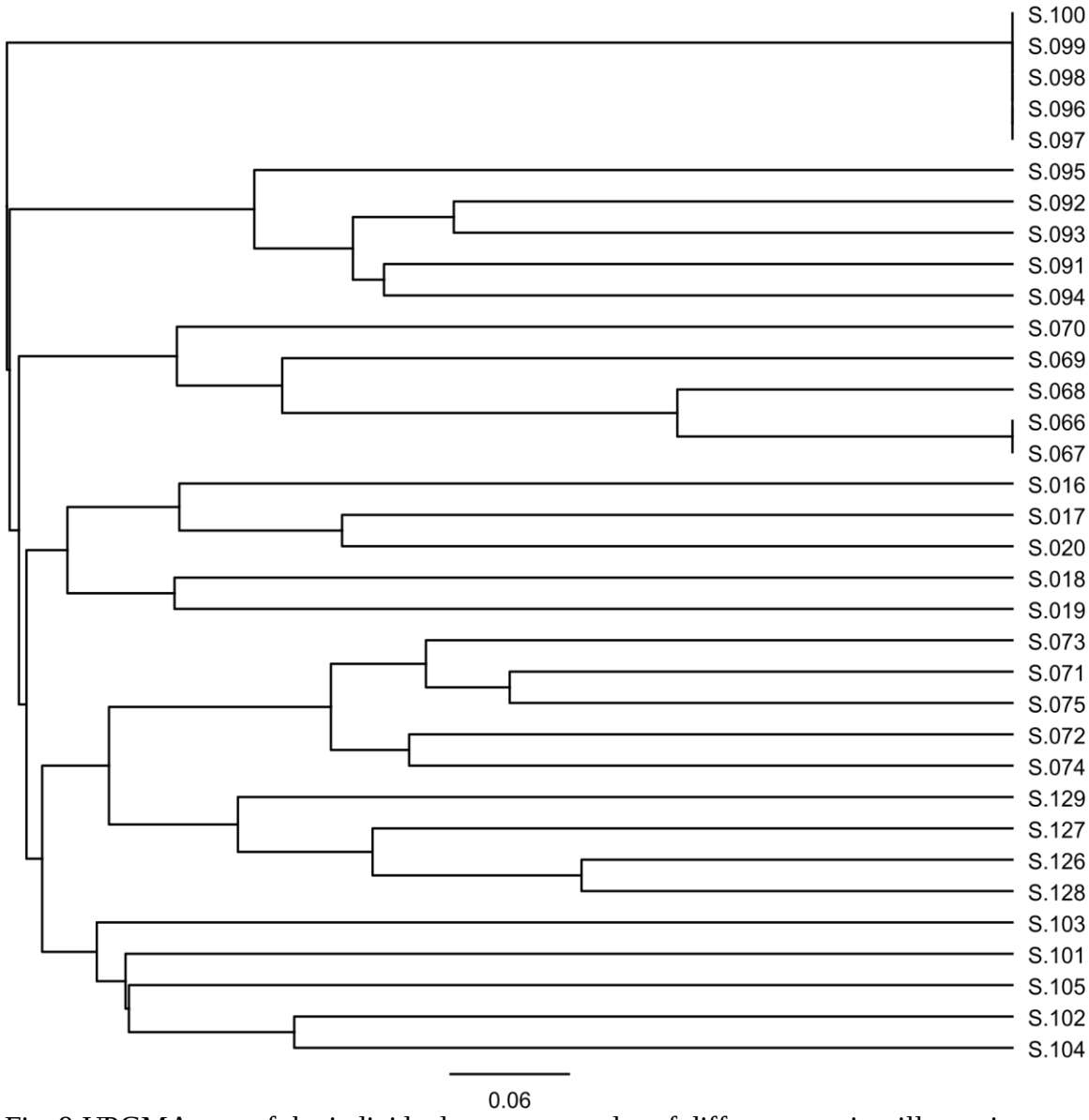

Fig. 9 UPGMA tree of the individual sponge samples of different species, illustrating similarity in community composition based on OTU presence (binary Jaccard diversity index). Figure was produced using QIIME. Sponge identifications are shown in Table 2.

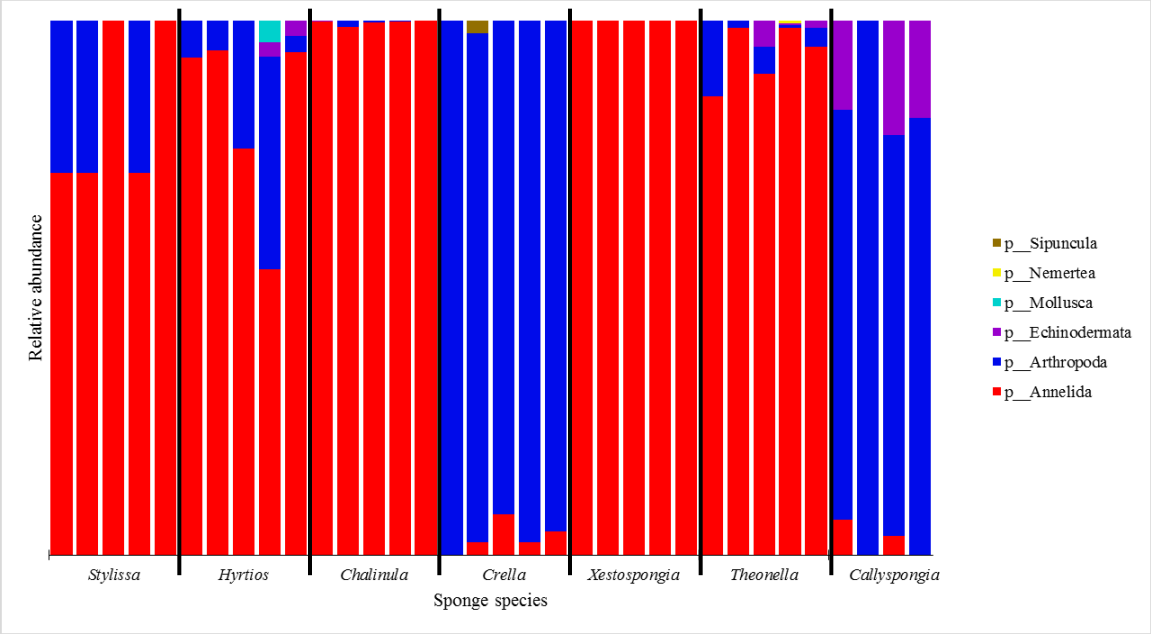

Fig. 10 Relative abundance of macrofauna found in the individual sponge samples of different species, categorized by phyla. Sponges are labeled by genus.

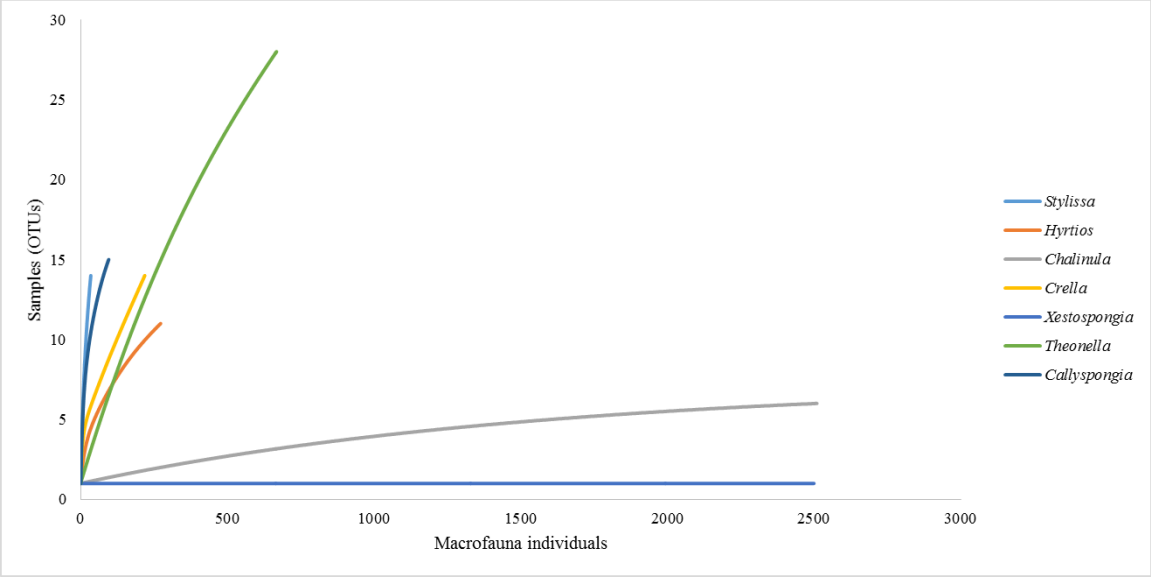

Fig. 11 Individual-based rarefaction curves representing the sampling effort of all sponge species, labeled by genus. Data were produced using EstimateS and the number of OTUs present was plotted vs. estimated diversity.

Of the 72 macrofaunal OTUs found in the sponges on the sheltered side of Al-Fahal, 47 are unique to only one sponge species (Table 6). For the purposes of this study, we will hereto refer to these OTUs as being species-specific. After removing singletons, there are still 24 species-specific OTUs divided among the seven species of sponges. *Stylissa carteri* and *Callyspongia* sp. are not dominated by any one OTU. *Crella* sp. contains four species-specific, non-singleton OTUs (56, 6, 26, 42), which are all arthropods, and is dominated by all four. *Hyrtios* sp. and *T. swinhoei* each contain four species-specific, non-singleton OTUs as well, but are each dominated by only one (OTUs 58 and 23, respectively), which are both annelids. *Xestospongia testudinaria* contains one species-specific, non-singleton OTU (58), which is an annelid and the species' only inhabitant. *Chalinula novo* also has only one OTU (137), which dominates its macrofaunal communities, but it is not certain whether this annelid species is distinct and species-specific. These extremely abundant worms were placed into a distinct classification because sequencing was not successful. Based on the species-specific, dominant OTUs present in the other sponge species, it is assumed that there is a similar situation for *Chalinula novo*.

Table 6 Summary of the OTUs found in the pooled samples of different sponge species from the sheltered side of Al-Fahal. Data for each sponge species include the total number of macrofaunal OTUs, the number of species-specific OTUs, the number of species-specific OTUs without singletons, the number of community-dominating, species-specific OTUs, and the phylum of the latter. The results for *Chalinula novo* are assumed based on visual observations and the results from the other sponge species.

Species	# OTUs	# unique	Non-singletons	Dominant	Taxa
Stylissa carteri	14	10	6	-	-
Crella sp.	14	9	4	OTUs 6, 26, 42, 56	Arthropoda
Chalinula novo	6	1	1	OTU 137	Annelida
Hyrtios sp.	11	7	4	OTU 58	Annelida
Xestospongia testudinaria	1	1	1	OTU 39	Annelida
Theonella swinhoei	28	14	4	OTU 23	Annelida
Callyspongia sp.	15	5	4	-	-

DISCUSSION

From a total of 1399 successfully-sequenced macrofauna individuals, 185 OTUs were distinguished. Only 16 of these OTUs received a species-level match in GenBank, indicating the large amount of work still needed to characterize this component of Red Sea macrofauna. Region-based diversity shows a significant difference between the macrofaunal communities associated with *Stylissa carteri* along the cross-shelf gradient, as hypothesized. The inshore reefs seem particularly different from the other regions. However, there is no significant difference shown between the exposed and sheltered sides of the reefs. Species-based diversity shows a significant difference between the macrofaunal communities associated with the seven sponge species from the sheltered side of Al-Fahal, as predicted. Five of the sponge species contain one or more community-dominating, species-specific (within this study) OTUs, indicating the possibility of host-specificity.

4.1 Region-Based Distinction

The significant change in macrofaunal communities of *S. carteri* across an offshore-inshore gradient may be attributed to a number of environmental factors. Observations show that offshore reefs in coastal Red Sea waters are normally characterized by vertical reef walls, as are some midshelf reefs. This may contribute to the close grouping of offshore and midshelf samples and their similar phyla-based, relative abundance taxa compositions. Inshore reefs of the central Red Sea tend to be much shallower and without prominent wall structures. The vertical structure of reef walls typically causes less light availability than a horizontal substrate (Bell and Barnes 2000b), along with less sedimentation. Sediments collect much more readily on a flat

surface, especially with the lower rate of water flow at inshore sites. The offshore sites are subject to stronger currents and higher wave action because they lack the protection of reef structures farther from shore. This is only made more prominent by the vertical structure of the reef walls. Midshelf reefs have some protection from the offshore sites, while inshore reefs have twice the protection and the benefits of more horizontal substrates. Whereas reef walls receive the full force of wave action and currents, flat reef tops have protrusions such as coral heads that may slow down the flow of water.

Stylissa carteri abundance in the study area generally follows the offshore-inshore gradient as well. The sponges collected at the lower end of the depth range tend to come from exposed and offshore sites due to the lower abundance of *S. carteri* at the average survey depth of ten meters in these regions. *Stylissa carteri* individuals tend to have a basal attachment of relatively small diameter, which may make it difficult to grow on a vertical reef structure (Bell and Barnes 2000a, Bell and Barnes 2000b). The small base may not be able to support the weight of the sponge growing outward from the wall, which would be accentuated by any sediment collection. Sedimentation rates may also influence sponge abundance. McClanahan and Obura (1997) found a higher abundance of sponges in areas with more sediment, which supports the observation of higher numbers of *S. carteri* with closer proximity to shore. Wilkinson and Cheshire (1989) attribute a lower biomass of sponges offshore to a lower level of organic matter, which may be carried away by the stronger flow of water.

The higher abundance of brittle stars and fish at inshore sites may be influenced by the more sheltered environment, horizontal substrate, or the occurrence of sponges at shallower depths. The higher abundance of organic matter inshore (Wilkinson and

Cheshire 1989) may be beneficial to the filter-feeding brittle stars that use sponges as an indirect food source. The trend in fish associated with *S. carteri* is supported by Pearse (1950), who found a greater variety of fishes associated with sponges in a shallow, enclosed sound of Bimini as opposed to the open ocean of the Tortugas. The molluscs found only at the miscellaneous reef (Qita' Al-Girsh) and the exposed sides of the inshore reefs are most likely affected by environmental conditions as well. Mastaller et al. (1978) found that molluscs in the waters of Port Sudan showed a lower abundance in areas of low water exchange, high sedimentation, and turbid waters. Qita' Al-Girsh is a small reef surrounded by deep water, so molluscs in this location, as well as on the exposed sides of the inshore reefs, would be subject to a high flow of water with little sedimentation and low turbidity. With these preferences met, they may favor the protection of the inshore reefs over the open nature of the offshore reef walls. The absolute abundance of macrofauna along the cross-shelf gradient has not yet been standardized to sponge mass, but the information to do so has been collected. The abundances will be standardized for future use.

Although there is a substantial difference in reef morphology, wave action, and various other environmental characteristics between reefs of the central Red Sea, little, if anything, is known about the relationships between *S. carteri* and its macrofauna. Location preferences may only be assumed. It may be true that some of the patterns observed are coincidental and more extensive sampling is required.

4.2 Species-Based Distinction

The distinct macrofaunal communities of the seven sponge species from the sheltered side of Al-Fahal may be attributed to the sponges' relation to one another.

Three of the sampled sponge species (*Chalinula novo*, *Xestospongia testudinaria*, and *Callyspongia* sp.) are of the order Haplosclerida. *Chalinula novo* and *Callyspongia* sp. are closely clustered in the OTU presence-based PCoA plots and the UPGMA tree, although *X. testudinaria* is very distinct. In addition, *Chalinula novo* and *Callyspongia* sp. are the only pair of sponge species with a Jaccard similarity value greater than 0.1 (0.3125). Looking at the individual-based rarefaction curves, *Chalinula novo* and *X. testudinaria* have very similar patterns. *Callyspongia* sp., on the other hand, has a different slope angle than the other two species of the same order.

Differences in macrofaunal communities may also be affected by the sponges' microbiota. Two of the study species from Al-Fahal (*S. carteri* and *Crella* sp.) have been described as LMA sponges (Giles et al. 2013) and three (*Hyrtios* sp., *Theonella swinhoei*, and *X. testudinaria*) have been described as HMA sponges (Yahel et al. 2003, Lee et al. 2011, Glosckner et al. 2014). *Stylissa carteri* is not dominated by any one macrofaunal OTU, while *Crella* sp. is dominated by arthropods. Each of the three HMA sponges is dominated by one species-specific annelid OTU. The dense tissues and small chambers of sponges with high bacterial abundances (Vacelet and Donadey 1977) may make them especially suitable for annelid symbionts of very small sizes. The less dense tissues of the LMA sponges may allow for a larger variety of macrofauna to gain access to the aquiferous systems. Weisz et al. (2008) found that LMA sponges exhibit an even lower tissue density inshore than offshore, which may be a contributing factor to the differences in *S. carteri* across all study sites.

The domination of each HMA sponge by a single OTU may be influenced by more than just the abundance of microbiota. It is possible that sponge chemicals play a

role in macrofauna colonization as well. Studies of bacterial communities have indicated that they play a role in the defense of their sponge hosts through production of toxins (Bultel-Ponce et al. 1999). For example, the cytotoxic effect of *T. swinhoei* tissue may be explained by the high concentration of cyanobacteria and filamentous endosymbionts (Magnino et al. 1999), which contain a unique β-amino acid (Bewley et al. 1996). This toxic compound may inhibit colonization of the sponge by many macrofaunal species, but have no negative effect on the one dominant annelid OTU discovered in the sponge's canals (Magnino et al. 1999). Secondary metabolites are described as the primary way sponges ward off predators (Wilson et al. 1999, Waddell and Pawlik 2000). However, the *T. swinhoei* samples collected in this study harbored the greatest diversity of macrofaunal communites, indicating further reasons for the observed patterns. Little is known on the subject of species-specific associations, warranting further study.

CONCLUSIONS

Sponges host a wide array of epifauna, infauna, and microbiota, acting as living hotels. This study used DNA barcoding techniques to examine the macrofaunal communities of central Red Sea sponges between regions and between species to gain knowledge of how these communities change on a fine scale. Significant differences were found on a regional basis along the cross-shelf gradient and on a species basis at the sheltered side of Al-Fahal, with several sponge taxa exhibiting species-specific symbiotic relationships. Abundance of *Stylissa carteri* followed the cross-shelf gradient as well, increasing from offshore to inshore reefs. A variety of physical, biological, and chemical factors may contribute to the findings, with many relating to one another. Reef

morphology and position affect light availability and sedimentation. Water flow affects turbulence and abundance of organic matter. Sponge morphology and microbial abundance, via differences in mesohyl density between LMA and HMA sponges, may affect ease of habitation. There is a network of factors contributing to the variation of macrofaunal communities of sponges and further study is required to understand species-specificity and regional differences. As shown by the low fraction of species-level matches of OTUs, there is still much to learn about the organisms that live in sponges in a general sense. As other space competitors in coral reef environments decline with the continuation of climate change and ocean acidification, understanding the ecology of sponges and their role as hosts to macrofauna and microbiota may become increasingly important.

APPENDIX 1

Table showing the number of macrofauna individuals of each operational taxonomic unit (OTU) found within each sponge sample. Taxonomic identifications to the phylum level are provided for each OTU.

This is an electronic supplemental file. It can be found in the KAUST Repository and

Digital Archive.

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