Coral Reefs
https://doi.org/10.1007/s00338-024-02568-8

REPORT

The impact of coral reef habitat and microbial abundance status on sponge-associated prokaryotic communities

D. F. R. Cleary[1] · N. C. M. Gomes[1] · R. Rappe-Ambo[2] · N. J. de Voogd[3,4]

Received: 25 September 2023 / Accepted: 9 September 2024

Abstract Sponges are one of the oldest lineages of animals on Earth and play key roles in shaping marine ecosystems. They are diverse, with more than 9600 species known to science, and come in a wide range of shapes, sizes, and colours. Sponges are, furthermore, known to host diverse communities of microbial symbionts, which play important roles in their physiology and ecology. In the present study, we sampled prokaryotic communities from 24 sponge species inhabiting coral reef flat and slope habitats off the coast of SW Celebes (Indonesia) in addition to sediment and seawater. The prokaryotic profiles of several sponge species were characterised for the first time. In line with previous studies, we revealed pronounced variation in diversity and composition among species with high microbial abundance (HMA) or low microbial abundance (LMA) status playing an important role in structuring prokaryotic communities across host sponge species. In addition to this, reef habitat (flat *versus* slope) also played a significant role in structuring prokaryotic communities. Most species in the reef slope habitat housed prokaryotic communities with a consistent profile of several cyanobacterial and prokaryotic OTUs, whereas these OTUs were largely absent from sponges inhabiting the reef flat habitat. Instead, they tended to house highly abundant bacterial populations related to the *Synechococcus spongiarum* group. We propose that specific strains of *S. spongiarum* may play a key role in enabling their host sponges to survive in an, otherwise, inhospitable environment (e.g., high irradiance and temperature) and, thus, help to explain differences in sponge composition between coral reef flat and slope habitats.

Keywords 16S · Bacteria · Indonesia · Porifera

Introduction

Depth plays a crucial role in structuring communities of marine organisms (Kahng et al. 2010; Lecchini et al. 2003). Environmental factors affected by depth include light availability, temperature, productivity, and water movement (Carmack et al. 2004; Leichter et al. 2007; Rio et al. 2014). As depth increases, the amount and quality of light changes due to the absorption and scattering by seawater. This light attenuation gradient profoundly affects the distribution and abundance of photosynthetic-dependent organisms and their symbionts, for example, corals and their symbiotic zooxanthellae (Mass et al. 2010). It also determines the depth limits at which photosynthesis can occur, restricting the vertical distribution of organisms and the zonation of benthic communities (Brown and Thatje 2014; Falkowski 1994).

D. F. R. Cleary, N. C. M. Gomes and N. J. de Voogd have contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00338-024-02568-8.

✉ D. F. R. Cleary
cleary@ua.pt

✉ N. J. de Voogd
n.j.de.voogd@biology.leidenuniv.nl

1 Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

2 Faculty of Marine Science and Fisheries, Department of Marine Science, Hasanuddin University, Makassar, Indonesia

3 Naturalis Biodiversity Center, Leiden, The Netherlands

4 Institute of Biology (IBL), Leiden University, Leiden, The Netherlands

Published online: 30 October 2024

As with light, there is a strong relationship between depth and temperature. As depth increases, water temperature decreases due to various factors such as reduced sunlight penetration and mixing with colder water masses. Temperature, furthermore, is a critical factor affecting physiological processes, growth rates, and reproduction of marine organisms with different species having adapted to specific temperature ranges (de Souza et al. 2023; Roberts et al. 2019; Thresher et al. 2014). Likewise, water movement, including currents and waves, is another important factor influenced by depth. Hydrodynamic forces can vary greatly with depth, affecting sedimentation rates, nutrient availability, and the removal of waste products. Water movement also plays a role in facilitating larval dispersal, which is crucial for connectivity and genetic exchange among benthic populations (Yahagi et al. 2017). As depth changes, water movement patterns can differ, leading to variations in community composition and structure. Previous studies have shown that depth significantly affects the composition of marine, benthic communities, including sponges and corals (Cleary et al. 2005; de Voogd et al. 2009; González-Murcia et al. 2022; van Soest et al. 2007). In addition to the above, organisms found in shallow water habitat may also inhibit recruitment by other organisms (Miller and Hay 1996).

In the present study, we assessed the effect of depth on communities of prokaryotes housed in sponges. To study this, sponges were sampled in reef flat (~ 1 m) and reef slope (> 3 m) habitats of the Spermonde Archipelago off the coast of Makassar, Southwest Celebes, Indonesia. Previous studies across a range of organisms including corals (Hoeksema 2012; Zhao et al. 2013), fishes (Meekan et al. 1995; Lecchini et al. 2003), molluscs (Zuschin et al. 2001), echinoderms (Mokady et al. 1996), and sponges (Bell and Smith 2004), have shown reef flats and slopes to house distinct communities with evident species-specific preferences. In addition to the above, sediment and seawater were also sampled.

Sponges are among the earliest branching metazoan lineages, or the earliest branching (Pick et al. 2010; Wainright et al. 1993). They are currently found across all types of aquatic ecosystems from the deep sea to shallow water, from fully marine to freshwater and from tropical to polar regions (van Soest et al. 2012). They also come in all shapes and forms from thinly-encrusting, bio-eroding taxa to large, massive species such as giant barrel sponges. Life spans vary from short lived to thousands of years (Bell and Barnes 2000; McGrath et al. 2018; Swierts et al. 2013). Water including dissolved organic matter (DOM), particulate organic matter (POM) and nutrients are drawn into the sponge body through openings known as ostia and transported throughout the sponge mesohyl. This process puts the sponges in intimate contact with the surrounding environment including contact with potentially harmful microorganisms such as pathogenic bacteria and viruses. Sponges have also been shown to play important roles in nutrient cycling (Maldonado et al. 2019; Pita et al. 2018; Southwell et al. 2008).

With respect to their microbial symbionts, sponges have been categorised as high microbial abundance (HMA) or low microbial abundance (LMA) based on the density of microbial cells they harbour with HMA sponges containing significantly more microbial cells per gram of tissue compared to LMA sponges (Vacelet and Donadey 1977; Reiswig 1981; Gloeckner et al. 2014). These categories are supported by differences in morphological and genetic traits between the two types. HMA species, for example, typically exhibit higher mesohyl densities and possess certain polyketide synthase genes, while LMA species have larger choanocyte chambers and higher pumping rates (Vacelet and Donadey 1977; Hochmuth et al. 2010). Studies have, furthermore, shown that HMA and LMA sponges tend to differ in their microbial diversity and composition with certain taxa, including Chloroflexi, Poribacteria, and Actinobacteria, indicative of HMA status, while Bacteroidetes and Firmicutes are more abundant in LMA sponges (Bayer et al. 2014; Moitinho-Silva et al. 2017).

In the present study, 24 sponge species were sampled belonging to 2 classes, 7 orders, and 12 families. The prokaryotic communities of most of these species were characterised for the first time. The sponge species sampled included both phototrophic and heterotrophic sponges in addition to demo- and calcareous sponges. We hypothesised that coral reef habitat (flat *versus* slope) and sponge microbial status would significantly predict sponge-associated prokaryotic composition. In addition to this, we compared diversity and composition of prokaryotic communities of sponges with those of sediment and seawater, and assessed the relative abundances of the most abundant phyla among the prokaryotic communities of sponges, sediment and seawater. We, furthermore, identified closely related organisms in GenBank to the most abundant OTUs in the present study.

Material and methods

Sampling

Sponges were sampled from shallow water, coral reef flat and slope habitats (Fig. 1) in the Spermonde archipelago, southwest Celebes, using a combination of snorkelling and SCUBA diving. A full list of the samples collected is presented in Supplementary Data 1. Once outside of the water, a section of sponge from each specimen was cut and immediately placed into a vial containing 96% alcohol. Care was taken to include surface and interior parts of each sponge specimen. In addition to sponge samples, sediment and water samples were collected. One litre

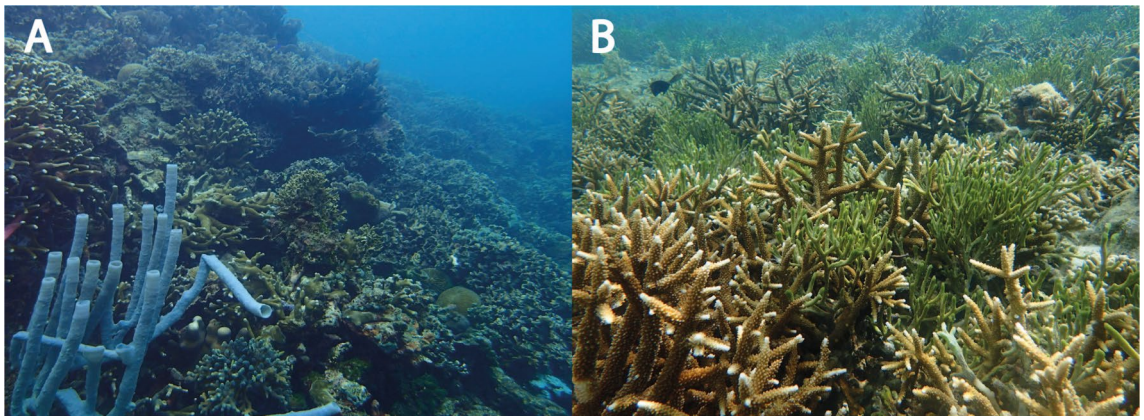

Fig. 1 Photographs of (**A**). Reef flat and (**B**). Reef slope habitats of the Spermonde Archipelago, Celebes Indonesia. The blue, tubular sponge in the foreground of A. is *Haliclona fascigera*. The green branching sponges in B. are specimens of *Halichondria cartilaginea*. Both photographs were taken by NJ de Voogd

of seawater was collected at ~ 1 m depth and subsequently filtered through a Millipore® White Isopore Membrane Filter (0.22 µm pore size); the filter was preserved in 96% ethanol. Sediment was collected by scraping surface sediment into a falcon tube. The vials were shipped to the Netherlands and stored in a -20 ºC freezer until DNA extraction. Samples from all species are stored at Naturalis Biodiversity Center, Leiden the Netherlands. A full list of all samples is presented in Supplementary Data 1.

The following sponge species were collected in coral reef slope habitat: *Acanthostrongylophora ingens* (Thiele 1899), *Petrosia hoeksemai* de Voogd and van Soest, 2002, *Petrosia nigricans* Lindgren 1897, *Xestospongia testudinaria* (Lamarck 1815), *Callyspongia biru* de Voogd 2004, *Haliclona fascigera* (Hentschel 1912), *Niphates olemda* (de Laubenfels, 1954), *Theonella swinhoei* Gray 1868, *Clathria basilana* Lévi 1961, *Clathria cervicornis* (Thiele 1903), *Clathria reinwardti* Vosmaer 1880, *Lamellodysidea herbacea* (Keller 1889), *Leucetta chagosensis* Dendy 1913, *Leucetta primigenia* Haeckel 1872, *Pericharax orientalis* van Soest and de Voogd 2015, and *Stylissa carteri* (Dendy 1889). The species *Neopetrosia chaliniformis* (Thiele 1899), *Xestospongia vansoesti* Bakus and Nishiyama, *Callyspongia samarensis* (Wilson 1925), *Haliclona cymaeformis* (Esper 1806), *Coelocarteria singaporensis* (Carter, 1883), *Phyllospongia foliascens* (Pallas 1766), and *Phyllospongia papyracea* (Esper 1806) were collected from coral reef flat habitat. The sponge species *Halichondria cartilaginea* (Esper 1797) was the only species sampled in both coral reef flat and slope habitat with two out of three specimens sampled in reef flat habitat. Pictures of selected sponge species are shown in Supplementary Fig. 1. Seven sponge species were classified as HMA and 17 as LMA based on TEM images of the sponge species in question (Cleary et al. unpublished data).

DNA extraction

DNA was extracted using the Qiagen DNeasy Powersoil extraction kit (Qiagen, Venlo, the Netherlands). The whole membrane filters, for water samples, were cut into small pieces and transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. Up to 250 mg of tissue was used from each sponge sample; tissue was taken from all sides of the specimen (outside to core, and if applicable top, middle and bottom of the sample). 250 mg of wet sediment was used for DNA extraction from sediment samples. The manufacturer's protocol was followed with the exception of vortex duration, which was divided into four steps of 90 seconds, turning the vortex adaptors around between each step in order to assure that all samples were equally vortexed. Sponge tissue was cut into small pieces using sterilised tweezers and scalpel blades and transferred to PowerBead Pro tubes containing ceramic and silica beads of different sizes. An extraction blank, in which no tissue was added to the PowerBead Pro tubes, was also included. The library preparation was conducted using a two-step PCR protocol for all samples in addition to two negative controls (mQ water instead of template DNA) and the extraction blank. For the first PCR, the V3-V4 regions of the 16S rRNA gene were targeted and amplified using the primers 314F/ S-D-Bact-0785-a-A-21 (5'-CCTACGGGNGGCWGCAG-3'/5'-GACTACHVGGGTATCTAATCC-3'; Klindworth et al. 2013) with added 5' Nextera transposase adaptors using the KAPA HiFi HotStart Ready Mix PCR Kit with a T100 Thermal Cycler (Bio-Rad, Hercules, CA, United States). The following PCR conditions were used: initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 30 s, followed by extension at 72 °C for 30 s. The final extension was carried out at 72 °C for 1 min. PCR success was confirmed

on an E-Gel™ (agarose gels at 2%), and the absence of amplification was validated for the negative controls and the extraction blank. PCR products were then cleaned with NucleoMag NGS-Beads (bead volume at 0.9 times the total volume of the sample, Macherey Nagel, Düren, Germany) using the VP 407AM-N 96 Pin Magnetic Bead Extractor stamp (V&P Scientific, San Diego, CA, United States). For the second PCR, the cleaned PCR products (1 µL each) were amplified and labelled using the MiSeq Nextera XT DNA library preparation kit (Illumina, San Diego, CA, United States) with the same thermal cycling scheme limited to 8 cycles. PCR products were then analysed with the Fragment Analyser Agilent 5300 using the DNF-910-33 dsDNA Reagent Kit (35–1500 bp) protocol (Agilent Technologies, Santa Clara, CA, United States) to confirm successful labelling of the DNA fragments. Negative controls and extraction blanks remained negative after this step. Pooling at equimolar concentration was performed with QIAgility 2 (Qiagen, Hilden, Germany). The final pool was then cleaned with NucleoMag NGSBeads, eluted in Milli-Q and the DNA concentration was verified using Tapestation 4150 (Kit HSD 5000, Agilent Technologies, Santa Clara, CA, United States). Paired-end sequence reads were generated with an Illumina MiSeq v3 PE300 platform at BaseClear B.V. (Leiden, Netherlands). FASTQ read sequence files were generated using bcl2fastq version 2.20 (Illumina). Initial quality assessment was based on data passing the Illumina Chastity filtering. Subsequently, reads containing PhiX control signal were removed using an in-house filtering protocol. In addition to this, reads containing (partial) adapters were clipped (up to a minimum read length of 50 bps). The second quality assessment was based on the remaining reads using the FASTQC quality control tool version 0.11.8.

Sequencing analysis

The 16S rRNA amplicon libraries were analysed using QIIME2 (version 2019.7; Bolyen et al. 2019). Raw data were imported yielding a demultiplexed 'qza' data file (artifact). The DADA2 plugin (Callahan et al. 2016) in QIIME 2 was subsequently used to trim sequences (final length 400 nt). The DADA2 analysis yielded output archives containing an OTU (at 100% sequence similarity and also known as amplicon sequence variant or 'ASV') table, denoising stats, and a fasta file of representative sequences. The feature-classifier plugin with the extract-reads method was then used with the i-sequences argument set to silva-138-99-seqs.qza. This was followed by the feature-classifier plugin with the fit-classifier-naive-bayes method and the i-reference-taxonomy method set to silva-138-99-tax.qza. Both silva-138 files can be obtained from https://docs.qiime2.org/2020.8/data-resources/?highlight=silva. The feature-classifier plugin was then used with the classify-sklearn method and the i-reads argument was set to the representative sequences file generated by the DADA2 analysis to produce a table with taxonomic classifications for all OTUs. Finally, mitochondria, chloroplasts, and Eukaryota were filtered out using the QIIME2 taxa plugin with the filter-table method. The OTU and taxonomy tables were later merged in R (R Core Team 2022). All OTUs unclassified at Domain and Phylum level were also removed. The OTU table is shown in Supplementary Data 2. Accession numbers of closely related organisms to selected OTUs were obtained using the NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). Sequences used in this study have been uploaded to the NCBI ShortRead Archive (BioProject nr: PRJNA865712).

Statistical analyses

Diversity and higher taxon abundance

Diversity indices were obtained using the rarefy and diversity functions from the vegan package in R (Oksanen et al. 2022). Evenness was calculated by dividing Shannon's H' by the log value of the number of OTUs in each sample. We tested for significant differences in the relative abundances of selected prokaryotic higher taxa, OTU richness, evenness, Shannon's H' and Fisher's alpha indices with habitat (reef flat *versus* slope) and microbial abundance status (HMA *versus* LMA) as predictors with an analysis of deviance using the glm function in R. For the diversity indices, we set the family argument to 'tweedie' using the tweedie function in R with var.power = 1.5 and link.power = 0 (a compound Poisson–gamma distribution). For the relative abundances of higher taxa, we set to family argument to quasibinomial.

Compositional analyses

Variation in composition was assessed with Principal Coordinates Analysis (PCO) using the phyloseq package in R (McMurdie and Holmes 2013) with the Bray–Curtis distance. The count data were first rarefied using the rarefy_even_depth function in phyloseq with the sample.size argument set to the minimum sample size ($n = 5748$). We tested for significant variation in OTU composition with habitat (reef flat *versus* slope) and microbial abundance status (HMA *versus* LMA) as predictors using the adonis function in vegan. The number of permutations was set at 999, all other arguments used the default values set in the function.

Results

After quality control, the dataset consisted of 984,755 sequences and 7640 OTUs. In terms of sequences, the most abundant phyla were Proteobacteria (396,825 sequences,

3226 OTUs), Cyanobacteria (243,567 sequences, 512 OTUs), Chloroflexi (79,630 sequences, 679 OTUs) and Actinobacteriota (66,803 sequences, 421 OTUs). The 50 most abundant OTUs are presented in Supplementary Data 3.

Evenness was significantly higher in HMA than LMA sponges and higher in slope than reef habitat; there was no significant interaction term (Fig. 2 and Table 1). OTU richness was significantly higher in HMA than LMA sponges and there was a significant interaction term with richness higher in LMA sponges from slope habitat than LMA sponges from reef flat habitat. Results of the Shannon's H' and Fisher's alpha indices reflected those of Evenness and OTU richness, respectively. Results for sponge species, sediment, and seawater are presented in Supplementary Fig. 2. Overall, evenness was highest in sediment and relatively high in seawater. There was also considerable variation in OTU richness among sediment samples.

The relative abundances of Proteobacteria, Cyanobacteria, and Bacteroidota were significantly greater in LMA than HMA sponges, while the relative abundances of Chloroflexi

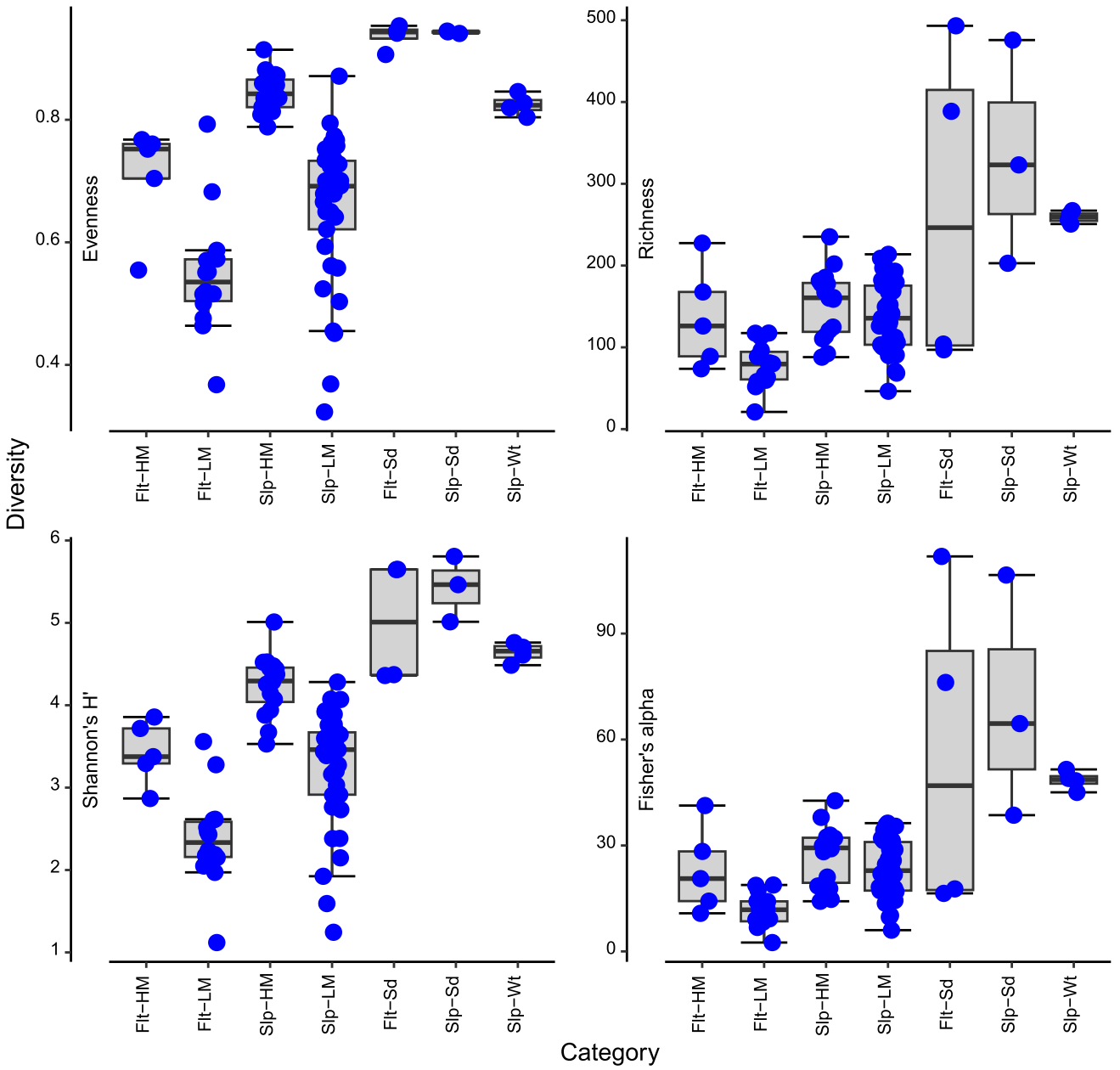

Fig. 2 Evenness, Richness, Shannon's H', and Fisher's alpha diversity indices for HMA (Flt-HM) and LMA (Flt-LM) sponges from reef flat habitat, HMA (Slp-HM) and LMA (Slp-LM) sponges from reef slope habitat, sediment from reef flat (Flt-Sd) and slope (Slp-Sd) habitats, and seawater from reef slope habitat (Slp-Wt)

Table 1 Results of GLM analyses for diversity indices and the relative abundances of the eight most abundant phyla and results of adonis analysis of OTU composition

Diversity and phylum relative abundance							
		Df	Deviance	Resid.Df	Resid.Dev	F	P
Evenness	NULL	71	2.71				
	Habitat	1	0.40	70	2.31	20.14	0.0000***
	Status	1	0.83	69	1.49	41.77	0.0000***
	Habitat:status	1	0.00	68	1.49	0.00	0.9684
Richness	NULL	71	124.69				
	Habitat	1	25.55	70	99.15	22.23	0.0000***
	Status	1	7.67	69	91.48	6.67	0.0120
	Habitat:status	1	6.37	68	85.11	5.54	0.0215*
Shannon's H'	NULL	71	9.68				
	Habitat	1	2.01	70	7.67	29.93	0.0000***
	Status	1	2.34	69	5.33	34.91	0.0000***
	Habitat:Status	1	0.05	68	5.28	0.71	0.4012
Fisher's alpha	NULL	71	70.16				
	Habitat	1	14.85	70	55.31	23.96	0.0000***
	Status	1	5.24	69	50.07	8.46	0.0049**
	Habitat:status	1	3.52	68	46.55	5.68	0.0200*
Proteobacteria	NULL	71	1870.10				
	Habitat	1	3.27	70	1866.80	0.20	0.6537
	Status	1	783.63	69	1083.20	48.65	0.0000***
	Habitat:status	1	18.10	68	1065.10	1.12	0.2928
Cyanobacteria	NULL	71	4139.50				
	Habitat	1	380.31	70	3759.10	12.68	0.0007***
	Status	1	1068.07	69	2691.10	35.62	0.0000***
	Habitat:status	1	337.64	68	2353.40	11.26	0.0013**
Chloroflexi	NULL	71	7054.80				
	Habitat	1	7.80	70	7047.00	0.05	0.8206
	Status	1	3545.80	69	3501.20	23.52	0.0000***
	Habitat:status	1	139.90	68	3361.30	0.93	0.3387
Actinobacteriota	NULL	71	2111.90				
	Habitat	1	2.85	70	2109.00	0.12	0.7336
	Status	1	36.15	69	2072.90	1.48	0.2275
	Habitat:status	1	24.26	68	2048.60	1.00	0.3220
NB1-j	NULL	71	6960.70				
	Habitat	1	1411.10	70	5549.60	35.68	0.0000***
	Status	1	1754.83	69	3794.80	44.37	0.0000***
	Habitat:status	1	1.74	68	3793.00	0.04	0.8343
Bacteroidota	NULL	71	1965.40				
	Habitat	1	139.57	70	1825.80	8.29	0.0053**
	Status	1	425.91	69	1399.90	25.29	0.0000***
	Habitat:status	1	4.41	68	1395.50	0.26	0.6105
Acidobacteriota	NULL	71	3526.00				
	Habitat	1	0.10	70	3525.90	0.00	0.9517
	Status	1	2304.17	69	1221.70	86.83	0.0000***
	Habitat:status	1	124.95	68	1096.80	4.71	0.0335*
Planctomycetota	NULL	71	3317.20				
	Habitat	1	779.71	70	2537.50	26.39	0.0000***
	Status	1	794.01	69	1743.40	26.87	0.0000***
	Habitat:status	1	128.34	68	1615.10	4.34	0.0409*

The independent predictor variables were habitat (reef versus slope) and status (HMA versus LMA). The interaction term (Habitat:Status) was included for each test. NULL referes to the deviance of the null model

Df, degrees of freedom; Resid.Df, residual degrees of freedom; F, F value; P, probability

*$0.01 < P < 0.05$, ** $0.001 < P < 0.01$, and *** $P < 0.001$

and Acidobacteriota were significantly greater in HMA sponges. Cyanobacterial relative abundance was, furthermore, significantly greater in reef flat sponges, whereas the reverse held for NB1-j, and Planctomycetota (Fig. 3 and Table 1). Results for sponge species, sediment, and seawater are presented in Supplementary Fig. 3 highlighting the considerable degree of variation among species. Proteobacterial abundance, for example, varied from 15.59% in *X. testudinaria* to 99.78% in *H. cymaeformis*. Proteobacterial abundance was, furthermore, higher in the reef slope inhabiting *S. carteri*, *N. olemda*, and *L. primigenia* than the reef flat inhabiting *C. singaporensis*, or reef slope inhabiting *P. hoeksemai*, *P. orientalis*, and *T. swinhoei*. Cyanobacterial relative abundance varied from 0.02% in *P. hoeksemai* to 63.89% in *L. herbacea*, both inhabitants of reef slope habitat. Cyanobacterial relative abundance was also depleted in the HMA sponges *P. hoeksemai*, *P. nigricans*, and *X. vansoesti* in addition to the aforementioned *H. cymaeformis*. Cyanobacterial relative abundance was relatively high in the reef flat species *N. chaliniformis*, *P. foliascens*, *C. singaporensis*, *C. samarensis*, and *P. papyracea* and the reef slope species *C. biru*, *H. fascigera*, and *C. basilana* in addition to the previously mentioned *L. herbacea*. Chloroflexi and Acidobacteriota abundances were relatively high in the reef flat inhabiting *C. singaporensis, N. chaliniformis*, and *X. vansoesti* and reef slope inhabiting *A. ingens*, *P. hoeksemai*,

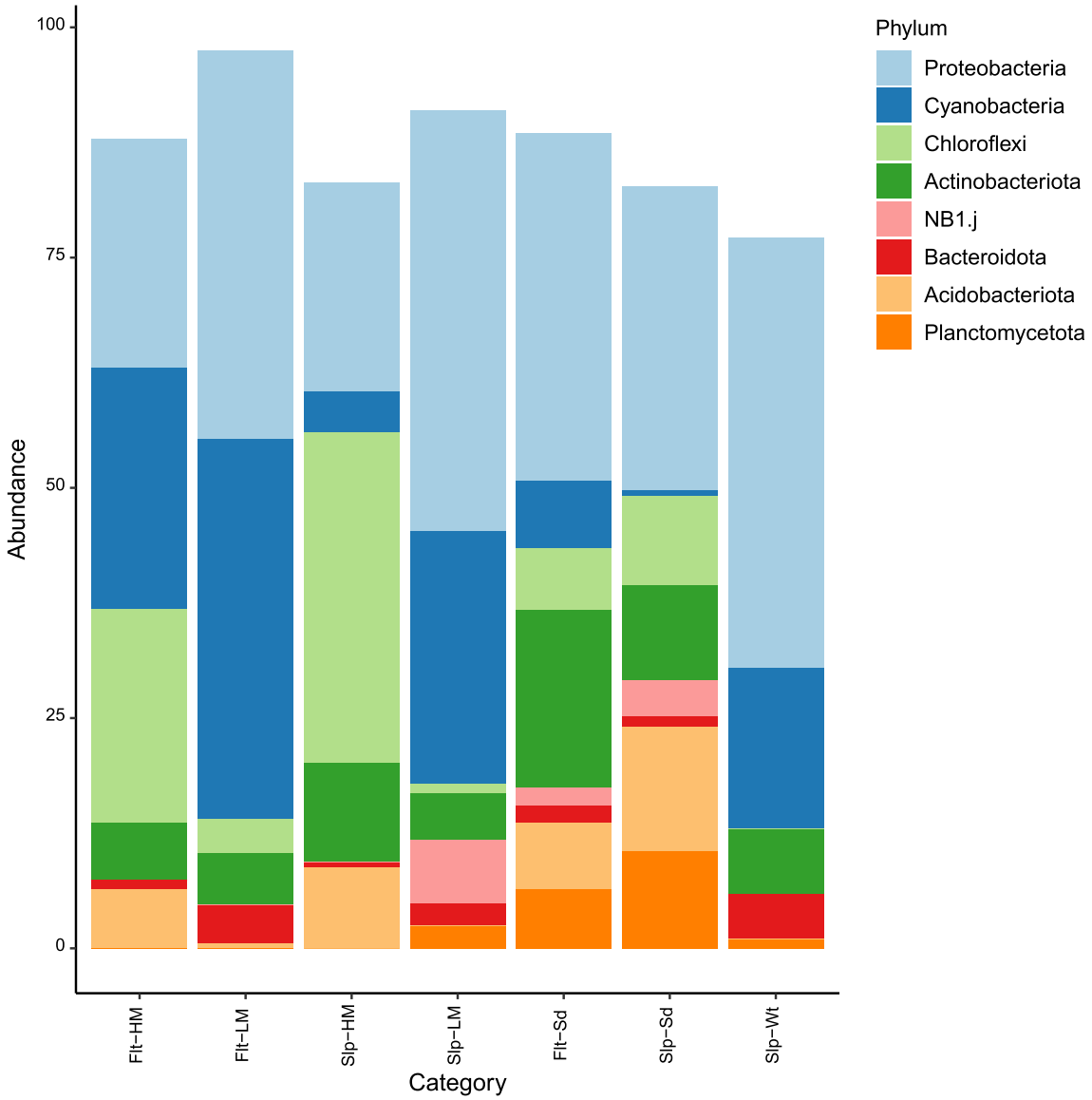

Fig. 3 Stacked barplots of the mean relative abundances of the eight most abundant phyla for HMA (Flt-HM) and LMA (Flt-LM) sponges from reef flat habitat, HMA (Slp-HM) and LMA (Slp-LM) sponges from reef slope habitat, sediment from reef flat (Flt-Sd) and slope (Slp-Sd) habitats, and seawater from reef slope habitat (Slp-Wt)

P. nigricans, *T. swinhoei,* and *X. testudinaria.* All of these, with the exception of *C. singaporensis*, were classified as HMA species. No Chloroflexi members were recorded in the reef flat inhabiting species *C. samarensis*, *H. cymaeformis*, *P. foliascens*, and *P. papyracea* or the reef slope inhabiting species *C. cervicornis*, *C. reinwardti*, and *L. herbacea*. Actinobacteriota members were recorded in all biotopes with the exception of *H. cymaeformis*, and in very low abundances in *C. samarensis*, *C. cervicornis*, *C. reinwardti* and *L. herbacea*. Actinobacteriotal relative abundance was highest in *P. papyracea* and sediment. Nb1-j members were only recorded in *P. hoeksemai*, *H. fascigera*, *C. singaporensis*, *L. herbacea*, *L. chagosensis*, *L. primigenia*, *P. orientalis*, and seawater at very low abundances. Nb1-j relative abundance was, by far, greatest in the sponge *S. carteri*. The difference in Nb1-j relative abundance due to habitat and HMA status is, thus, mainly due to this LMA species, which was only recorded in slope habitat highlighting the limitations of the present study. Seawater samples were characterised by high relative abundances of Proteobacteria, Cyanobacteria, Actinobacteriota, and Bacteroidota, whereas sediment samples had comparatively high abundances of Acidobacteriota and Acidobacteriota.

Both reef habitat ($F_{1, 68} = 6.57$, $R^2 = 0.076$, $P < 0.001$) and microbial abundance status ($F_{1, 68} = 8.03$, $R^2 = 0.093$, $P < 0.001$) were significant predictors of variation in OTU composition as was the interaction term ($F_{1, 68} = 4.12$, $R^2 = 0.048$, $P < 0.001$). Reef habitat, thus, explained 7.6% of the variation in OTU composition, while microbial status explained 9.3% and the interaction term 4.8%. In the PCO ordination (Fig. 4), the main axis separated water and LMA sponge samples from reef slope habitat at low axis-1 values from sediment, LMA sponge specimens from reef flat habitat, and HMA sponge specimens from both reef flat and slope habitats. A PCO ordination showing all sponge species is presented in Supplementary Fig. 4. One specimen of *T. swinhoei* clustered separate from the other specimens and closer to the LMA species from reef slope habitat. The reef slope species *L. herbacea*, in turn, clustered with reef flat sponge species. The second axis of variation separated the reef flat species *P. foliascens* and *P. papyracea* from the other reef flat sponge species.

The distribution of abundant OTUs differed markedly between LMA sponges from reef flat and slope habitats with few OTUs shared between both groups (Fig. 5). This was particularly the case for abundant cyanobacterial OTUs, which were often restricted to one or two reef flat species (Supplementary Fig. 5). For example, the dominant cyanobacterial OTUs in reef flat sponges consisted of OTUs 3 (*P. papyracea* and *P. foliascens*), 12 (*C. samarensis*), 13 (*N. chaliniformis*), 20 (*C. singaporensis*), and 44 (*C. singaporensis*). All of these were classified to the *Synechococcus spongiarum* group and were closely related (sequence similarities > 99%) to sequences in GenBank obtained from sponge species identified as *Phyllospongia foliascens* (as *Carteriospongia foliascens)*, *Haliclona* sp., *Aplysina fulva*, *Coelocarteria singaporensis*, and *Aplysina cauliformis*. Another OTU (OTU-78) classified to the *S. spongiarum* group was relatively abundant in the reef slope inhabiting *A. ingens* and had 100% sequence similarity to an organism in GenBank obtained from the sponge species *Aplysina cauliformis* (Supplementary Data 3).

In line with the results for Cyanobacteria, several abundant proteobacterial OTUs and a single abundant actinobacteriotal OTU were restricted to sponge species sampled from reef flat habitat. As with the cyanobacterial OTUs, these were also largely restricted to one or two sponge species, namely, OTUs 25 (*C. samarensis*), 30 (*H. cymaeformis*), 33 (*P. papyracea* and *P. foliascens*), 46 (*H. cartilaginea*), 53 (*P. papyracea* and *P. foliascens*), 77 (*H. cartilaginea*), 109 (*P. papyracea*), and 112 (*H. cartilaginea*) (Supplementary Fig. 5). Several of these OTUs were closely related (sequence similarity > 98%) to organisms in GenBank recorded from coral (*Porites lutea*) and sponge (*Callyspongia vaginalis*, *Carteriospongia foliascens*, and *Ircinia* sp.) host species. OTU-112, however, only had 94.6% sequence similarity to an organism recorded in the sponge *Tsitsikamma favus* (Supplementary Data 3).

In contrast to the above, the cyanobacterial OTUs, 1, 5, 19, 31, 41 and 60 were consistently abundant in several reef slope species, namely, *C. basilana*, *C. biru, C. cervicornis, C. reinwardti, H. cartilaginea, H. fascigera, L. chagosensis, L. primigenia*, *N. olemda*, *P. orientalis*, and *S. carteri* (Supplementary Fig. 5). OTU-1 was also present in reef flat species and OTUs 31 and 60 (but not 19 and 41) in the reef flat specimen of *H. cartilaginea*. Likewise, OTUs 9, 15, 28, 35, 57, 91, and 92, all classified to Proteobacteria, were relatively abundant in the same sponge species with the exception of *P. orientalis*. All of these OTUs had very high sequence similarities (> 99%) to organisms in GenBank obtained from seawater and a single OTU (15) to an organism obtained from the coral *Astrangia poculata* (Supplementary Data 3). Most of these OTUs were absent from reef flat species in addition to the reef slope species *A. ingens*, *L. herbacea*, *P. hoeksemai*, *P. nigricans*, *T. swinhoei* and *X. testudinaria* (Supplementary Fig. 5).

With respect to Cyanobacteria, the order Synechococcales dominated most reef flat and slope sponges in addition to HMA and LMA sponges (Supplementary Figs. 6 and 7). The order Cyanobacteriales, however, was also relatively abundant in the sponge species *H. cartilaginea* (*Prochloron* sp.) and sediment (*Xenococcus* sp.) sampled from reef flat habitat and *L. herbacea* sampled from reef slope habitat. The dominant OTU (OTU-10 classified to the genus *Hormoscilla*) in *L. herbacea* accounted for > 50% of all sequences and was not recorded. It had 100% sequence similarity to an

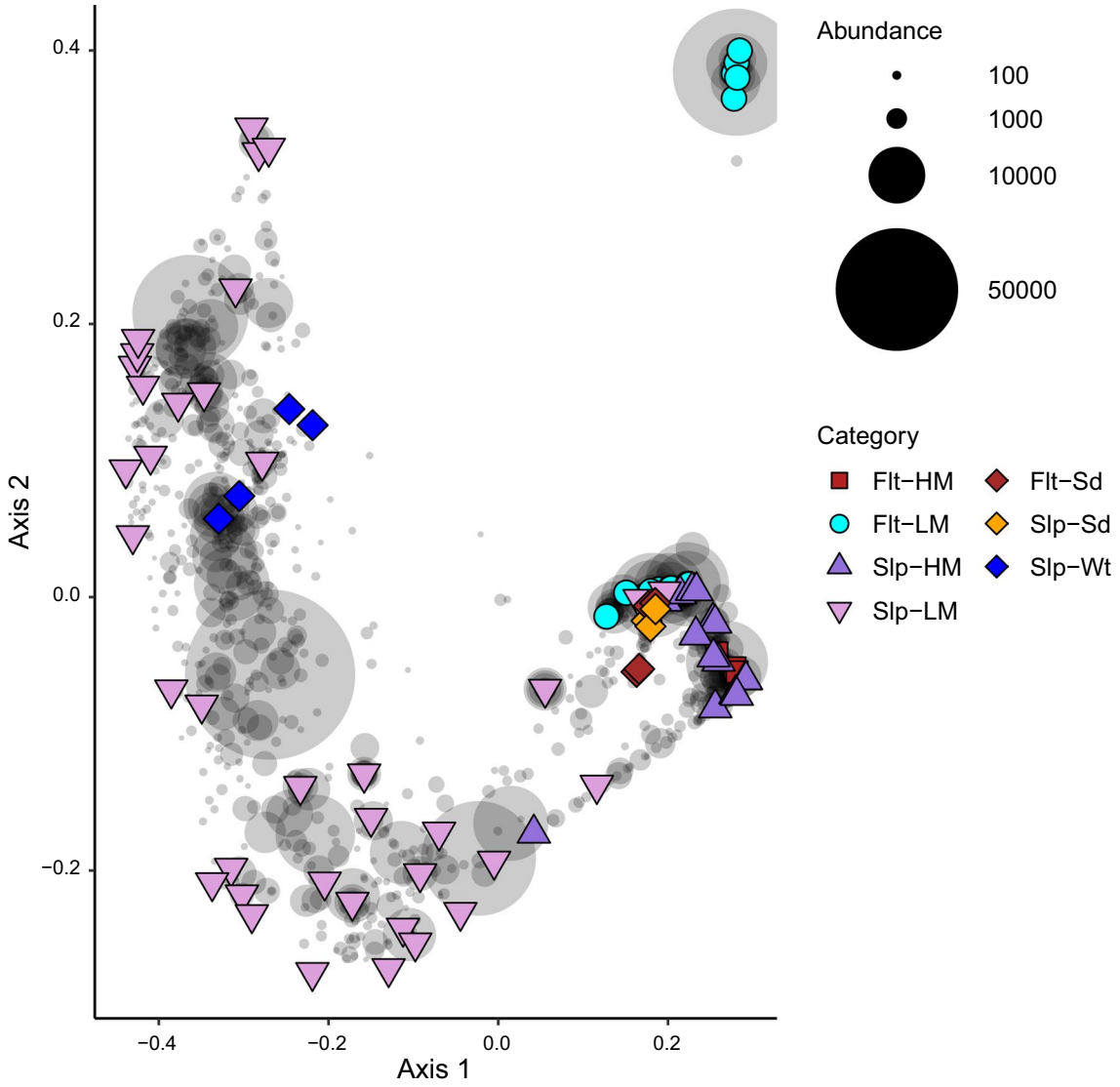

Fig. 4 Ordination showing the first two axes of the principal coordinates analysis (PCO) of OTU composition. Symbols are colour coded and represent samples of HMA (Flt-HM) and LMA (Flt-LM) sponges from reef flat habitat, HMA (Slp-HM) and LMA (Slp-LM) sponges from reef slope habitat, sediment from reef flat (Flt-Sd) and slope (Slp-Sd) habitats, and seawater from reef slope habitat (Slp-Wt) as shown in the legend on the right side of the figure. Grey symbols represent weighted averages scores for OTUs. The symbol size is proportional to group abundance (number of sequence reads)

organism obtained from a microbial mat (Supplementary Data 3).

Discussion

Coral reef habitat and microbial abundance status were both significant predictors of variation in sponge-associated prokaryotic composition. We also identified substantial variation in diversity, higher taxon abundance, and composition among sponge species, sediment and seawater. Differences in diversity were more apparent with the evenness than the richness component. Evenness was highest in HMA sponge species including several Haplosclerid species in addition to the Tetractinellid *T. swinhoei*. Evenness was lowest in *H. fascigera*, *N. olemda*, and *L. herbacea*. In the calcareous species, evenness was higher in *P. orientalis* than *L. primigenia*. In previous studies (Cleary et al. 2020, 2021), we showed that evenness was consistently higher in HMA than LMA species. Overall, our results with respect to microbial abundance status align with several previous studies (Cleary et al. 2019, 2020, 2021; Erwin et al. 2015; Ribes et al. 2015; Moitinho-Silva et al. 2017; Schmitt et al. 2011). HMA sponges from reef flat and slope habitats also clustered together, in contrast to LMA sponges. All HMA sponges were also enriched with Chloroflexi and Acidobacteriota,

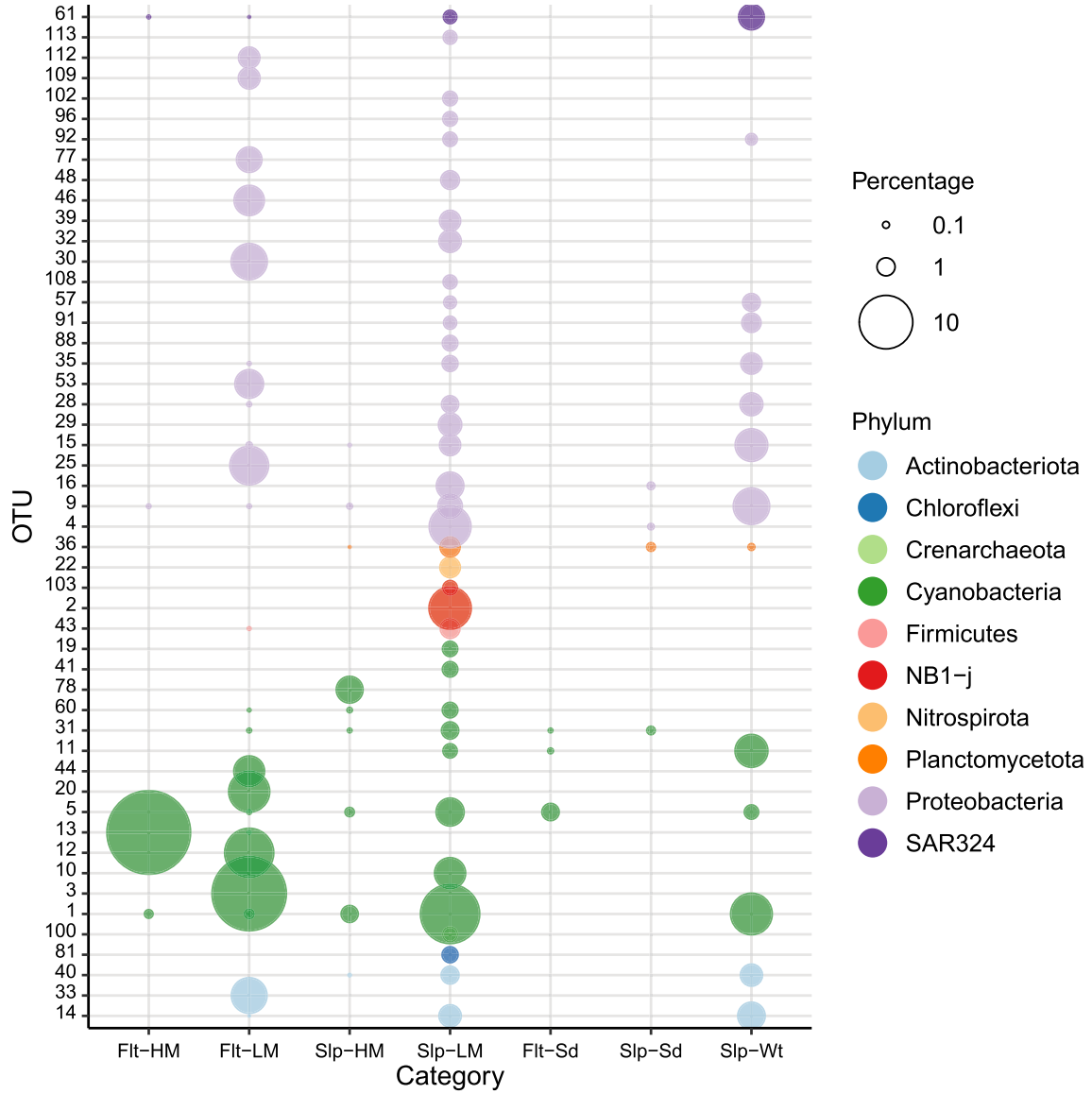

Fig. 5 Relative abundances of the most abundant OTUs (shown along the y-axis) recorded in HMA (Flt-HM) and LMA (Flt-LM) sponges from reef flat habitat, HMA (Slp-HM) and LMA (Slp-LM) sponges from reef slope habitat, sediment from reef flat (Flt-Sd) and slope (Slp-Sd) habitats, and seawater from reef slope habitat (Slp-Wt). The OTU symbols are colour-coded according to prokaryotic phylum classification. The circle size of the OTU is proportional to the mean percentage of sequences per category as indicated by the symbol legend in the upper right corner of the figure

taxa previously found to predict HMA status (Moitinho-Silva et al. 2017; Schmitt et al. 2011).

With respect to our hypothesis that the very high light intensities of the shallow water reef flat environment favour a distinct prokaryotic profile, our results suggested that this effect was particularly pertinent to LMA sponge species with several reef slope sponge species housing consistent sets of abundant cyanobacterial and proteobacterial OTUs, whereas reef flat species were dominated by different, highly abundant cyanobacterial or proteobacterial OTUs. The cyanobacterial OTUs and several proteobacterial populations, e.g. those classified as SAR11, which were abundant in reef slope sponges were also highly similar to organisms previously sampled from seawater. These OTUs may represent bacterial food particles consumed by the sponges. Maldonado and Young (1998) previously observed phagocytosis and digestion of cyanobacteria and heterotrophic bacteria in specimens of the sponge species *Aplysina fistulans* and *Ircinia felix*.

In contrast to the LMA sponge species in reef slope habitat, the reef flat species *C. singaporensis*, *C. samarensis*, *N. chaliniformis*, *P. foliascens*, and *P. papyracea* all contained

highly abundant cyanobacterial OTUs, all of which were classified as *Synechococcus spongiarum*. These sponge species belong to three different taxonomic orders (Poecilosclerida, Haplosclerida and Dictyoceratida) and also are very different in outer morphology (sub-hemispherical, branching and flabellate), however, they were all locally very abundant and some were observed overgrowing other reef organisms. *Coelocarteria singaporensis* is known to form dense aggregations in the intertidal zone (Indonesia, Singapore, and Micronesia), where the fistules of the main body even emerge out of water (Hajdu and Lôbo-Hajdu 2002; Lim et al. 2008). *Callyspongia samarensis* is a poorly-known species, but was recently reported to prevail and overgrow corals in shallow lagoon areas, where coral cover was relatively low due to blast fishing (Cabaitan et al. 2016). *Phyllospongia papyracea* and *P. foliascens* are phototropic species, which thrive in shallow water lagoonal habitats and they can acquire up to 50% of their energy from their cyanobacterial symbionts (Abdul Wahab et al. 2020, Pineda et al. 2016; Wilkinson, 1988).

Erwin and Thacker (2008a) observed *Synechococcus spongiarum* members across a range of sponge taxa and environments from temperate to tropical. Simister et al. (2012), furthermore, identified *Synechococcus spongiarum* as the largest sponge specific cluster. It is also one of the most widespread (Burgsdorf et al. 2015). In a study of two sponge species, *Aplysina fulva* and *Neopetrosia subtriangularis*, Erwin and Thacker (2008b) demonstrated experimentally that shading adversely affected the growth of *A. fulva*, but not *N. subtriangularis*. Both sponge species were, furthermore, shown to be heterotrophic at low irradiances and phototrophic at high irradiances. They also identified multiple *S. spongiarum* clades in both species; specific clades were, however, only observed in single sponge species.

In a study of the sponge species *Ircinia fasciculata*, associated with higher light environments, and *Ircinia variabilis*, associated with lower light environments, Erwin et al. (2012) showed that both were dominated by a novel *S. spongiarum* clade. *Ircinia fasciculata* had more of the dominant symbiont and had more stored energy from photosynthesis in the form of glycogen granules. Both species, furthermore, had the dominant symbionts interacting with host archeocytes, but *I. variabilis* presumably derived less nutritional benefit due to lower cyanobacterial photosynthetic activity.

In *S. spongiarum* clades, Burgsdorf et al. (2015) observed enrichment of DNA modification and recombination genes, but reduced levels of genes involved in signal transduction, cell wall biogenesis, and inorganic ion transport. They, furthermore, suggested that *S. spongiarum* exploited a novel mechanism to evade sponge predation by the absence of genes involved in residue synthesis characteristic of the O antigen of free-living *Synechococcus* species. Finally, in the sponge *Chondrilla australiensis*, Usher et al. (2005) confirmed vertical transfer of *S. spongiarum* from adult to larval sponge stages. This, however, does not preclude potential horizontal symbiont transfer.

For example, using transplantation experiments, Britstein et al. (2020) observed horizontal transfer of Ca. *Synechococcus feldmannii* although they were unable to identify the source; similar sequences were not recorded in seawater or sediment samples suggesting that it may be part of the rare biosphere. Given the filtering ability of the sponge, however, it should be able to select for even extremely rare components of the environment. Other modes of transfer may include transport via animal vectors such as nudibranchs, which were shown to transfer microbes to sponges during feeding (Wecker et al. 2015). A previous study of the Ca. *S. feldmannii* genome, furthermore, revealed adaptations to both symbiotic and free-living stages (Burgsdorf et al. 2019).

Gao et al. (2014) previously showed that healthy and diseased specimens of the sponge *Phyllospongia foliascens* (as *Carteriospongia foliascens)* from the Red Sea coast hosted highly distinct bacterial communities. Healthy specimens housed lower diversity communities dominated by Cyanobacteria, Bacteroidetes and Proteobacteria with the most abundant OTUs assigned to *S. spongiarum* followed by *Nitrosococcus*, *Donghicola* (Rhodobacteraceae), and the JTB23 group (Proteobacteria). Unhealthy specimens, in contrast, housed more diverse communities enriched with Planctomycetes members and other cyanobacterial taxa (*Rivularia*, *Calothrix*, *Oscillatoria*, and *Phormidium*). Here, *P. foliascens* was also dominated by *S. spongiarum* followed by an abundant OTU assigned to the Rhodobacteraceae. As in the study of Gao et al. (2014), the abundance of *S. spongiarum* in the sponge *Aplysina cauliformis* also declined in specimens affected by a disease known as *Aplysina* red band syndrome (Olson et al. 2014).

The highly dominant *S. spongiarum* OTUs found in reef flat sponge species in the present study accounted for between 32 and 49% of all sequences. Less abundant *S. spongiarum* OTUs were observed in reef slope taxa, for example, the HMA sponges *X. testudinaria* and *A. ingens*, but were most abundant in *A. ingens* at 12%. The reef flat habitat from which the sponge specimens were sampled in the Spermonde was shallow and warm with high irradiance levels compared to reef slope habitat. Recently, Curdt et al. (2022) studied the impact of light availability on *S. spongiarum* abundance and host performance of the sponge *Lendenfeldia chondrodes* using a controlled aquarium experiment. They showed that *S. spongiarum* abundance changed with light conditions, affecting sponge growth. Lack of light, furthermore, prevented sponge growth and led to the expulsion of all cyanobacteria by the end of the experiment. Higher light conditions, in contrast, allowed rapid sponge growth and high cyanobacterial densities. Furthermore, exposure to

high levels of photosynthetically active radiation, initiated an increase in lutein levels, a protein, which absorbs UV radiation and protects symbionts and host from UV-related damage.

The high relative abundance of *S. spongiarum* in *N. chaliniformis* in the present study contrasts with another study in the Maldives (Cleary et al. 2021), where total cyanobacterial abundance was only around 5%. Noteworthy is that these samples were not collected from reef flat habitat, but at depths of 10–12 m. In a study of *C. singaporensis* in control and low pH seep sites, Morrow et al. (2015) showed that the percentage of Synechococcaceae increased from 35% at control sites to 70% at seep sites. This result aligned with other studies, which observed increased abundances of Synechococcaceae members in low pH environments suggesting that they conferred an advantage in helping their host to adapt to these otherwise adverse conditions (Cleary et al. 2013, 2018; Morrow et al. 2015). Along these lines, Britstein et al. (2020) observed remarkable stability in the microbiome of the sponge *Petrosia ficiformis* during the acquisition of *Candidatus Synechococcus feldmannii* (Burgsdorf et al. 2019). The main functions acquired were photosynthesis, genes involved in carotene production, and oxidative stress tolerance. These suggest a role of the symbiont in protecting itself and possibly its host from harmful radiation.

Although most sponges in the present study were enriched with Synechococcaceae members including a clear trend of elevated abundances of *S. spongiarum* in LMA sponge hosts from reef flat habitat, there were some exceptions. In contrast to the other reef flat species, the LMA *Haliclona cymaeformis* and HMA *X. vansoesti* did not house abundant cyanobacterial OTUs. The dominant OTUs in *X. vansoesti* were classified to the Acidobacteriota and Chloroflexi phyla, both of which are HMA indicator taxa (Moitinho-Silva et al. 2017). *Haliclona cymaeformis*, in turn, was dominated by a single OTU (OTU-30), classified to the Nitrosococcaceae family and AqS1 genus. Unfortunately, only a single specimen was sampled. In a previous study (Cleary et al. 2019) of *H. cymaeformis* from Taiwan, its prokaryotic community was dominated by OTUs assigned to the EC94 (Betaproteobacteriales) and AqS1 genera in similar proportions followed by OTUs classified to the SAR86 clade and another, less abundant, OTU classified to the AqS1 genus. The present study, thus, does confirm the predominance of symbionts belonging to the AqS1 genus in the microbiome of *H. cymaeformis*.

A draft genome of AqS1, from the sponge *Amphimedon queenslandica*, contained genes involved in sulfur oxidation, carbon monoxide oxidation, inorganic phosphate assimilation, coenzyme A synthesis, and the synthesis of several B vitamins (Gauthier et al. 2016). Gauthier et al. (2016) also identified a single clustered, regularly interspaced, short, palindromic repeat (CRISPR) in the genome of AqS1 and showed it to be enriched in ankyrin-repeat containing proteins. CRISPRs are an important means of controlling the introduction of foreign DNA and are found in a wide range of prokaryotic organisms (Makarova et al. 2011). Finally, AqS1 was enriched in ankyrin-repeat containing proteins, which promote host-microbe symbiotic interactions. In addition to the above, *H. cymaeformis* is distinct in having an obligate symbiotic association with the filamentous red alga *Ceratodictyon spongiosum*. *Halichondria cartilaginea* also lives in association with a filamentous alga, namely the green alga *Cladophoropsis vaucheriiformsis*. These sponges can easily be confused and although *H. cymaeformis* is known to be very abundant in shallow water habitats in Australia (Trautman et al. 2000), *H. cartilaginea* was observed forming dense sponge aggregations in the reef flat habitat and not *H. cymaeformis*. It is thought that photosymbiotic sponges are more common in the intertidal, because these sponges are not able to filter feed during air exposure and therefore depend on their autotrophic symbionts. Also, photosymbionts might provide protection in highly illuminated environments by producing UV absorbing mycosporine-like amino-acids (Steindler et al. 2002). These compounds are also produced by cyanobacteria upon exposure to UV radiation.

One exception to the impact of habitat on LMA species concerned *L. herbacea*, which clustered close to sediment samples and was dominated by a cyanobacterial OTU classified to *Hormoscilla-SI04-45*. In addition to *S. spongiarum*, *Hormoscilla* SI04-45 is another major sponge clade (Usher 2008). *Hormoscilla* SI04-45, also known as *Hormoscilla spongeliae*, was previously classified as *Oscillatoria spongeliae*. Cleary et al. (2024), in Taiwan, and other studies (Flatt et al. 2005; Ridley et al. 2005) also identified *Hormoscilla* as a dominant component of the prokaryotic communities of *L. herbacea*. *Lamellodysidea herbacea* is well-known as a phototrophic sponge with a broad geographical distribution and has been found across varying, often shallow water, habitats including reef flats and lagoons (Faisal et al. 2021). Biggerstaff et al. (2017) observed rapid growth of *L. herbacea* in apparent response to sedimentation in line with previous results, (Powell et al. 2014) while de Voogd et al. (2009) found it to be associated with areas of relatively high rubble and turf algal cover in NE Borneo, Indonesia. Biggerstaff et al. (2017), furthermore, cited mechanisms of sediment clearance and photoacclimation to turbidity as mechanisms enabling it to proliferate under perturbed environmental conditions. In Taiwan, *L. herbacea* was observed in shallow water, low turbidity, pool habitat (Cleary et al. 2024) and, although recorded in the reef slope in the present study, it is typically associated with shallow water habitats (Faisal et al. 2021).

Conclusion

In the present study, both reef habitat and microbial abundance status proved significant predictors of variation in sponge-associated prokaryotic composition. There was, furthermore, a pattern of increased cyanobacterial abundance in reef flat sponges with particular enrichment of OTUs classified as *S. spongiarum* although there were some exceptions to this pattern. The results of the present study suggest that specific clades of *S. spongiarum* may help certain sponge species to survive and thrive in otherwise inhospitable, shallow reef flat habitats, which are often subject to intense UV radiation and temperature fluctuations. This may, furthermore, help to explain the markedly different composition of sponge communities in shallow reef flat *versus* slope habitat. Future experimental studies including translocation, and shading experiments, however, are needed to confirm this observation.

Acknowledgements Research permits were issued by the Indonesian State Ministry of Research and Technology (RISTEK). We thank our colleagues from Universitas Hasanuddin and especially Prof. Dr. Jamaluddin Jompa, and their students for their invaluable support in arranging the fieldwork permits and assistance in the field. We thank Martin van der Plas and Rob Langelaan for their support in the lab.

Funding Openaccess funding provided by FCT|FCCN (b-on). This work is part of the research programme NWO-VIDI with project number 16.161.301 by the Netherlands Organization for Scientific Research (NWO). This work was also supported by European Funds through COMPETE [FCOMP-01-0124-FEDER-008657] and by National Funds through the Portuguese Foundation for Science and Technology (FCT) within the LESS CORAL [PTDC/AAC-AMB/115304/2009] project. We also acknowledge financial support to CESAM from FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdul Wahab MA, Wilson NG, Prada D, Gomez O, Fromont J (2020) Molecular and morphological assessment of tropical sponges in the subfamily Phyllospongiinae, with the descriptions of two new species. Zool J Linn Soc 193(1):319–335

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Bayer K, Kamke J, Hentschel U (2014) Quantification of bacterial and archaeal symbionts in high and low microbial abundance sponges using real-time PCR. FEMS Microbol Ecol 89:679–690

Bell JJ, Barnes DKA (2000) Sponge morphological diversity: a qualitative predictor of species diversity? Aquat Conserv 11:109–121. https://doi.org/10.1002/aqc.436

Bell JJ, Smith D (2004) Ecology of sponge assemblages (*Porifera*) in the Wakatobi region, south-east Sulawesi. Indonesia J Mar Biolog Assoc UK 84(3):581–591. https://doi.org/10.1017/S0025315404009580h

Biggerstaff A, Jompa J, Bell JJ (2017) Increasing benthic dominance of the phototrophic sponge Lamellodysidea herbacea on a sedimented reef within the coral triangle. Mar Biol 164:220. https://doi.org/10.1007/s00227-017-3253-3

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37(8):852–857. https://doi.org/10.1038/s41587-019-0209-9

Britstein M, Cerrano C, Burgsdorf I, Zoccarato L, Kenny NJ, Riesgo A, Lalzar M, Steindler L (2020) Sponge microbiome stability during environmental acquisition of highly specific photosymbionts. Environ Microbiol 22(8):3593–3607. https://doi.org/10.1111/1462-2920.15165

Brown A, Thatje S (2014) Explaining bathymetric diversity patterns in marine benthic invertebrates and demersal fishes: physiological contributions to adaptation of life at depth. Biol Rev Camb Philos Soc 89(2):406–426. https://doi.org/10.1111/brv.12061

Burgsdorf I, Slaby BM, Handley KM, Haber M, Blom J, Marshall CW, Gilbert JA, Hentschel U, Steindler L (2015) Lifestyle evolution in cyanobacterial symbionts of sponges. Mbio 6(3):10–1128. https://doi.org/10.1128/mBio.00391-15

Burgsdorf I, Handley KM, Bar-Shalom R, Erwin PM, Steindler L (2019) Life at home and on the roam: genomic adaptions reflect the dual lifestyle of an intracellular, facultative symbiont. mSystems 4(4):10–1128. https://doi.org/10.1128/mSystems.00057-19

Cabaitan PC, Gomez ED, Yap HT (2016) The spaghetti sponge *Callyspongia samarensis* (Wilson, 1925) provides temporary habitat for reef fish recruits. Mar Biodiv 46:541–542

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583

Carmack EC, Macdonald RW, Jasper S (2004) Phytoplankton productivity on the Canadian Shelf of the Beaufort Sea. Mar Ecol Prog Ser 277:37–50. https://doi.org/10.3354/meps277037

Cleary DFR, Becking LE, de Voogd NJ, Renema W, de Beer M, van Soest RWM, Hoeksema BW (2005) Variation in the diversity and composition of benthic taxa as a function of distance offshore, depth and exposure in the Spermonde Archipelago, Indonesia. Estuar Coast Shelf Sci 65:557–570

Cleary DFR, Becking LE, Pires ACC, de Voogd NJ, Egas C, Gomes NCM (2013) Habitat and host related variation in sponge bacterial communities in Indonesian coral reefs and marine lakes. FEMS Microbiol Ecol 85:465–482

Cleary DFR, Polónia ARM, de Voogd NJ (2018) Bacterial communities inhabiting the sponge Biemna fortis, sediment and water in marine lakes and the open sea. Microb Ecol 76:610–624. https://doi.org/10.1007/s00248-018-1156-6

Cleary DFR, Polónia ARM, Huang YM, Gomes NCM, de Voogd NJ (2019) A comparison of prokaryote communities inhabiting sponges, bacterial mats, sediment and seawater in Southeast Asian coral reefs. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz169

Cleary DFR, Polónia ARM, Reijnen BT, Berumen ML, de Voogd NJ (2020) Prokaryote communities inhabiting endemic and newly discovered sponges and octocorals from the Red Sea. Microb Ecol 80:103–119. https://doi.org/10.1007/s00248-019-01465-w

Cleary DFR, Polónia ARM, de Voogd NJ (2021) Composition and diversity of Prokaryotic communities sampled from sponges and soft corals in Maldivian waters. Mar Ecol 42:e12638. https://doi.org/10.1111/maec.12638

Cleary DFR, Huang YM, Polónia ARM, van der Plas M, Gomes NCM, de Voogd NJ (2024) Sponges and their prokaryotic communities sampled from a remote karst ecosystem. Mar Biodivers 54:8. https://doi.org/10.1007/s12526-023-01387-4

Curdt F, Schupp PJ, Rohde S (2022) Light availability affects the symbiosis of sponge specific cyanobacteria and the Common Blue Aquarium Sponge (*Lendenfeldia chondrodes*). Animals 12(10):1283. https://doi.org/10.3390/ani12101283

de Souza MR, Caruso C, Ruiz-Jones L, Drury C, Gates RD, Toonen RJ (2023) Importance of depth and temperature variability as drivers of coral symbiont composition despite a mass bleaching event. Sci Rep 13(1):8957. https://doi.org/10.1038/s41598-023-35425-9

de Voogd NJ, Becking LE, Cleary DFR (2009) Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia. Mar Ecol Prog Ser 396:219–230. https://doi.org/10.3354/meps08349

Erwin PM, Thacker RW (2008a) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. Mol Ecol 17(12):2937–2947. https://doi.org/10.1111/j.1365-294X.2008.03808.x

Erwin PM, Thacker RW (2008b) Phototrophic nutrition and symbiont diversity of two Caribbean sponge-cyanobacteria symbioses. Mar Ecol Prog Ser 362:139–147. https://doi.org/10.3354/meps07464

Erwin PM, Pita L, López-Legentil S, Turon X (2012) Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. Appl Environ Microbiol 78(20):7358–7368. https://doi.org/10.1128/AEM.02035-12

Erwin PM, Coma R, López-Sendino P, Serrano E, Ribes M (2015) Stable symbionts across the HMA-LMA dichotomy: low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts. FEMS Microbiol Ecol 91(10):fiv115. https://doi.org/10.1093/femsec/fiv115

Faisal MR, Kellermann MY, Rohde S, Putra MY, Murniasih T, Risdian C, Mohr KI, Wink J, Praditya DF, Steinmann E, Köck M, Schupp PJ (2021) Ecological and pharmacological activities of polybrominated diphenyl ethers (PBDEs) from the Indonesian marine sponge *Lamellodysidea herbacea*. Mar Drugs 19(11):611. https://doi.org/10.3390/md19110611

Falkowski PG (1994) The role of phytoplankton photosynthesis in global biogeochemical cycles. Photosynth Res 39(3):235–258. https://doi.org/10.1007/BF00014586

Flatt PM, Gautschi JT, Thacker RW, Musafija-Girt M, Crews P, Gerwick WH (2005) Identification of the cellular site of polychlorinated peptide biosynthesis in the marine sponge *Dysidea* (*Lamellodysidea*) *herbacea* and symbiotic cyanobacterium *Oscillatoria spongeliae* by CARD-FISH analysis. Mar Biol 147:761–774. https://doi.org/10.1007/s00227-005-1614-9

Gao ZM, Wang Y, Lee OO, Tian RM, Wong YH, Bougouffa S, Batang Z, Al-Suwailem A, Lafi FF, Bajic VB, Qian PY (2014) Pyrosequencing reveals the microbial communities in the Red Sea sponge *Carteriospongia foliascens* and their impressive shifts in abnormal tissues. Microb Ecol 68(3):621–632. https://doi.org/10.1007/s00248-014-0419-0

Gauthier M-EA, Watson JR, Degnan SM (2016) Draft genomes shed light on the dual bacterial symbiosis that dominates the microbiome of the coral reef sponge *Amphimedon queenslandica*. Front Mar Sci 3:196. https://doi.org/10.3389/fmars.2016.00196

Gloeckner V, Wehrl M, Moitinho-Silva L, Gernert C, Schupp P, Pawlik JR, Lindquist NL, Erpenbeck D, Wörheide G, Hentschel U (2014) The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. Biol Bull 227(1):78–88. https://doi.org/10.1086/BBLv227n1p78

González-Murcia S, Coppock AG, Ekins M, Battershill CN, Jones GP (2022) Effects of exposure, depth and aspect on sponge communities on a coral reef. Mar Ecol Prog Ser 685:111–126

Hajdu E, Lôbo Hajdu G (2002) Family Isodictyidae Dendy 1924. In: Hooper JNA, van Soest RWM (eds) Systema *Porifera*: a guide to the classification of sponges. Springer, Boston, pp 703–706

Hochmuth T, Niederkrüger H, Gernert C, Siegl A, Taudien S, Platzer M, Crews P, Hentschel U, Piel J (2010) Linking chemical and microbial diversity in marine sponges: possible role for poribacteria as producers of methyl-branched fatty acids. ChemBioChem 11:2572–2578. https://doi.org/10.1002/cbic.201000510

Hoeksema BW (2012) Distribution patterns of Mushroom corals (*Scleractinia*: *Fungiidae*) across the Spermonde Shelf. South Sulawesi Raffles Bull Zool 60(1):183–212. https://doi.org/10.5281/zenodo.5347208

Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275. https://doi.org/10.1007/s00338-010-0593-6

Lecchini D, Adjeroud M, Pratchett MS, Cadoret L, Galzin R (2003) Spatial structure of coral reef fish communities in the Ryukyu Islands, southern Japan. Oceanol Acta 26:537–547. https://doi.org/10.1016/S0399-1784(03)00048-3

Leichter JJ, Paytan A, Wankel S, Hanson K (2007) Nitrogen and oxygen isotopic signatures of subsurface nitrate seaward of the Florida Keys reef tract. Limnol Oceanogr 52:1258–1267. https://doi.org/10.4319/lo.2007.52.3.1258

Lim SC, de Voogd NJ, Tan KS (2008) A guide to sponges of Singapore. Science Centre Singapore, Singapore, pp 1–173

Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, Moineau S, Mojica FJ, Wolf YI, Yakunin AF, van der Oost J, Koonin EV (2011) Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol 9(6):467–477. https://doi.org/10.1038/nrmicro2577

Maldonado M, Young CM (1998) Limits on the bathymetric distribution of keratose sponges: a field test in deep water. Mar Ecol Prog Ser 174:123–139

Maldonado M, López-Acosta M, Sitjà C, García-Puig M, Galobart C, Ercilla G, Leynaert A (2019) Sponge skeletons as an important sink of silicon in the global oceans. Nat Geosci 12:815–822. https://doi.org/10.1038/s41561-019-0430-7

Mass T, Kline DI, Roopin M, Veal CJ, Cohen S, Iluz D, Levy O (2010) The spectral quality of light is a key driver of photosynthesis and photoadaptation in *Stylophora pistillata* colonies from different depths in the Red Sea. J Exp Biol 213(Pt 23):4084–4091. https://doi.org/10.1242/jeb.039891

McGrath EC, Woods L, Jompa J, Haris A, Bell JJ (2018) Growth and longevity in giant barrel sponges: redwoods of the reef or Pines in the Indo-Pacific? Sci Rep 8(1):15317. https://doi.org/10.1038/s41598-018-33294-1

McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8(4):e61217

Meekan MG, Steven ADL, Fortin MJ (1995) Spatial patterns in the distribution of damselfishes on a fringing coral reef. Coral Reefs 14:151–161

Miller MW, Hay ME (1996) Coral-seaweed-grazer-nutrient interactions on temperate reefs. Ecol Monogr 66:323–344

Moitinho-Silva L, Steinert G, Nielsen S, Hardoim CCP, Wu Y-C, Mccormack GP, López-Legentil S, Marchant R, Webster N, Thomas T, Hentschel U (2017) Predicting the HMA-LMA status in marine sponges by machine learning. Front Microbiol 8:752. https://doi.org/10.3389/fmicb.2017.00752

Mokady O, Lazar B, Loya Y (1996) Echinoid bioerosion as a major structuring force of Red Sea coral reefs. Biol Bull 190:367–372. https://doi.org/10.2307/1543029

Morrow KM, Bourne DG, Humphrey C, Botté ES, Laffy P, Zaneveld J, Uthicke S, Fabricius KE, Webster NS (2015) Natural volcanic CO_2 seeps reveal future trajectories for host-microbial associations in corals and sponges. ISME J 9(4):894–908. https://doi.org/10.1038/ismej.2014.188

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, and de Caceres M, Durand S, and Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette MH, Ribeiro Cunha E, Smith T, Stier A, ter Braak CJF, Weedon J (2022) Vegan: community ecology package. R package version 2.6-4. https://cran.r-project.org/web/packages/vegan/index.html.

Olson JB, Thacker RW, Gochfeld DJ (2014) Molecular community profiling reveals impacts of time, space, and disease status on the bacterial community associated with the Caribbean sponge *Aplysina cauliformis*. FEMS Microbiol Ecol 87(1):268–279. https://doi.org/10.1111/1574-6941.12222

Pick KS, Philippe H, Schreiber F, Erpenbeck D, Jackson DJ, Wrede P, Wiens M, Alié A, Morgenstern B, Manuel M, Wörheide G (2010) Improved phylogenomic taxon sampling noticeably affects nonbilaterian relationships. Mol Biol Evol 27(9):1983–1987. https://doi.org/10.1093/molbev/msq089

Pineda MC, Strehlow B, Duckworth A, Doyle J, Jones R, Webster NS (2016) Effects of light attenuation on the sponge holobiont– implications for dredging management. Sci Rep 6:39038

Pita L, Rix L, Slaby BM, Franke A, Hentschel U (2018) The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome 6(1):46. https://doi.org/10.1186/s40168-018-0428-1

Powell A, Smith DJ, Hepburn LJ, Jones T, Berman J, Jompa J, Bell JJ (2014) Reduced diversity and high sponge abundance on a sedimented Indo-Pacific reef system: Implications for future changes in environmental quality. PLoS ONE 9(1):e85253. https://doi.org/10.1371/journal.pone.0085253

R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reiswig HM (1981) Partial carbon and energy budgets of the bacteriosponge *Verongia fistularis* (*Porifera*: *Demospongiae*) in Barbados. Mar Ecol 2:273–293. https://doi.org/10.1111/j.1439-0485.1981.tb00271.x

Ribes M, Dziallas C, Coma R, Riemann L (2015) Microbial diversity and putative diazotrophy in high- and low-microbial-abundance mediterranean sponges. Appl Environ Microbiol 81(17):5683–5693. https://doi.org/10.1128/AEM.01320-15

Ridley CP, John Faulkner D, Haygood MG (2005) Investigation of *Oscillatoria spongeliae*-dominated bacterial communities in four dictyoceratid sponges. Appl Environ Microbiol 71(11):7366–7375. https://doi.org/10.1128/AEM.71.11.7366-7375.2005

Rio M-H, Mulet S, Picot N (2014) Beyond GOCE for the ocean circulation estimate: Synergetic use of altimetry, gravimetry, and in situ data provides new insight into geostrophic and Ekman currents. Geophys Res Lett 41:8918–8925. https://doi.org/10.1002/2014GL061773

Roberts TE, Bridge TCL, Caley MJ, Madin JS, Baird AH (2019) Resolving the depth zonation paradox in reef-building corals. Ecology 100(8):e02761. https://doi.org/10.1002/ecy.2761

Schmitt S, Deines P, Behnam F, Wagner M, Taylor MW (2011) Chloroflexi bacteria are more diverse, abundant, and similar in high than in low microbial abundance sponges. FEMS Microbiol Ecol 78:497–510. https://doi.org/10.1111/j.1574-6941.2011.01179.x

Simister RL, Deines P, Botté ES, Webster NS, Taylor MW (2012) Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. Environ Microbiol 14(2):517–524. https://doi.org/10.1111/j.1462-2920.2011.02664.x

Southwell MW, Weisz JB, Martens CS, Lindquist NL (2008) In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnol Oceanogr 53:986–996. https://doi.org/10.4319/lo.2008.53.3.0986

Steindler L, Beer S, Ilan M (2002) Photosymbiosis in intertidal and subtidal tropical sponges. Symbioses 33:263–273

Swierts T, Peijnenburg KT, de Leeuw C, Cleary DF, Hörnlein C, Setiawan E, Wörheide G, Erpenbeck D, de Voogd NJ (2013) Lock, stock and two different barrels: comparing the genetic composition of morphotypes of the indo-pacific sponge *Xestospongia testudinaria*. PLoS ONE 8(9):e74396. https://doi.org/10.1371/journal.pone.0074396

Thresher R, Althaus F, Adkins J, Gowlett-Holmes K, Alderslade P, Dowdney J, Cho W, Gagnon A, Staples D, McEnnulty F, Williams A (2014) Strong depth-related zonation of megabenthos on a rocky continental margin (~700–4000 m) off southern Tasmania. Australia Plos ONE 9(1):e85872. https://doi.org/10.1371/journal.pone.0085872

Trautman DA, Hinde R, Borowitzka MA (2000) Population dynamics of an association between a coral reef sponge and a red macroalga. J Exp Mar Biol Ecol 244(1):87–105

Usher KM (2008) The ecology and phylogeny of cyanobacterial symbionts in sponges. Mar Ecol 29(2):178–192

Usher KM, Sutton DC, Toze S, Kuo J, Fromont, (2005) Inter-generational transmission of microbial symbionts in the marine sponge *Chondrilla australiensis* (*Demospongiae*). Mar Freshw Res 56:125–131. https://doi.org/10.1071/MF04304

Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Biol Ecol 30:301–314. https://doi.org/10.1016/0022-0981(77)90038-7

van Soest RWM, Cleary DFR, de Kluijver MJ, Lavaleye MSS, Maier C, van Duyl FC (2007) Sponge diversity and community composition in Irish bathyal coral reefs. Contrib Zool 76:121–142

van Soest RW, Boury-Esnault N, Vacelet J, Dohrmann M, Erpenbeck D, de Voogd NJ, Santodomingo N, Vanhoorne B, Kelly M, Hooper JN (2012) Global diversity of sponges (*Porifera*). PLoS ONE 7(4):e35105. https://doi.org/10.1371/journal.pone.0035105

Wainright PO, Hinkle G, Sogin ML, Stickel SK (1993) Monophyletic origins of the metazoa: an evolutionary link with fungi. Science 260(5106):340–342. https://doi.org/10.1126/science.8469985

Wecker P, Fournier A, Bosserelle P, Debitus C, Lecellier G, Berteaux-Lecellier V (2015) Dinoflagellate diversity among nudibranchs and sponges from French Polynesia: insights into associations and transfer. C R Biol 338(4):278–283. https://doi.org/10.1016/j.crvi.2015.01.005

Wilkinson CR (1988) Foliose *Dictyoceratida* of the Australian Great Barrier Reef: II. Ecology and distribution of these prevalent sponges. Mar Ecol 9:321–327

Yahagi T, Kayama Watanabe H, Kojima S, Kano Y (2017) Do larvae from deep-sea hydrothermal vents disperse in surface waters? Ecology 98(6):1524–1534. https://doi.org/10.1002/ecy.1800

Zhao MX, Yu KF, Shi Q, Chen TR, Zhang HL, Chen TG (2013) Coral communities of the remote atoll reefs in the Nansha Islands, southern South China Sea. Environ Monit Assess 185(9):7381–7392. https://doi.org/10.1007/s10661-013-3107-5

Zuschin M, Hohenegger J, Steininger F (2001) Molluscan assemblages on coral reefs and associated hard substrata in the northern Red Sea. Coral Reefs 20:107–116. https://doi.org/10.1007/s003380100140