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Research Article

RESEARCH ARTICLE

Stable symbionts across the HMA-LMA dichotomy: low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts

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One sentence summary: Temporal monitoring of microbial symbionts in six diverse sponge hosts reveals that sponge-associated bacteria are unique to their host and remarkably stable across seasons and years in a temperate sea.

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ABSTRACT

Marine sponges host bacterial communities with important ecological and economic roles in nature and society, yet these benefits depend largely on the stability of host–symbiont interactions and their susceptibility to changing environmental conditions. Here, we investigated the temporal stability of complex host–microbe symbioses in a temperate, seasonal environment over three years, targeting sponges across a range of symbiont density (high and low microbial abundance, HMA and LMA) and host taxonomy (six orders). Symbiont profiling by terminal restriction fragment length polymorphism analysis of 16S rRNA gene sequences revealed that bacterial communities in all sponges exhibited a high degree of host specificity, low seasonal dynamics and low interannual variability: results that represent an emerging trend in the field of sponge microbiology and contrast sharply with the seasonal dynamics of free-living bacterioplankton. Further, HMA sponges hosted more diverse, even and similar symbiont communities than LMA sponges and these differences in community structure extended to core members of the microbiome. Together, these findings show clear distinctions in symbiont structure between HMA and LMA sponges while resolving notable similarities in their stability over seasonal and inter-annual scales, thus providing insight into the ecological consequences of the HMA-LMA dichotomy and the temporal stability of complex host–microbe symbioses.

Keywords: porifera; microbiome; symbiosis; temporal variation; T-RFLP; 16S rRNA

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INTRODUCTION

Symbiotic microorganisms inhabit nearly all forms of multicellular life and play critical roles in our understanding of animal ecology and evolution (Fraune and Bosch 2010; McFall-Ngai *et al.* 2013). In the marine environment, sessile invertebrates harbor diverse microbial symbiont taxa that confer considerable advantages to their immobile hosts, including enhanced growth rates via supplemental autotrophic nutrition (Erwin and Thacker 2008; Freeman *et al.* 2013) and reduced disease susceptibility when residential symbionts remain intact (Cebrian *et al.* 2011; Fan *et al.* 2013). Further, microbial symbionts have been implicated in the production of secondary metabolites from marine invertebrates (Flowers *et al.* 1998; Kwan *et al.* 2012): natural products that play important ecological (e.g. defense against predation; Pawlik 2011) and economic (e.g. pharmaceutical value; Erwin, López-Legentil and Schuhmann 2010) roles in nature and society. Such potential and realized benefits depend largely on the stability of these symbiotic partnerships and their impact on host resilience under changing environmental conditions (Webster 2007). Understanding the dynamics of host–symbiont interactions is a critical first step in assessing symbiont stability, host resilience and how the interaction of these two factors may determine larger shifts in natural marine communities in the face of growing anthropogenic disturbances.

Marine sponges represent an ideal system to study the dynamics and impact of host–symbiont interactions across broad ecological scales: they are distributed worldwide in marine environments (Van Soest *et al.* 2012), form abundant components of past and present benthic communities (Bell *et al.* 2013) and contribute to benthic–pelagic coupling by actively filtering large volumes of seawater (McMurray, Pawlik and Finelli 2014). Sponge also host complex consortia of prokaryotic and eukaryotic microorganisms that are generally dominated by bacterial symbionts (Simister *et al.* 2012) and contributed greatly to overall genetic diversity within the holobiont (Li *et al.* 2014). Within the sponge microbiome, microbial-mediated nutrient cycling pathways add to the metabolic repertoire of the host sponge, including key transformation in the carbon (e.g. carbon fixation) and nitrogen (e.g. nitrification) cycles (Hoffmann *et al.* 2009; Freeman and Thacker 2011; Ribes *et al.* 2012). Coupling symbiont physiological capabilities with the abundance and high filtration rates of host sponges, these holobionts may transform the chemical composition of seawater in these habitats and play important roles in marine nutrient cycles of coastal ecosystems (Jiménez and Ribes 2007).

A major conceptual development in the field of sponge microbiology has been the recognition of high microbial abundance (HMA) and low microbial abundance (HMA) host sponge species and the ecological implications of the HMA-LMA dichotomy (Gloeckner *et al.* 2014). Early observations of sponge-associated bacteria revealed two distinct categories of host sponges: those that host a remarkably high biomass of bacteria in their body (up to 35% of the total sponge volume; Vacelet 1975; Wilkinson 1978) and those with few or no bacterial cells in their tissues (Reiswig 1974, 1981; Vacelet and Donadey 1977). These two categories were originally termed bacteriosponges and non-bacteriosponges and later studies changed the nomenclature to HMA and LMA sponges, more accurately reflecting the diversity of archaeal and eukaryotic microbes also present within sponge tissues (Hentschel *et al.* 2003). In addition to the namesake differences in microbial symbiont abundance, distinctions between HMA and LMA sponges extend to symbiont composition (Blanquer, Uriz and Galand 2013), diversity and specificity (Weisz *et al.* 2007; Kamke, Taylor and Schmitt 2010; Moitinho-Silva *et al.* 2014), as well as host sponge morphology and physiology (Vacelet and Donadey 1977; Weisz, Lindquist and Martens 2008; Schläppy *et al.* 2010; Ribes *et al.* 2012; Poppell *et al.* 2014).

A growing body of research suggests that the sponge microbiome, for both LMA and HMA species, exhibits a high degree of host specificity and temporal stability. The sponge microbiome is comprised of unique bacterial and archaeal lineages, including novel candidate phyla (e.g. Poribacteria; Fieseler *et al.* 2004), that are absent ('sponge-specific' *sensu* Hentschel *et al.* 2002) or rare ('sponge-enriched' *sensu* Moitinho-Silva *et al.* 2014) in free-living communities (Simister *et al.* 2012; Taylor *et al.* 2013). At the community level, these symbionts exhibit a high degree of host specificity (e.g. Easson and Thacker 2014), even among congeneric and sympatric host sponges (Erwin *et al.* 2012a; Hardoim *et al.* 2012). These host-specificity patterns are generally maintained over time and across seasons, as investigations of temporal variability in sponge-associated microorganisms report high symbiont stability (Taylor *et al.* 2004; Thiel *et al.* 2007; Anderson, Northcote and Page 2010; Erwin *et al.* 2012b; Björk *et al.* 2013; Simister *et al.* 2013; Hardoim and Costa 2014), although some exceptions exist (Wichels *et al.* 2006; Cao *et al.* 2012; White *et al.* 2012). As a diverse metazoan phylum consisting of over 8500 species (Van Soest *et al.* 2012), a greater breadth of host sponge coverage is required to assess general trends in the stability of sponge-associated microbial communities. In this study, a replicated, hierarchical sampling design was applied to six taxonomically and phylogenetically diverse host sponge species, representing three HMA and three LMA sponges, sampled over a three-year time period. The targeted species represent all three major phylogenetic lineages of Demospongiae (Morrow and Cárdenas 2015): Keratosa (*Dysidea avara*; Redmond *et al.* 2013), Verongimorpha (*Chondrosia reniformis*; Redmond *et al.* 2013) and Heteroscleromorpha (*Agelas oroides*, *Axinella damicornis*, *Petrosia ficiformis*, *Spriastrella cunctatrix*; Riesgo *et al.* 2014). To accommodate this broad, replicated study design, we employed the high-throughput DNA fingerprinting technique terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu *et al.* 1997), a high-resolution tool for profiling complex microbial communities in nature (Van Dorst *et al.* 2014), including the sponge microbiome (Erwin, Olson and Thacker 2011; Lee *et al.* 2011).

The Mediterranean Sea represents an ideal system to study the temporal dynamics of sponge–microbe associations. Sponges comprise an important ecological component of infralittoral benthic communities (Ballesteros 2006) and often dominate low irradiance habitats in coastal Mediterranean ecosystems (e.g. caves, vertical walls). Further, the Mediterranean Sea is subject to seasonal fluctuations in abiotic factors (e.g. temperature, irradiance) that directly and indirectly dictate the structure of free-living microorganisms (Pinhassi *et al.* 2006; Alonso-Sáez *et al.* 2007), providing a comparative framework for assessing the stability of host-associated microbiomes. Previous work has shown that Mediterranean sponges harbor diverse microbial symbiont communities (Erwin *et al.* 2012a; Blanquer, Uriz and Galand 2013), that these symbionts can contribute to local nutrient cycling (Jiménez and Ribes 2007) and that sponge–microbe interactions are largely stable across seasons (Erwin *et al.* 2012b; Björk *et al.* 2013). Here, we extend our knowledge of temporal stability in Mediterranean sponges by expanding host sponge coverage and sampling design to examine the structure, dynamics and core microbial communities of six phylogenetically diverse HMA and LMA sponge species across both seasonal and annual scales.

METHODS

Sample collection

The HMA sponge species *Agelas oroides* (Schmidt, 1864), *Chondrosia reniformis* Nardo, 1847 and *Petrosia (Petrosia) ficiformis* (Poiret, 1789) and the LMA sponge species *Axinella damicornis* (Esper, 1794), *Dysidea avara* (Schmidt, 1862) and *Spirastrella cunctatrix* Schmidt, 1868 were collected close to the Medes Islands marine reserve in the NW Mediterranean Sea (42°3′0″ N, 3°13′0″ E) by SCUBA at depths between 5 and 10 m. Representing common Mediterranean sponges with distinct morphologies, these species were identified based on their characteristic gross morphological features. The exception was *S. cunctatrix*, an encrusting red-orange sponge with superficial similarity to the sympatric species *Crambe crambe*, where micromorphological features (spicule preparations) were used to confirm identification by gross morphology (Rützler 2002). The HMA or LMA status of each species was taken from the literature (Vacelet and Donadey 1977; Uriz, Martin and Rosell 1992; Ribes *et al.* 2012). Separate (i.e. different sponges each sampling time) replicate individuals ($n = 4$) from each species and ambient seawater samples ($n = 2$) were collected quarterly (February, May, August and November) for three consecutive years beginning in May 2009. For *S. cunctatrix*, four sampling months yielded insufficient replicates and individuals collected during the previous month (October 2011, January 2012) or following month (March 2010, March 2011) were used. Specimens were sublethally sampled and excised fragments collected in separate plastic bottles, brought to the surface and carefully transferred to separate 2 L jars containing filtered seawater (0.22-μm filter) to remove food microbes or loosely associated microbes from the sponges. Samples of the ambient water were taken at 5 m depth in two separate 5 L jars. All jars were transported in an insulated cooler to the laboratory (<2 h) where small pieces (ca. 2 mm^2) of endosome tissue were dissected with sterilized scalpels and frozen in liquid nitrogen, and then stored at −80°C until DNA extraction. Aliquots of seawater (300–500 mL each, 1 aliquot per sample jar) were concentrated on 0.2-μm polycarbonate filters, submerged in lysis buffer and stored at −80°C until DNA extraction.

DNA extraction and PCR amplification

DNA extracts were prepared from sponge samples ($n = 288$) and seawater filters ($n = 24$) using the DNeasy Blood & Tissue kit (Qiagen), following the manufacturers Animal Tissue protocol. Full strength and 1:10 dilutions of DNA extracts were used as templates for PCR amplification of 16S rRNA gene sequences (ca. 1500 bp) with the universal bacterial forward primer 8F (Turner *et al.* 1999), with a 5′-end 6-carboxyfluorescein label attached to the 5′ end, and reverse primer 1509R (Martínez-Murcia, Acinas and Rodriguez-Valera 1995), as described previously (Erwin *et al.* 2012b).

T-RFLP analysis

Triplicate PCR products were gel purified and cleaned using the QIAquick Gel Extraction kit (Qiagen). Purified PCR products (ca. 50 ng) were digested separately with the restriction endonucleases HaeIII and MspI (Promega) for 8 h at 37°C. Following incubation, samples were ethanol precipitated to remove excess salts from enzyme buffers, eluted in 10 μl highly deionized formamide with 0.5 μl GeneScan 600-LIZ size standard (Applied Biosystems). Samples were heated for 2 min at 94°C, cooled on ice and analyzed by capillary electrophoresis on an automated sequencer (ABI 3730 Genetic Analyzer; Applied Biosystems) at the Scientific and Technical Services of the University of Barcelona. The lengths of individual terminal restriction fragments (T-RFs or peaks) were determined by comparison with the internal size standards using the program PeakScanner (Applied Biosystems) and T-RFs beyond the resolution of the size standard (50–600 bp) were removed to avoid inaccurate sizing calculations. To discriminate between 'true' T-RFs and background signal, T-RFs with peak areas less than 50 fluorescence units were discarded and the objective filtering algorithm of Abdo *et al.* (2006) was applied to the remaining datasets using TREX (cutoff value = 2 standard deviations; Culman *et al.* 2009). The remaining T-RFs were aligned across samples using a 1-bp clustering threshold and standardized to relative abundance (percentage of total fluorescence) within each sample.

Statistical analyses of T-RFLP data

Bacterial community similarity was compared among samples using Bray–Curtis similarity matrices constructed from square-root transformations of relative T-RF abundance data and visualized in non-metric multidimensional scaling (nMDS) and cluster plots. Permutational multivariate analyses of variance (PERMANOVA) were used to determine significant differences in bacterial community structure across three factors: source (sponge species or seawater), season within source (nested analysis) and year within source (nested analysis). Estimated components of variation (CV) were calculated to determine the variability among bacterial communities attributable to each factor. Significant PERMANOVA outcomes were followed with multiple pairwise comparisons among levels of each factor, with *P*-values corrected based on the Benjamini–Yekutieli (B-Y) false discovery rate control (Benjamini and Yekutieli 2001), and visualized in heat maps. nMDS plots and PERMANOVA calculations were performed using Primer v6 and PERMANOVA+ (Plymouth Marine Laboratory, UK).

Bacterial community structure was compared among sponge and seawater sources using common ecological metrics for richness (number of T-RFs) and evenness (relative abundance of T-RFs) calculated for each sample. Species richness (*S*) calculates the total number of unique T-RFs, while Simpson's evenness ($E_{1/D}$) provides a measure independent of richness and ranging from 0 to 1. Simpson's inverse index (1/D) is a reciprocal version of Simpson's heterogeneity index ($D = \sum p_i^2$, where p_i is the proportion of individuals in species *i*) that incorporates both dimensions of diversity (richness and evenness) into the calculation. The Berger Parker index (d) is a simple dominance metric that calculates the relative abundance of the most dominant taxon (i.e. max p_i). One-way analyses of variance (ANOVA) were performed on ranked values (Kruskal–Wallis), due to failed normality of the raw data (Shapiro–Wilk, $P < 0.05$). Pairwise multiple comparisons were conducted using Dunn's method, due to uneven sample sizes among groups (sponges vs. seawater). Statistical analyses were performed using the software Sigmaplot v11.0.

Determination of core bacterial communities

Core bacterial communities were strictly defined as bacterial T-RFs that were present in all samples ($n = 48$) within a species across all seasons and years. Rare members of core communities were identified as those exhibiting <1% average relative abundance across samples within a species (Pita *et al.* 2013).

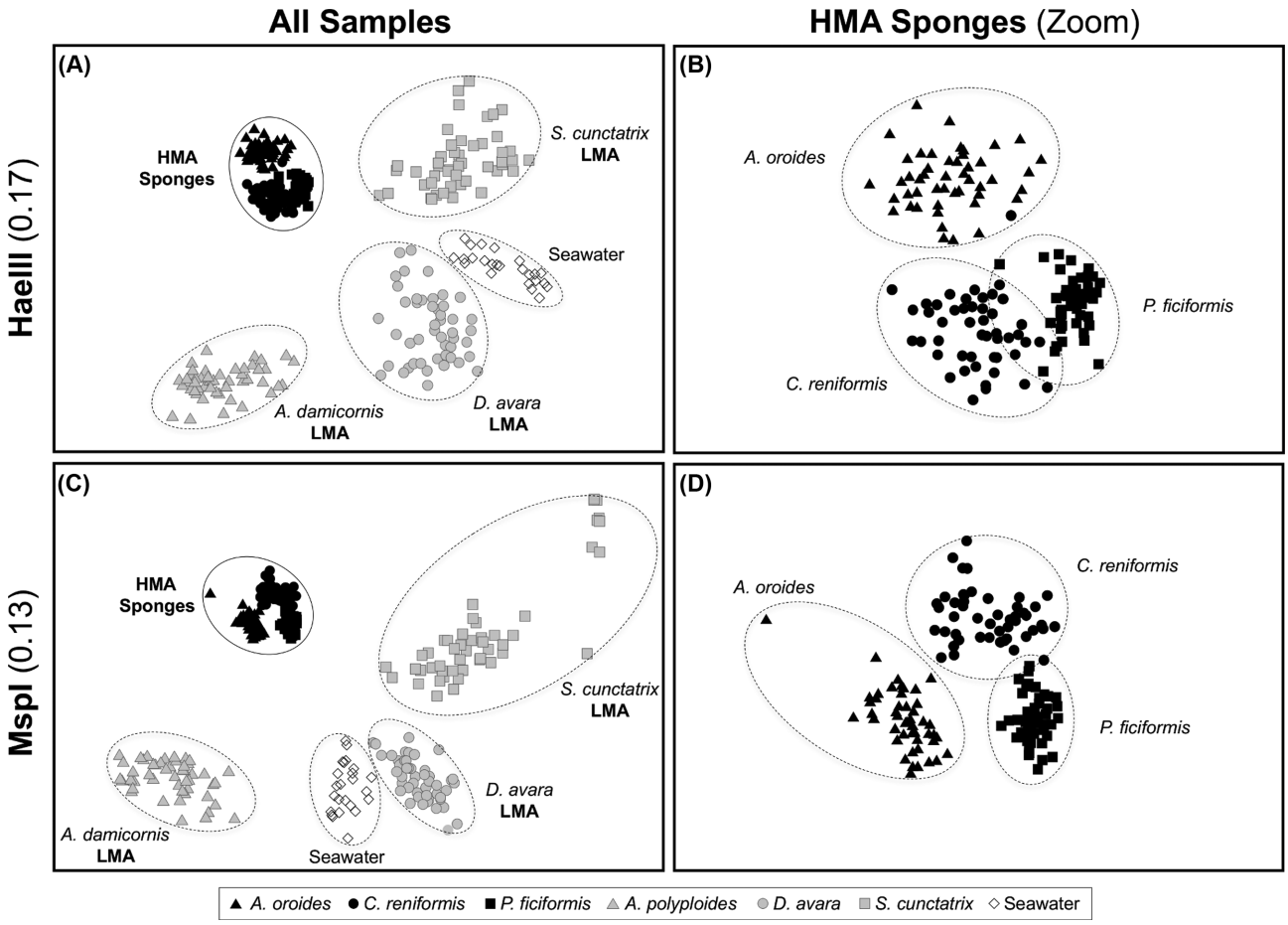

Figure 1. nMDS plots of bacterial community similarity from three HMA sponges (black symbols), three LMA sponges (gray symbols) and ambient seawater (white symbols) over the three-year sampling period. nMDS ordination based on Bray–Curtis similarity of T-RFLP profiles for HaeIII (**A**, **B**) and MspI (**C**, **D**) data sets, with stress values for 2D ordination shown in parentheses. Data points are coded by source with dashed circles encompassing all samples from each source (sponge species or seawater) and solid circles encompassing all HMA sponges.

RESULTS

Host-related variation in bacterial communities

T-RFLP analyses provided high-resolution profiles of bacterial communities inhabiting six sponge species and ambient seawater, recovering 213 distinct T-RFs (105–130 per source) with the HaeIII enzyme and 223 distinct T-RFs (108–143 per source) with the MspI enzyme. nMDS plots and dendrograms constructed from T-RFLP profiles consistently clustered bacterial communities by their source for both enzymes across the three-year sampling period (Figs 1 and 2). Accordingly, statistical analyses revealed a significant effect of source on bacterial community similarity (PERMANOVA, $P < 0.001$, Table 1), including significant pairwise comparisons among all sponge species and seawater ($P < 0.002$, Table S1, Supporting Information). In fact, half of the observed variation among bacterial communities was explained by the source of each sample (sponge species or seawater, Table 1).

While each sponge species hosted a unique bacterial symbiont community, relationships among sponge hosts revealed different specificity patterns between HMA and LMA sponge species. HMA species formed a distinct group based on bacterial community similarity in nMDS (Fig. 1) and cluster plots (Fig. 2), clearly separated from LMA sponge and seawater communities. Within the HMA species cluster, *C. reniformis* and *P. ficiformis* consistently hosted more similar bacterial communities compared to *A. oroides* (Figs 1 and 2). In contrast, the bacterial communities in LMA hosts did not cluster together based on similarity. Two LMA species (*D. avara* and *S. cunctatrix*) hosted bacterial communities more similar to seawater than to the third LMA host (*A. damicornis*) or any of the HMA sponges (Figs 1 and 2), with the microbiome of *D. avara* exhibiting the greatest similarity to seawater bacterioplankton.

Bacterial community structure

The bacterial symbiont communities present in HMA and LMA sponge hosts were also differentiated based on common ecological diversity metrics. HMA sponges hosted significantly more species rich, diverse and even symbiont communities compared to LMA sponges (ANOVA, $P < 0.05$). Notably, these symbiont community trends were consistent across enzyme datasets and among all pairwise comparisons by host species (Dunn's multiple comparisons tests, $P < 0.05$; Fig. 3), indicating that a few, dominant symbionts occur in LMA hosts and many similarly abundant symbionts occur in HMA hosts. For example, the three most abundant bacterial taxa in LMA sponges accounted for over 50% of the total symbiont community, while the three most abundant bacterial taxa in HMA

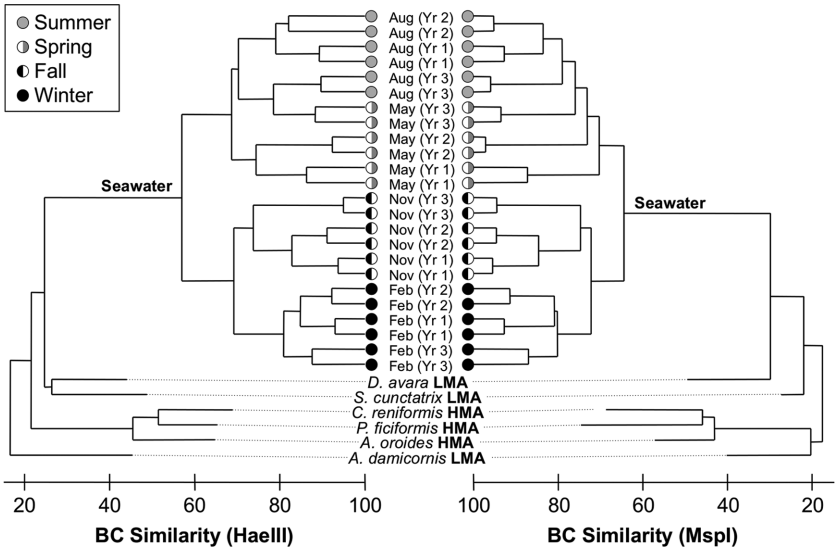

Figure 2. Seasonal changes in the structure of bacterioplankton communities over the three-year monitoring period, showing consistent clustering of seawater samples by season: summer (gray circles), spring (gray half-circles), fall (black half-circles) and winter (black circles). Dendrograms based on Bray–Curtis similarity values from T-RFLP profiles with HaeIII (left) and MspI (right) data sets. Sponge samples exhibited no seasonal structure and were collapsed at host-specific nodes for clarity. See full (uncollapsed) dendrogram in Fig. S1 (Supporting Information).

Table 1. Permutational statistical analyses of variation in T-RFLP profiles by source (sponge species or seawater), season (month) and year[a].

		HaeIII			MspI		
Factor	df	Pseudo-F	P (perm)	CV (%)	Pseudo-F	P (perm)	CV (%)
Source	6	28.127	0.001	49.94	24.13	0.001	48.43
Month (source)	21	2.2534	0.001	9.98	1.7473	0.001	8.50
Year (source)	14	2.3684	0.001	9.03	2.4486	0.001	10.25
Month (source) × year (source)	42	2.3845	0.001	11.77	3.0113	0.001	13.91
Residual	228	–	–	19.28	–	–	18.91

[a]df, degrees of freedom; Pseudo-F, multivariate analog of Fisher's F statistic (i.e. ratio of variance); P (perm), *P*-values based on 999 permutations; CV, component of variation (i.e. percentage of variation among samples explained by each factor level).

sponges accounted for only 22–27% of the total symbiont community.

Seasonal and annual variation in bacterial communities

Some variability was evident across temporal scales, as statistical analyses revealed a significant effect of season, year and an interaction term (season × year) on bacterial community similarity (PERMANOVA, $P < 0.001$, Table 1). Together, these two factors and their interaction term accounted nearly one third of the observed variation among bacterial communities in sponges and seawater (Table 1). Pairwise comparisons within each sponge host were used to further investigate these statistical outcomes and revealed some significant differences in bacterial community similarity across sampling times (Fig. 4). However, these differences were not consistent across years and were often detected with a single restriction enzyme dataset. Further, no consistent clustering of bacterial communities by season or year was observed in similarity dendrograms for any sponge host (Fig. S1, Supporting Information). In contrast, seawater bacteria exhibited clear seasonal patterns in community similarity, with samples from spring and summer months and from fall and winter months forming distinct clusters (Figs 2 and S1, Supporting Information) and significant differences observed among months for nearly all pairwise comparisons (Fig. 4). Even within the spring/summer and fall/winter seawater bacteria groups, samples clustered consistently by month across all three years, with the exception of spring and summer samples during the third year (Fig. 2).

Compared to the seasonal structure observed in ambient seawater bacteria, sponge-associated bacterial communities exhibited a high degree of seasonal stability. In particular, HMA sponge hosts showed high bacterial community similarity (73.6–83.1%) across all seasons and years, with no significant differences detected across months for *A. oroides* and *P. ficiformis* and no consistent differences detected for *C. reniformis* (i.e. both enzyme datasets; Fig. 4, Table S2, Supporting Information). Lower bacterial community similarity was observed in LMA sponges (48.4–64.7%) across the sampling period and all three LMA hosts showed significant differences in some pairwise comparisons by month, although these differences were not consistently detected each year, indicating the presence/absence of transient bacterial symbionts unexplained by temporal factors included in the multivariate analyses.

Core bacterial communities and symbiont overlap

The repeated sampling of sponge hosts and ambient seawater over time also allows for the analysis of core members of

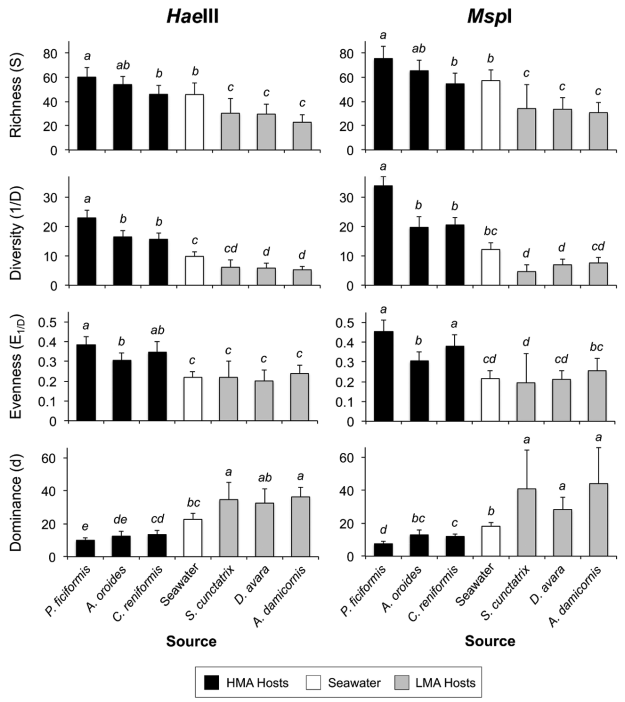

Figure 3. Diversity metrics comparing the richness and evenness of bacterial communities in three HMA sponges (black bars), three LMA sponges (gray bars) and ambient seawater (white bars). Different letters above bars indicate significantly different means among sources ($P < 0.05$) and error bars represent ±1 standard deviation.

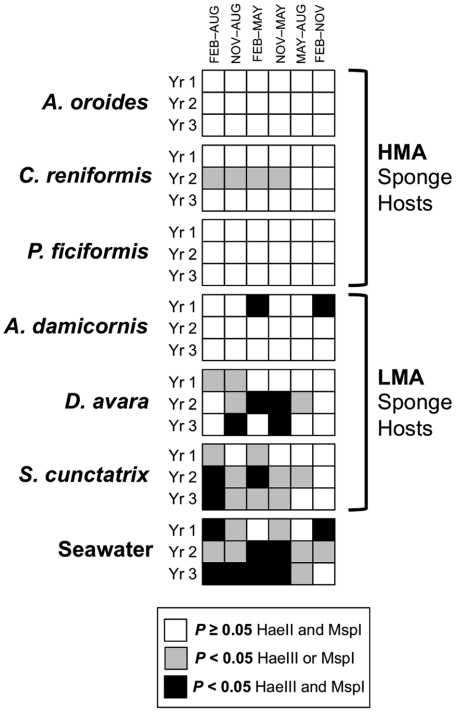

Figure 4. Heatmap summary of temporal variation in bacterial communities of six sponge species and ambient seawater, highlighting the stability of sponge-associated communities and high variability in ambient bacterioplankton. Results of pairwise comparisons of community structure (PERMANOVA) are shown as black (significant for both enzyme datasets), gray (significant for one dataset) and white (non-significant) boxes following correction based on the B-Y false discovery rate control. Exact *P*-values are provided in Table S2 (Supporting Information).

bacterial symbiont communities, thereby distinguishing stable from transient bacterial groups. Consistent with diversity metrics of the entire symbiont communities, a higher number of core bacterial groups were present in HMA sponges (10–35 T-RFs) compared to LMA sponges (1–6 T-RFs). These core bacterial groups accounted for the majority of symbiont communities in each host sponge species, based on one or both enzyme datasets (Table 2). Surprisingly, several members of the core microbiota in HMA hosts were rare symbionts taxa, particularly in the hosts *A. oroides* and *P. ficiformis*. These rare symbionts were detected in all samples of each sponge host yet averaged <1% relative abundance (Table 2). None of the rare members of the core microbiota reached a relative abundance above 2.89% in any sample from any time point.

While considerable overlap was observed among sponge and seawater communities, restricting host-specificity analysis to core bacteria revealed further differences between LMA and HMA sponges (Fig. 5). Among HMA sponge hosts, several core members were shared across all three (5–6 T-RFs) or two of three (7–11 T-RFs) host species, indicating some overlap in the membership of each hosts' symbiont community. In stark contrast, no core bacteria were shared among any of the LMA host species, indicating a higher degree of host specificity of these dominant symbiont groups (Fig. 5). It is also noteworthy that HMA hosts shared more core bacterial members with seawater (2–5 T-RFs) compared to LMA hosts (1–2 T-RFs) and that several core members were shared between LMA and HMA hosts (4–6 T-RFs).

DISCUSSION

In this study, we assessed the temporal stability of complex host–microbe associations in six taxonomically diverse Mediterranean sponges, revealing low seasonal and interannual dynamics in bacterial communities of both HMA and LMA hosts. This remarkable temporal stability contrasted sharply with the clear seasonal fluctuations in free-living bacterioplankton present in ambient seawater surrounding the investigated sponges. The microbiomes of HMA sponges were also clearly differentiated from LMA sponges based on community structure and diversity metrics, with HMA sponges hosting more diverse, even and similar symbiont communities than LMA sponges. These differences extend to core members of the microbiome (i.e. present in all samples of a sponge species), which were more diverse and exhibited greater overlap between HMA sponge species than LMA sponges species. In fact, no core symbiont taxa were shared between LMA sponge species. Together, these findings highlight clear distinctions in the structure of symbiont communities between HMA and LMA sponges while resolving notable similarities in their temporal stability over seasonal and interannual scales.

Source was the main driver of symbiont structure across all investigated sponge species and seawater, accounting for over half of the variation observed among microbial communities. The role of the host in dictating symbiont structure is emerging as a paradigm in sponge microbiology, reported in numerous LMA and HMA host species collected from diverse geographic regions (Blanquer, Uriz and Galand 2013; Webster *et al.* 2013; Easson and Thacker 2014). More surprising, we observed high similarity (>40%) between microbial communities of HMA hosts. Several studies have shown that HMA sponges are separate from LMA sponges based on symbiont community

Table 2. Core bacterial communities in six Mediterranean sponge species, showing the total number of core T-RFs (present in all samples within a species) and rare core T-RFs (average relative abundance <1%) for both restriction enzyme datasets (HaeIII and MspI). Percentages of total symbiont communities accounted for by core T-RFs (% RA) and minimum and maximum relative abundances (RA Range) for rare T-RFs are shown.

		HaeIII				MspI			
		Core T-RFs		Rare core T-RFs		Core T-RFs		Rare core T-RFs	
Host sponge	Type	No.	% RA	No.	RA Range	No.	% RA	No.	RA range
A. oroides	HMA	17	66.90	2	0.12–2.37%	21	66.71	5	0.17–2.34%
C. reniformis	HMA	10	49.51	0	–	19	58.81	1	0.17–2.89%
P. ficiformis	HMA	27	78.83	3	0.15–1.47%	35	69.19	8	0.13–2.37%
A. damicornis	LMA	3	51.08	0	–	4	31.07	0	–
D. avara	LMA	3	47.66	0	–	6	62.25	0	–
S. cunctatrix	LMA	5	63.05	0	–	1	40.78	0	–
Seawater	–	13	73.83	0	–	15	72.32	3	0.15–2.88%

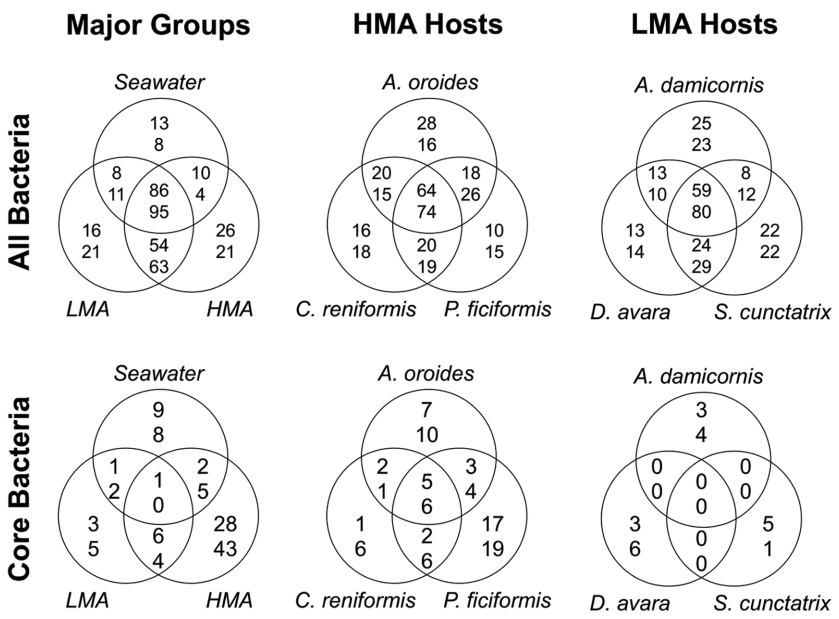

Figure 5. Venn diagrams depicting the host specificity of total (top row) and core (bottom row) bacterial communities in three HMA sponges, three LMA sponges and ambient seawater. Circles represent bacterial communities by group (LMA or HMA) or host species, with overlapping portions indicating the number of shared bacterial T-RFs and non-overlapping portions the number of host-specific T-RFs (top values = HaeIII, bottom values = MspI).

similarity (Gerçe *et al.* 2011; Blanquer, Uriz and Galand 2013; Schöttner *et al.* 2013; Poppell *et al.* 2014), though generally clustering at low similarity values (<10%). Clustering of HMA sponges is a pattern that likely reflects a higher percentage of shared, generalist symbiont taxa in HMA hosts compared to LMA hosts (Kamke, Taylor and Schmitt 2010; Erwin, Olson and Thacker 2011; Schmitt *et al.* 2011; Giles *et al.* 2013; Simister *et al.* 2013). The comparison of core symbiont communities herein provides a clear illustration of this pattern, with several shared core taxa among HMA hosts and no shared core taxa among LMA hosts.

Our study also yielded consistent differences in symbiont richness and evenness that underlie these overall patterns of community structure. HMA sponges hosted symbiont communities with greater richness and evenness compared to LMA hosts, a result observed for both total and core symbiont communities. Indeed, the microbiomes of LMA sponges are typically dominated by a few specialist (i.e. host-specific) symbiont taxa (Kamke, Taylor and Schmitt 2010; Erwin, Olson and Thacker 2011; Giles *et al.* 2013). However, exceptions to these general trends have been reported (Schöttner *et al.* 2013; Easson and Thacker 2014) and potentially ascribed to specific habitats (cold water) or host characteristics (phototrophic sponges). Given the broad taxonomic and ecological diversity of species in the phylum Porifera (Van Soest *et al.* 2012), such exceptions are not unexpected and the HMA-LMA dichotomy remains a useful framework for comparative analyses in sponge microbiology (Gloeckner *et al.* 2014).

The host specificity and diversity patterns of symbiont communities in HMA and LMA sponge hosts were maintained across seasons and years, despite the large temporal fluctuations in abiotic and biotic factors that characterize the Mediterranean Sea. The temporal stability of the sponge microbiome, even in seasonal environments, is also emerging as a common theme in the field. To date, at least 22 sponge species have been investigated for *in situ* symbiont stability over time, representing 11 HMA and 11 LMA hosts from 15 families, 11 orders and all three major subclasses (Morrow and Cárdenas 2015) of

Table 3. Taxonomic diversity[a] and microbial abundance category (LMA vs. HMA) of sponge species used in this study[b] and previous investigations of temporal variation in sponge-associated microbial communities[c].

Subclass	Order	Family	Species	Category	Citation
Keratosa	Dictyoceratida	Dysideidae	***Dysidea avara***	LMA	This study, (Björk *et al.* 2013)
		Irciniidae	*Ircinia fasciculata*	HMA	(Erwin *et al.* 2012b)
			Ircinia oros	HMA	(Erwin *et al.* 2012b)
			Ircinia variabilis	HMA	(Erwin *et al.* 2012b)
			Sarcotragus spinosulus	HMA	(Hardoim and Costa 2014)
		Spongiidae	*Spongia officinalis*	HMA	(Bauvais *et al.* 2015)
Verongimorpha	Chondrosida	Chondrillidae	*Chondrilla nucula*	HMA	(Thiel *et al.* 2007)
		Chondrosidae	***Chondrosia reniformis***	HMA	This study, (Björk *et al.* 2013)
	Verongida	Aplysinidae	*Aplysina cauliformis*	HMA	(Olson, Thacker and Gochfeld 2013)
Heteroscleromorpha	Agelasida	Agelasidae	***Agelas oroides***	HMA	This study, (Björk *et al.* 2013)
	Axinellida	Axinellidae	*Axinella corrugata*	LMA	(White *et al.* 2012)
			Axinella damicornis	LMA	This study
			Cymbastela concentrica	LMA	(Taylor *et al.* 2004)
	Clionaida	Spirastrellidae	***Spirastrella cunctatrix***	LMA	This study
	Haplosclerida	Callyspongidae	*Callyspongia* sp.	LMA	(Taylor *et al.* 2004)
		Petrosiidae	***Petrosia ficiformis***	HMA	This study
	Poecilosclerida	Mycalidae	*Mycale hentscheli*	LMA	(Anderson *et al.* 2010)
	Subertida	Halichondridae	*Halichondria panicea*	LMA	(Wichels *et al.* 2006)
			Hymeniacidon sinapium	LMA	(Cao *et al.* 2012)
			Stylinos sp.	LMA	(Taylor *et al.* 2004)
	Tethyida	Tethyidae	*Tethya stolonifera*	LMA	(Simister *et al.* 2013)
	Tetractinellida	Ancorinidae	*Ancorina alata*	HMA	(Simister *et al.* 2013)

[a]Classifications based on the recent, revised taxonomic framework for Demospongiae (Morrow and Cárdenas 2015).
[b]Species targeted in the current study are shown in bold.
[c]References cited refer to studies examining symbiont community variability *in situ* using molecular (culture-independent) techniques. Additional temporal studies have focused on sponges maintained *ex situ* (Friedrich *et al.* 2001; Mohamed *et al.* 2008a,b; Webster *et al.* 2011) and used culture-dependent techniques (Webster and Hill 2001).

Demospongiae (Table 3). High temporal stability was reported for most (82%) sponge hosts, including the six species herein, indicating high stability of symbiont communities in both HMA and LMA sponges from diverse taxonomic groups. Further, these studies were conducted in different geographic locations (South Pacific Ocean, Western Atlantic Ocean, Mediterranean Sea) using different sampling schemes (monthly, quarterly, yearly) and symbiont characterization methodologies (multiple DNA fingerprinting techniques, first- and second-generation DNA sequencing). The consistency of these diverse studies suggests an overall high degree of host–symbiont fidelity in the complex marine sponge–bacteria symbioses.

Exceptions to the general trend of high stability of the sponge microbiome include reports of temporal variation in sponge-associated microbial communities in four sponge species: *Halichondria panicea* (Wichels *et al.* 2006), *Hymeniacidon sinapium* (Cao *et al.* 2012), *Ax. corrugata* (White *et al.* 2012) and *Aplysina cauliformis* (Olson, Thacker and Gochfeld 2013). The sole HMA host in this species list, *A. cauliformis*, also exhibited significant variability in symbiont communities across locations and disease status, yet maintained a common core of stable bacterial taxa (Olson, Thacker and Gochfeld 2013). The remaining host species represent LMA sponges and two are closely related (*H. panicea*, *Hy. sinapium*, family Halichondridae), leading to the preliminary hypothesis that some sponge lineages within the LMA category may exhibit greater flexibility in host–symbiont interactions over time. While our study reported greater symbiont variability across replicates of LMA sponges, these differences did not follow any seasonal or interannual pattern and the factors collection month and year had low explanatory power compared to host sponge species. Similarly, three of the aforementioned studies reported temporal variability, rather than clear seasonal shifts (Wichels *et al.* 2006; White *et al.* 2012; Olson, Thacker and Gochfeld 2013). Indeed, it is difficult to assess whether these temporal changes reflect responses of the symbiont community to periodic fluctuations in environmental factors (i.e. seasonal dynamics), due to the limited ability of the sampling designs (two time points; White *et al.* 2012) and statistical methods (no quantitative comparisons of symbiont fingerprint data; Wichels *et al.* 2006) to detect seasonal trends. In contrast, the Japanese sponge *Hy. sinapium* was shown to exhibit seasonal shifts in symbiont communities over 1.5 years in Yellow Sea (Cao *et al.* 2012), an area characterized by drastic changes in environmental conditions throughout the year, including sedimentation rate and large temperature fluctuations (<0° to 30°C). Thus, exceptions to the trend of high temporal stability of sponge microbiomes may occur in particular host species (e.g. *A. cauliformis*) or environments where extreme seasonal fluctuations may exceed the physiological tolerance levels of specific symbionts, resulting in the observed seasonal community shifts.

The investigation of microbial communities over temporal scales also allows for the detection of core microbial taxa, those that occur consistently in particular habitats. Such core taxa are hypothesized to have ecological relevance in the functioning of the overall microbial communities (Shade and Handelsman 2012) and are a particularly relevant aspect of sponge microbiomes, given that bacteria detected in the sponge body may represent permanent symbionts or transient microbes (Olson, Thacker and Gochfeld 2013), such parasites (Webster *et al.* 2002) or food source bacterioplankton (Reiswig 1975). Accordingly, a large proportion (61–66%) of non-core (transient) bacteria were shared between sponges and seawater while most (74–78%) of the core bacteria were present only in sponges. The

analysis of core communities in the six host sponges investigated herein revealed parallel patterns with analyses of total symbiont communities, including a higher diversity and greater overlap of core taxa in HMA hosts compared to LMA hosts. The comparatively low complexity of the LMA microbiome (one to six core taxa, herein) offers a tractable model for in-depth characterization of symbiont community structure and function. For example, a recent study utilized next-generation sequencing and fluorescence *in situ* hybridization techniques to identify and localize a single bacterial species that dominates the microbiome of the LMA sponge *Crambe crambe*, suggesting a putative role of this symbiont in the production of bioactive secondary metabolites isolated from the host sponge (Croué *et al.* 2013). In contrast to the LMA hosts, the core microbiomes of HMA hosts were not only more diverse (10–35 core taxa) but included rare symbiont taxa (averaging <1% relative abundance), indicating that not all rare members of the sponge microbiome are transient symbionts. Notably these core taxa remained rare throughout the three-year monitoring period, rather than exploiting temporal windows of favorable conditions and increasing in abundance ('conditionally' rare) as reported in marine bacterioplankton (Alonso-Sáez, Díaz-Pérez and Morán 2015). Additional studies focusing on the rare components of the sponge microbiome are required to assess their role in overall symbiont community function, which may be disproportionate to their abundance as observed in other rare environmental taxa (Musat *et al.* 2008; Pester *et al.* 2010).

Monitoring the stability of the sponge microbiome over seasonal and annual scales provides baseline levels of natural variation and aids in the development of sponge–microbe symbioses as biomonitoring tools. Given the complexity of the sponge microbiome, it is necessary to define baseline shifts in symbiont communities prior to detecting abnormal shifts and disease incidence in response to changing environment conditions, such as acute anthropogenic disturbances or chronic climate change (Webster 2007). In addition to studies of symbiont structure, future studies targeting specific functional guilds of symbionts (Fan *et al.* 2012) and their temporal fluctuations (Zhang, Vicente and Hill 2014) will provide insight into the consequences of structural shifts, the potential for functional redundancy and cascading effects of host sponge health and resilience. Currently, sponges are being developed as biomonitors of toxic metal pollutants (Cebrian, Uriz and Turon 2007; Davis *et al.* 2014) and coupling microbiome data with toxicological analyses may prove an even more sensitive measure of sublethal stress in marine organisms (Fan *et al.* 2013). In the face of growing anthropogenic disturbances, further development of sponges and their microbiome as models for basic ecological research and applied biomonitoring initiatives will enhance our understanding of host–symbiont dynamics and clarify the role of symbiont stability and host resilience in determining larger shifts in natural marine communities.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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REFERENCES

Abdo Z, Schüette UME, Bent SJ, *et al.* Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ Microbiol* 2006;**8**: 929–38.

Alonso-Sáez L, Balagué V, Sà EL, *et al.* Seasonality in bacterial diversity in north-west Mediterranean coastal waters: assessment through clone libraries, fingerprinting and FISH. *FEMS Microbiol Ecol* 2007;**60**:98–112.

Alonso-Sáez L, Díaz-Pérez L, Morán XAG. The hidden seasonality of the rare biosphere in coastal marine bacterioplankton. *Environ Microbiol* 2015, DOI: 10.1111/1462-2920.12801.

Anderson SA, Northcote PT, Page MJ. Spatial and temporal variability of the bacterial community in different chemotypes of the New Zealand marine sponge *Mycale hentscheli*. *FEMS Microbiol Ecol* 2010;**72**:328–42.

Ballesteros E. Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Oceanogr Mar Biol* 2006;**44**: 123–95.

Bauvais C, Zirah S, Piette L, *et al.* Sponging up metals: bacteria associated with the marine sponge *Spongia officinalis*. *Mar Environ Res* 2015;**104**:20–30.

Bell JJ, Davy SK, Jones T, *et al.* Could some coral reefs become sponge reefs as our climate changes? *Glob Change Biol* 2013;**19**:2613–24.

Benjamini Y, Yekutieli D. The control of false discovery rate in multiple testing under dependency. *Ann Stat* 2001;**29**: 1165–88.

Björk JR, Díez-Vives C, Coma R, *et al.* Specificity and temporal dynamics of complex bacteria-sponge symbiotic interactions. *Ecology* 2013;**94**:2781–91.

Blanquer A, Uriz MJ, Galand PE. Removing environmental sources of variation to gain insight on symbionts vs. transient microbes in high and low microbial abundance sponges. *Environ Microbiol* 2013;**15**:3008–19.

Cao H, Cao X, Guan X, *et al.* High temporal variability in bacterial community, silicatein and hsp70 expression during the annual life cycle of *Hymeniacidon sinapium* (Demospongiae) in China's Yellow Sea. *Aquaculture* 2012;**358-359**: 262–73.

Cebrian E, Uriz MJ, Garrabou J, *et al.* Sponge mass mortalities in a warming Mediterranean Sea: are cyanobacteria-harboring species worse off? Thrush S (ed.). *PLoS One* 2011;**6**: e20211.

Cebrian E, Uriz M-J, Turon X. Sponges as biomonitors of heavy metals in spatial and temporal surveys in northwestern Mediterranean: multispecies comparison. *Environ Toxicol Chem* 2007;**26**:2430–9.

Croué J, West NJ, Escande M-L, *et al.* A single betaproteobacterium dominates the microbial community of the crambescidine-containing sponge *Crambe crambe*. *Sci Rep* 2013;**3**:2583.

Culman SW, Bukowski R, Gauch HG, *et al.* T-REX: software for the processing and analysis of T-RFLP data. *BMC Bioinformatics* 2009;**10**:171.

Davis AR, de Mestre C, Maher W, *et al.* Sponges as sentinels: metal accumulation using transplanted sponges across a metal gradient. *Environ Toxicol Chem* 2014;**33**:2818–25.

Easson CG, Thacker RW. Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. *Front Microbiol* 2014;**5**:532.

Erwin P, Thacker R. Phototrophic nutrition and symbiont diversity of two Caribbean sponge–cyanobacteria symbioses. *Mar Ecol Prog Ser* 2008;**362**:139–47.

Erwin PM, López-Legentil S, Schuhmann PW. The pharmaceutical value of marine biodiversity for anti-cancer drug discovery. *Ecol Econ* 2010;**70**:445–51.

Erwin PM, Olson JB, Thacker RW. Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern Gulf of Mexico. López-García P (ed.). *PLoS One* 2011;**6**:e26806.

Erwin PM, López-Legentil S, González-Pech R, *et al.* A specific mix of generalists: bacterial symbionts in Mediterranean *Ircinia* spp. *FEMS Microbiol Ecol* 2012a;**79**:619–37.

Erwin PM, Pita L, Lopez-Legentil S, *et al.* Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Appl Environ Microb* 2012b;**78**:7358–68.

Fan L, Liu M, Simister R, *et al.* Marine microbial symbiosis heats up: the phylogenetic and functional response of a sponge holobiont to thermal stress. *ISME J* 2013;**7**:991–1002.

Fan L, Reynolds D, Liu M, *et al.* Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. *P Natl Acad Sci USA* 2012;**109**: E1878–87.

Fieseler L, Horn M, Wagner M, *et al.* Discovery of the novel candidate phylum '*Poribacteria*' in marine sponges. *Appl Environ Microb* 2004;**70**:3724–32.

Flowers AE, Garson MJ, Webb RI, *et al.* Cellular origin of chlorinated diketopiperazines in the dictyoceratid sponge *Dysidea herbacea* (Keller). *Cell Tissue Res* 1998;**292**:597–607.

Freeman CJ, Thacker RW. Complex interactions between marine sponges and their symbiotic microbial communities. *Limnol Oceanogr* 2011;**56**:1577–86.

Freeman CJ, Thacker RW, Baker DM, *et al.* Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? *ISME J* 2013;**7**: 1116–25.

Fraune S, Bosch TCG. Why bacteria matter in animal development and evolution. *BioEssays* 2010;**32**:571–80.

Friedrich AB, Fischer I, Proksch P, *et al.* Temporal variation of the microbial community associated with the Mediterranean sponge *Aplysina aerophoba*. *FEMS Microbiol Ecol* 2001;**38**: 105–13.

Gerçe B, Schwartz T, Syldatk C, *et al.* Differences between bacterial communities associated with the surface or tissue of Mediterranean sponge species. *Microb Ecol* 2011;**61**:769–82.

Giles EC, Kamke J, Moitinho-Silva L, *et al.* Bacterial community profiles in low microbial abundance sponges. *FEMS Microbiol Ecol* 2013;**83**:232–41.

Gloeckner V, Wehrl M, Moitinho-Silva L, *et al.* The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. *Biol Bull* 2014;**227**:78–88.

Hardoim CCP, Costa R. Temporal dynamics of prokaryotic communities in the marine sponge *Sarcotragus spinosulus*. *Mol Ecol* 2014;**23**:3097–112.

Hardoim CCP, Esteves AIS, Pires FR, *et al.* Phylogenetically and spatially close marine sponges harbour divergent bacterial communities. Harder T (ed.). *PLoS One* 2012;**7**:e53029.

Hentschel U, Fieseler L, Wehrl M, *et al.* Microbial diversity of marine sponges. *Prog Mol Subcell Biol* 2003;**37**:59–88.

Hentschel U, Hopke J, Horn M, *et al.* Molecular evidence for a uniform microbial community in sponges from different oceans. *Appl Environ Microb* 2002;**68**:4431–40.

Hoffmann F, Radax R, Woebken D, *et al.* Complex nitrogen cycling in the sponge *Geodia barretti*. *Environ Microbiol* 2009;**11**:2228–43.

Jiménez E, Ribes M. Sponges as a source of dissolved inorganic nitrogen: nitrification mediated by temperate sponges. *Limnol Oceanogr* 2007;**52**:948–58.

Kamke J, Taylor MW, Schmitt S. Activity profiles for marine sponge-associated bacteria obtained by 16S rRNA vs 16S rRNA gene comparisons. *ISME J* 2010;**4**:498–508.

Kwan JC, Donia MS, Han AW, *et al.* Genome streamlining and chemical defense in a coral reef symbiosis. *P Natl Acad Sci USA* 2012;**109**:20655–60.

Lee OO, Wang Y, Yang J, *et al.* Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *ISME J* 2011;**5**:650–64.

Li Z-Y, Wang Y-Z, He L-M, *et al.* Metabolic profiles of prokaryotic and eukaryotic communities in deep-sea sponge *Lamellomorpha* sp. indicated by metagenomics. *Sci Rep* 2014;**4**: 3895.

Liu W-T, Marsh TL, Cheng H, *et al.* Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microb* 1997;**63**:4516–22.

McFall-Ngai M, Hadfield MG, Bosch TC, *et al.* Animals in a bacterial world, a new imperative for the life sciences. *P Natl Acad Sci USA* 2013;**110**:3229–36.

McMurray S, Pawlik J, Finelli C. Trait-mediated ecosystem impacts: how morphology and size affect pumping rates of the Caribbean giant barrel sponge. *Aquat Biol* 2014;**23**:1–13.

Martínez-Murcia AJ, Acinas SG, Rodriguez-Valera F. Evaluation of prokaryotic diversity by restrictase digestion of 16S rDNA directly amplified from hypersaline environments. *FEMS Microbiol Ecol* 1995;**17**:247–55.

Mohamed NM, Enticknap JJ, Lohr JE, *et al.* Changes in bacterial communities of the marine sponge *Mycale laxissima* on transfer into aquaculture. *Appl Environ Microb* 2008a;**74**: 1209–22.

Mohamed NM, Rao V, Hamann MT, *et al.* Monitoring bacterial diversity of the marine sponge *Ircinia strobilina* upon transfer into aquaculture. *Appl Environ Microb* 2008b;**74**:4133–43.

Moitinho-Silva L, Bayer K, Cannistraci CV, *et al.* Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea. *Mol Ecol* 2014;**23**:1348–63.

Morrow C, Cárdenas P. Proposal for a revised classification of the Demospongiae (Porifera). *Front Zool* 2015;**12**:7.

Musat N, Halm H, Winterholler B, *et al.* A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *P Natl Acad Sci USA* 2008;**105**:17861–6.

Olson JB, Thacker RW, Gochfeld DJ. Molecular community profiling reveals impact of time, space, and disease status on the bacterial community associated with the Caribbean sponge *Aplysina cauliformis*. *FEMS Microb Ecol* 2013;**87**:268–79.

Pawlik JR. The chemical ecology of sponges on Caribbean reefs: natural products shape natural systems. *BioScience* 2011;**61**:888–98.

Pester M, Bittner N, Deevong P, *et al.* A 'rare biosphere' microorganism contributes to sulfate reduction in a peatland. *ISME J* 2010;**4**:1591–602.

Pinhassi J, Gómez-Consarnau L, Alonso-Sáez L, *et al.* Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea. *Aquat Microb Ecol* 2006;**45**:241–52.

Pita L, Turon X, López-Legentil S, *et al.* Host rules: spatial stability of bacterial communities associated with marine sponges (Ircinia spp.) in the Western Mediterranean Sea. *FEMS Microbiol Ecol* 2013;**86**:268–76.

Poppell E, Weisz J, Spicer L, *et al.* Sponge heterotrophic capacity and bacterial community structure in high- and low-microbial abundance sponges. *Mar Ecol* 2014;**35**:414–24.

Redmond NE, Morrow CC, Thacker RW, *et al.* Phylogeny and systematics of Demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. *Integr Comp Biol* 2013;**53**: 388–415.

Reiswig HM. Water transport, respiration and energetics of three tropical marine sponges. *J Exp Mar Biol Ecol* 1974;**14**:231–49.

Reiswig HM. Bacteria as food for temperate-water marine sponges. *Can J Zool* 1975;**53**:582–9.

Reiswig HM. Partial carbon and energy budgets of the bacteriosponge *Verongia fistularis* (Porifera: Demospongiae) in Barbados. *Mar Ecol* 1981;**2**:273–93.

Ribes M, Jiménez E, Yahel G, *et al.* Functional convergence of microbes associated with temperate marine sponges. *Environ Microbiol* 2012;**14**:1224–39.

Riesgo A, Novo M, Sharma PP, *et al.* Inferring the ancestral sexuality and reproductive condition in sponges (Porifera). *Zool Scripta* 2014;**43**:101–17.

Rützler K. Family Spirastrellidae Ridley & Dendy, 1886. In: Hooper JNA, Van Soest RWM (eds). *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer Academic/Plenum Publishers, 2002, 220–3.

Schläppy M-L, Schöttner SI, Lavik G, *et al.* Evidence of nitrification and denitrification in high and low microbial abundance sponges. *Mar Biol* 2010;**157**:593–602.

Schöttner S, Hoffmann F, Cárdenas P, *et al.* Relationships between host phylogeny, host type and bacterial community diversity in cold-water coral reef sponges. Gilbert JA (ed.). *PLoS One* 2013;**8**:e55505.

Schmitt S, Deines P, Behnam F, *et al.* *Chloroflexi bacteria* are more diverse, abundant, and similar in high than in low microbial abundance sponges. *FEMS Microbiol Ecol* 2011;**78**: 497–510.

Shade A, Handelsman J. Beyond the Venn diagram: the hunt for a core microbiome. *Environ Microbiol* 2012;**14**:4–12.

Simister RL, Deines P, Botté ES, *et al.* Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. *Environ Microbiol* 2012;**14**:517–24.

Simister R, Taylor MW, Rogers KM, *et al.* Temporal molecular and isotopic analysis of active bacterial communities in two New Zealand sponges. *FEMS Microbiol Ecol* 2013;**85**:195–205.

Taylor MW, Tsai P, Simister RL, *et al.* 'Sponge-specific' bacteria are widespread (but rare) in diverse marine environments. *ISME J* 2013;**7**:438–43.

Taylor MW, Schupp PJ, Dahllöf I, *et al.* Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. *Environ Microbiol* 2004;**6**: 121–30.

Thiel V, Leininger S, Schmaljohann R, *et al.* Sponge-specific bacterial associations of the Mediterranean sponge *Chondrilla nucula* (Demospongiae, Tetractinomorpha). *Microb Ecol* 2007;**54**:101–11.

Turner S, Pryer KM, Miao VP, *et al.* Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J Eukaryot Microbiol* 1999;**46**:327–38.

Uriz MJ, Martin D, Rosell D. Relationships of biological and taxonomic characteristics to chemically mediated bioactivity in Mediterranean littoral sponges. *Mar Biol* 1992;**113**:287–97.

Vacelet J. Etude en microscopie electronique de l'assocation entre bacteries et spongiaires du genre *Verongia* (Dictyoceratida). *J Microsc Biol Cell* 1975;**23**:271–88.

Vacelet J, Donadey C. Electron microscope study of the association between some sponges and bacteria. *J Exp Mar Biol Ecol* 1977;**30**:301–14.

Van Dorst J, Bissett A, Palmer AS, *et al.* Community fingerprinting in a sequencing world. *FEMS Microbiol Ecol* 2014;**89**:316–30.

Van Soest RWM, Boury-Esnault N, Vacelet J, *et al.* Global diversity of sponges (Porifera). Roberts JM (ed.). *PLoS One* 2012;**7**:e35105.

Webster NS. Sponge disease: a global threat? *Environ Microbiol* 2007;**9**:1363–75.

Webster NS, Cobb RE, Soo R, *et al.* Bacterial community dynamics in the marine sponge *Rhopaloeides odorabile* under *in situ* and ex situ cultivation. *Mar Biotechnol* 2011;**13**:296–304.

Webster NS, Hill RT. The culturable microbial community of the Great Barrier Reef sponge *Rhopaloeides odorabile* is dominated by an α-Proteobacterium. *Mar Biol* 2001;**138**:843–51.

Webster NS, Luter HM, Soo RM, *et al.* Same, same but different: symbiotic bacterial associations in GBR sponges. *Front Microbiol* 2013;**3**:444.

Webster NS, Negri AP, Webb RI, *et al.* A spongin-boring α-proteobacterium is the etiological agent of disease in the Great Barrier Reef sponge *Rhopaloeides odorabile*. *Mar Ecol Prog Ser* 2002;**232**:305–9.

Weisz JB, Hentschel U, Lindquist N, *et al.* Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Mar Biol* 2007;**152**: 475–83.

Weisz JB, Lindquist N, Martens CS. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia* 2008;**155**:367–76.

White JR, Patel J, Ottesen A, *et al.* Pyrosequencing of bacterial symbionts within *Axinella corrugata* sponges: diversity and seasonal variability. *PLoS One* 2012;**7**:e38204.

Wichels A, Würtz S, Döpke H, *et al.* Bacterial diversity in the breadcrumb sponge *Halichondria panicea* (Pallas). *FEMS Microbiol Ecol* 2006;**56**:102–18.

Wilkinson CR. Microbial associations in sponges. III. Ultrastructure of the *in situ* associations in coral reef sponges. *Mar Biol* 1978;**49**:177–85.

Zhang F, Vicente J, Hill RT. Temporal changes in the diazotrophic bacterial communities associated with Caribbean sponges *Ircinia stroblina* and *Mycale laxissima*. *Front Microbiol* 2014;**5**:561.