environmental microbiology reports

Environmental Microbiology Reports (2016) **8**(4), 536–544 doi:10.1111/1758-2229.12430

Restructuring of the sponge microbiome favors tolerance to ocean acidification

M. Ribes,[1]* E. Calvo,[1] J. Movilla,[1] R. Logares,[1] R. Coma[2] and C. Pelejero[1,3]

[1]*Institut de Ciències del Mar, ICM-CSIC, Passeig Marítim de la Barceloneta 37-49, Barcelona, Catalunya 08003, Spain.*

[2]*Centre d'Estudis Avançats de Blanes, CEAB-CSIC, Accès Cala Sant Francesc 14, Blanes, Girona, Catalunya 17300, Spain.*

[3]*Institució Catalana de Recerca i Estudis Avançats, ICREA, Barcelona, Catalunya 08010, Spain.*

Summary

Ocean acidification is increasing and affects many marine organisms. However, certain sponge species can withstand low-pH conditions. This may be related to their complex association with microbes. We hypothesized that species with greater microbial diversity may develop functional redundancy that could enable the holobiont to survive even if particular microbes are lost at low-pH conditions. We evaluated the effects of acidification on the growth and associated microbes of three ubiquitous Mediterranean sponges by exposing them to the present pH level and that predicted for the year 2100. We found marked differences among the species in the acquisition of new microbes, being high in *Dysidea avara*, moderate in *Agelas oroides* and null in *Chondrosia reniformis*; however, we did not observe variation in the overall microbiome abundance, richness or diversity. The relative abilities to alter the microbiomes contributes to survivorship in an OA scenario as demonstrated by lowered pH severely affecting the growth of *C. reniformis*, halving that of *A. oroides*, and unaffecting *D. avara*. Our results indicate that functional stability of the sponge holobiont to withstand future OA is species-specific and is linked to the species' ability to use horizontal transmission to modify the associated microbiome to adapt to environmental change.

*For correspondence: E-mail mribes@icm.csic.es; Tel. +34 93 2309522; Fax +34 93 2309555.

Introduction

Ocean acidification (OA), the reduction in global oceanic pH due to the absorption of anthropogenic atmospheric carbon dioxide (CO_2), is severely affecting marine ecosystems (e.g., Gattuso and Hansson, 2011). In addition to the present surface decrease of 0.1 pH units since the industrial revolution, an unprecedented decline of 0.3–0.4 units is expected by 2100 (IPCC, 2013). This predicted change in pH has been shown to cause detrimental effects on the performance of many organisms, particularly those phyla with structural components based on calcium carbonate (e.g., Eyre *et al*., 2014). In contrast, it has been suggested that some sponge species may remain unaffected by OA (Durkworth *et al*., 2012; Goodwin *et al*. 2013; Morrow *et al*., 2015) and, overall, sponge taxa could benefit from acidic conditions (Bell *et al*., 2013; Morrow *et al*., 2015). However, the lack of knowledge about the mechanisms by which sponges respond to altered pH has become a limiting step in understanding how some sponge species resist OA.

Sponges, the most ancient metazoans, are key components of hard-bottom systems due to their abundance (Hooper and van Soest, 2002), ability to filter water and capacity to display multiple pathways for transforming nutrients, an ability primarily conferred by their microbial partners (e.g., Maldonado *et al*., 2012). Marine sponges establish both permanent and temporary associations with a large variety and contrasting diversity of microorganisms, and these sets of microorganisms are characteristic of each sponge species and contribute to the performance of the holobiont (Schmitt *et al*., 2011; Easson and Thacker, 2014). Because marine water column microbes can tolerate the natural variations in pH that occur seasonally, with depth, and along productivity gradients, the processes mediated by microbes are not expected to be fundamentally affected under future higher CO_2/lower pH conditions (Olivier *et al*., 2014). Thus, due to the crucial role of the microbe-animal interaction in the performance of the holobiont (McFall-Ngai *et al*., 2013) and to the evidence supporting that core microbiome taxa can adapt to local conditions (Kelly *et al*., 2014), the associated microbes could confer environmental stress tolerance to the holobiont. In this context, short-term experiments provide evidence regarding

whether the associated microbial communities are sensitive to near-future OA without obvious signs of holobiont stress (Meron *et al*., 2011; Webster *et al*., 2013; Lesser *et al*., 2015).

Facing environmental changes, conceptual models of microbial communities related to diversity and function predict higher functional redundancy and higher ecosystem stability at higher diversity; this is because there is a greater probability of the presence of stress-resistant taxa (Konopka, 2009). Compensatory dynamics between functionally similar species has been a key issue in the field of theoretical ecology dealing with environmental perturbations (Tilman, 1999, Loreau, 2000). Furthermore, it has been proposed that the network of interactions between microbes can provide a buffer effect against environmental change because functionally different microbes occupy a network of complementary niches (Konopka *et al*., 2015). In this framework, we hypothesize that marine sponges that harbor a richer microbiota would be less affected by OA than those that harbor a lower richness of associated microbes because of the higher potential functional redundancy and/or network complexity in more diverse microbiota.

To test this hypothesis, we targeted three sympatric sponge species that are abundant and widely distributed in Mediterranean sublittoral rocky-bottom habitats and that host different microbial diversity: *Dysidea avara*, with the lowest diversity, *Agelas oroides*, with intermediate diversity, and *Chondrosia reniformis*, with the highest diversity (Ribes *et al*., 2015; Erwin *et al*., 2015). We investigated the three sponge species under both current and future acidification conditions as follows: a) the holobiont performance was assessed by measuring growth rate; b) the surface area of the sponge occupied by associated bacteria was measured by CARD-FISH and confocal microscopy, and c) the microbiome composition was analyzed through 16S rDNA pyrosequencing.

Results and discussion

The experimental set-up allowed the precise adjustment of the selected pH conditions, which were maintained throughout the experiment at approximately 8.10 for the control condition and 7.82 for the acidified condition. Seawater chemistry is summarized in Supporting Information Table S1. Due to logistical limitations regarding the aquarium systems and space availability, the experiments were performed at different times (January to March for *A. oroides* and *C. reniformis* and July to September for *D. avara*), limiting the between-species comparisons. Temperature in the tanks was maintained at the natural levels for the time of year (15 ± 2°C for *C. reniformis* and *A. oroides* and 23 ± 1°C for *D. avara*, Supporting Information Table S1).

After 66 days of exposure, significant differences between treatments were observed in the growth of the sponge species, with the growth rate severely affected in *C. reniformis*, halved in *A. oroides*, unaffected in *D. avara* (Fig. 1). However, only *C. reniformis* showed a significant effect of treatment after the first 24 days of exposure, indicating a faster negative response of this species to changing conditions (Fig. 1). There were large differences in growth rate among the genotypes of each species, and the colonies exhibiting faster growth experienced greater effects of low pH (Supporting Information Fig. S1). Under the control conditions, growth rates differences ranged from −0.7 to 15% in *C. reniformis*, −1.5 to 36.5% in *A. oroides* and −0.8 to 6.1% in *D. avara*. This pattern of growth variability is similar to that previously observed in the field (de Caralt *et al*., 2007; Teixidó *et al*., 2011). Together, the variability in growth and the trend that the effect of low pH increased with the growth of the two affected species suggest that isolated genotypes may persist even though the species population may be negatively influenced under acid conditions. However, it is unknown whether other processes, such as gametogenesis, may also be affected by OA, thereby affecting the ultimate success of these species.

Under both conditions, the mean growth of *D. avara* did not differ from zero. This was unexpected because *D. avara* is the species with the highest recorded growth (10% month^{-1}, de Caralt *et al*., 2007). Nevertheless, this result is consistent with field observations because an ongoing study has found that spawning of this species occurs in late June and that the species subsequently exhibits a period of tissue readjustment with little or no net increase in biomass (de Caralt unpublished). To evaluate whether the lack of an observed OA effect on *D. avara* was related to the lack of growth, we contrasted the growth of the species exposed to both treatments using only the specimens that exhibited growth. In this case, the specimens displayed a mean growth rate of 2.9%, with no significant low-pH effects (as determined using one-way ANOVA of arctangent-transformed data, $F_{1,8} = 0.1097$, $p = 0.7489$), suggesting that the growth rate of *D. avara* was not affected by the simulated future OA scenario.

The three species hosted very different abundances of microbial components estimated as the proportion of sponge tissue occupied by microbes, with high abundances in both *C. reniformis* (40.8 ± 3.3%, mean ± SE) and *A. oroides* (30.2 ± 2.3%, mean ± SE) and low abundances in *D. avara* (0.1 ± 0.01%, mean ± SE; Supporting Information Fig. S2). Similarly, significant initial differences in the Chao1 index, which estimates total richness, and the Shannon index of diversity (H), which considers richness and evenness, were observed between the low

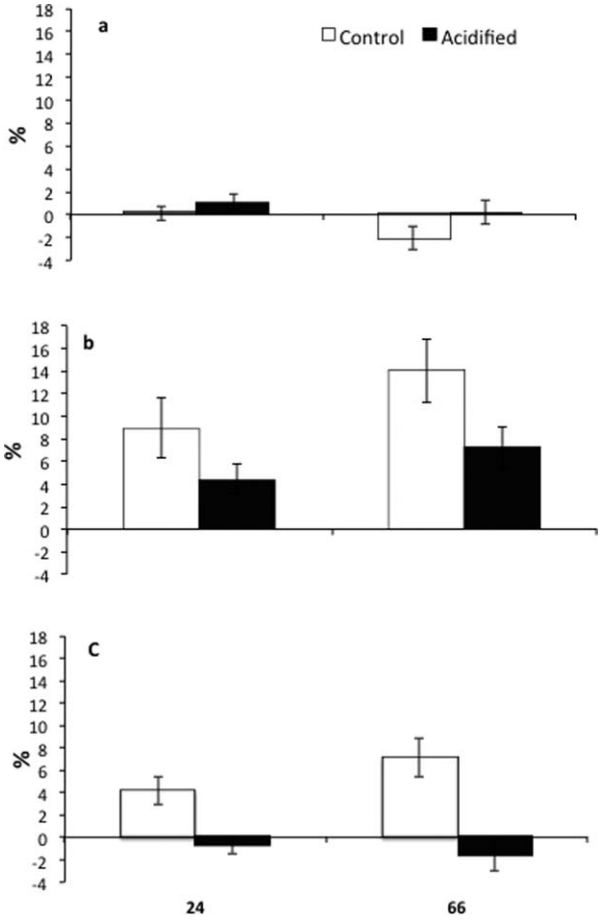

Fig. 1. Sponge growth rate expressed as the percentage of buoyant weight gained (positive %) or lost (negative %) relative to the initial buoyant weight in the control and acidified conditions at days 24 and 66 of the experiment. a: *D. avara*, b: *A. oroides*, c: *C. reniformis*.

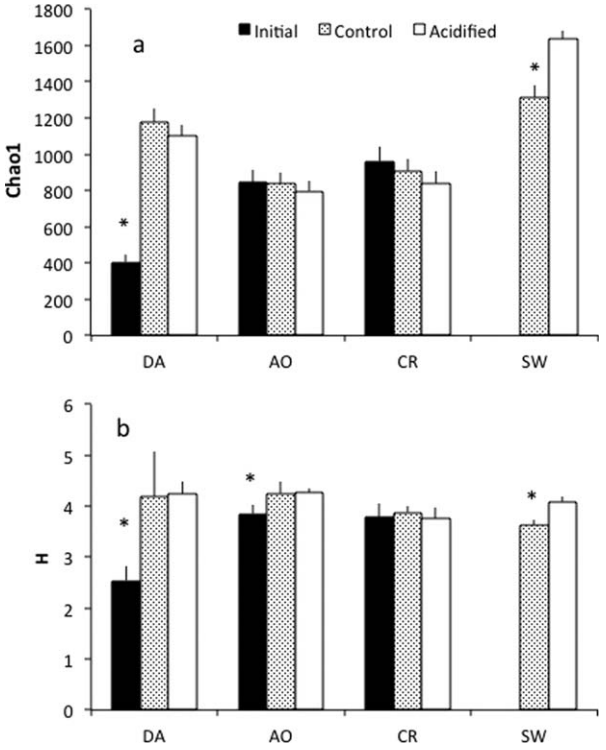

Fig. 2. Bacterial richness (a, Chao1) and diversity (b, Shannon index, H) estimates based on 16S rRNA gene pyrosequencing for *D. avara* (DA), *A. oroides* (AO), *C. reniformis* (CR) and seawater (SW). Colored bars represent initial and final samples under control and acidified conditions, respectively. * indicates significant differences between initial (T_0) and final (T_2) conditions.

values exhibited by *D. avara* for both indices and those from the other two species (Welch's test, $p = 0.036$ and $p = 0.046$ for *C. reniformis* and *A. oroides*, respectively) (Fig. 2a and b). This fact was consistent with previous reports (Schmitt *et al.*, 2011; Björk *et al.*, 2013; Ribes *et al.*, 2015). The ambient water exhibited a higher richness and diversity in the acidified conditions than in the control conditions at the end of the experiment (Welch's test, $p = 0.038$; Welch's test, $p = 0.036$; respectively) (Fig. 2a). Unfortunately, the initial water samples were lost. This is consistent with the recently observed pattern of change in pH homeostasis genes from distinct bacterial groups under low-chlorophyll conditions, such as that of our study, where the relative bacterioplankton transcript abundance was observed to change between acidified and control conditions without affecting bacterial production (Bunce *et al.*, in press).///

The abundance of associated microbes did not vary among the initial, control and low-pH conditions for any of the three species (*C. reniformis*: $F_{2,27} = 0.8783$, $p = 0.4270$; *A. oroides*: $F_{2,28} = 0.1051$, $p = 0.9006$; *D. avara*: $F_{2,21} = 1.6916$, $p = 0.2084$) (Supporting Information Fig. S2). Both the control and acidification conditions promoted a significant increase in richness (Welch's test, $p = 0.019$) in *D. avara*, but no such effect was observed in the other species (Fig. 2a). In terms of diversity, both the control and acidification conditions resulted in significant increases in the Shannon index values for *D. avara* (Welch's test, $p = 0.015$) and for *A. oroides* (Welch's test, $p = 0.015$) but not for *C. reniformis* (Fig. 2b). Then, the observed changes in the microbiome in terms of the microbe abundance, richness and diversity relative to the initial conditions were similar between the control and acidified treatments. In accordance with these results, the Bray-Curtis measure of dissimilarity among bacterial communities showed a treatment-independent clustering of sponge species (Supporting Information Fig. S3). Therefore, the changes in the microbiome parameters did not contribute to explain the differences in sponge growth rates obtained between the treatments. These changes can be interpreted as a response to the aquaria-laboratory

conditions unrelated to the acidification condition and should be taken into account in future laboratory studies. Thus, our results are consistent with the increases in the richness and evenness of the bacterial communities associated with sponges upon transfer into aquarium conditions that have previously been identified by clone library analysis and DGGE (e.g., Mohamed *et al.*, 2008;).

From the perspective of major taxonomic group composition, a few changes were observed among the initial condition and the control and acidified experimental treatments (Supporting Information Fig. S4). *Prochlorococcus* increased in abundance, and Proteobacterial sequences decreased for *D. avara*; Alphaproteobacteria increased, and Chloroflexi decreased in abundance for *A. oroides*. No changes were observed for *C. reniformis*. However, these changes occurred in both experimental treatments (except for Alphaproteobacteria in *A. oroides*, Supporting Information Fig. S4); therefore, most of these changes were also interpreted as adaptations to laboratory conditions.

We found marked restructuring of the microbiomes, understood as acquisitions and losses of OTUs, among the three sponge species. *C. reniformis* exhibited almost no changes in the associated microbiome in terms of either losses or acquisitions of OTUs in either treatment (Tables 1 and 2). Restructuring of the *D. avara* and *A. oroides* microbiomes was characterized by the acquisition of a large number of new OTUs that were exclusively found in one of the experimental treatments. *D. avara* acquired 255 new OTUs only under the acidified condition and 342 only under the control condition. *A. oroides* exhibited acquisition of a notable number of new OTUs (114) but only under the acidified condition. For both species, most of the lost OTUs were shared between the control and acidified conditions. Taxonomically, the newly acquired OTUs in both treatment conditions mostly belonged to the phyla Bacteroidetes and Proteobacteria (mainly Alphaproteobacteria), which were also found in the ambient water.

The documented lack of variation in microbiome abundance, richness, diversity and major taxonomic group composition between treatments prevented the examination of the potential role of network complexity; at the same time, it highlighted the sponges' ability to restructure their microbial components, through exchange with the ambient water, as the primary factor contributing to the differential responses of sponges to OA. Of the three species, *D. avara* exhibited the highest rates of microbial exchange, primarily involving a differential acquisition between the acidified and control conditions. This differential microbial reorganization occurred in conjunction with a similar growth pattern under both conditions (i.e., no effect of the acidic conditions). *A. oroides* exhibited a certain degree of microbial restructuring characterized by the acquisition of microbial components only under the acidified conditions. This lower degree of microbial reorganization occurred in conjunction with the species' growth under the acidic conditions decreasing by half. The absence of microbial restructuring in *C. reniformis* was accompanied by a failure to grow under acidified conditions. These results suggest that the ability of marine sponges to tolerate OA may be related to their capacity to exchange microbial components with the environment. This is consistent with the recognition that the ecological interactions of animals and microbes play a crucial role in the metabolic and regulatory networks that characterize an organism's health (McFall-Ngai *et al.*, 2013). The results support the need to improve our mechanistic understanding of the host-microbe interaction in order to interpret the effects of environmental change on the complex sponge holobiont system.

Microbial restructuring to ensure the holobiont's success in response to change may occur through compensatory dynamics among functionally similar microbes within the sponge (i.e., the decline of one species is offset by the increase of another species), as has been documented in other systems (i.e., Tilman, 1999). In this case, the species exhibiting a high abundance, richness and diversity of associated microbes would be expected to exhibit a better functional response to environmental changes such as acidification stress. However, our observations do not support this hypothesis. This is consistent with the documented lack of functional redundancy in microbes associated with the nitrification process in these species (Ribes *et al.*, 2012). Furthermore, our results may be related to the high interdependence of the microbiome-sponge host association, which can be conducive to highly specialized niches that may constrain functional redundancy (e.g., Fan *et al.*, 2013, Erwin *et al.*, 2015).

Our results show that the exchange of a large number of abundant microbes provides experimental evidence of horizontal transmission that has been conducive to the development of distinct microbiomes in *D. avara* and *A. oroides*, which may have contributed to their ability to withstand exposure to OA conditions. Previous pyrosequencing studies had observed changes in rare taxa but not in members of the core microbiome community when evaluating the influence of environmental variations in light levels and water movements on sponges (Erwin *et al.*, 2012; Cardenas *et al.*, 2014). Both low- and high-level exchanges of associated microbes in response to environmental change are consistent with the proposed conceptual model of sponge-microbe interactions, indicating that the balance between beneficial and harmful outcomes is controlled by environmental conditions. Although sponges may also be able to

Table 1. Restructuring of sponge microbiomes considered as acquisitionof abundant OTUs (i.e., more than 10 sequences)

Bacteria			Dysidea avara						Agelas oroides						Chondrosia reniformis						Ambient water			
			ACID		CONTROL		ACID +CONTROL		ACID		CONTROL		ACID +CONTROL		ACID		CONTROL		ACID +CONTROL		ACID		CONTROL	
Phylum	Class	Order	NOTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTU	sN Seq	N OTU	sN Seq	N OTUs	N Seq	N OTUs	N Seq	N OUT	sN Seq	N OTUs	N Seq	N OTUs	N Seq
Acidobacteria			5	21 (10)	6	59 (82)	1	16 22	2	20 (1)		–		–		–		–		–	–	–	–	–
Actinobacteria	Acidimicrobiia		2	13 (0.7)	–	–	–	–	–	–		–		–		–		–		–	6	44 (41)	9	33 (33)
	Actinobacteria		3	19 (11)	4	65 (59)	–	–	–	–		–		–		–		–		–	6	85 (128)	1	14
Bacteroidetes	Flavobacteriia		23	21 (14)	17	24 (16)	3	142 (192) 19 (12)	27	193 (684)	4	11 (1)		–		–		–		–	42	68 (104)	43	75 (123)
	Sphingobacteriia		9	49 (74)	11	20 (18)	3	17 (9) 15 (6)	3	28 (12)		–		–		–		–		–	–	–	–	–
Chloroflexi			1	12	3	35 (15)	–	–	–	–		–		–		–		–		–	–	–	–	–
Cyanobacteria			6	18 (8)	10	21 (11)	2	22 (7) 64 (9)	–	–		–		–		–		–		–	–	–	–	–
	Synechoco-ccophycideae	Pseudanabaenales	1	130	–	–	–	–	–	–		–		–		–		–		–	–	–	–	–
		Synechococcales	–	–	7	13 (2)	4	23 (7) 19 (6)	–	–		–		–		–		–		–	3	113 (126)	2	92 (13)
Firmicutes	Bacilli		2	19 (4)	4	42 (20)	–	–	–	–		–		–		–		–		–	–	–	–	–
	Clostridia		1	13	7	16 (6)	–	–	–	–		–		–		–		–		–	–	–	–	–
Nitrospirae	Nitrospira	Nitrospirales	1	11	6	25(16)	–	–	–	–		–		–		–		–		–	–	–	–	–
Planctomycetes	Phycisphaerae	Phycisphaerales	1	31	7	14 (7)	3	14 (1) 17 (6)	–	–		–		–		–		–		–	–	–	–	–
	Planctomycetia	Pirellulales	9	19 (6)	16	26 (17)	–	–	8	17 (10)		–		–		–		–		–	–	–	–	–
		Planctomycetales	–	–	2	16 (4)	–	–	–	–		–		–		–		–		–	–	–	–	–
Proteobacteria	Alphaproteo-bacteria		31	21 (12)	27	34 (51)	15	52 (114) 52 (74)	8	61 (101)		–		–	1	13		–		–	27	31 (26)	36	38 (50)
		Rhizobiales	22	24 (17)	27	25 (22)	10	24 (20) 17 (6)	–	41 (21)		–		–		–		–		–	–	141 (18)	3	154 (121)
		Rhodobacterales	36	18 (11)	15	29 (22)	10	77 (21) 66 (38)	21	91 (268)		–		–		–		–		–	47	69 (149)	58	84 (217)
		Rhodospirillales	14	16 (7)	27	25 (30)	6	39 (23) 57 (52)	3	29 (17)		–		–		–		–		–	4	12 (1)	6	16 (9)
		Rickettsiales	7	24 (22)	11	59 (70)	5	51 (41) 34 (19)	3	20 (6)		–		–		–		–		–	54	280 (1213)	74	357 (1797)
	Deltaproteo-bacteria		6	23 (18)	18	22 (18)	–	–	3	41 (41)		–		–		–		–		–	2	11 (0)		
	Gamma proteo bacteria		15	15 (7)	20	19 (10)	5	29 (26) 44 (58)	14	26 (28)		–		–		–	4	11 (1)		–	5	13 (3)	4	15 (5)
		Alteromonadales	8	28 (34)	13	36 (33)	4	22 (17) 77 (74)	4	29 (24)		–		–		–		–		–	15	50 (90)	14	61 (121)
		Chromatiales	4	20 (14)	7	17 (6)	5	30 (26) 42 (53)	2	52 (54)		–		–		–		–		–	2	31 (11)	2	45 (13)
		HTCC2188	1	10	8	26 (16)	–	–	–	–		–		–		–		–		–	–	–	1	19
		Legionellales	15	17 (7)	25	28 (23)	3	19 (3) 26 (13)	5	17 (9)		–		–		–		–		–	3	39 (24)	1	11
		Oceanospirillales	5	27 (14)	8	32 (25)	8	22 (15) 25 (11)							1	71					30	127 (219)	53	116 (254)
		Xanthomonadales	6	13 (2)	8	23 (9)	1	10 13	3	28 (19)		–		–		–		–		–	–	–	–	–
SBR1093			6	62 (103)	12	30 (37)	10	44 (43) 118 (112)	–	–		–		–		–		–		–	1	12	1	11
TM6			2	11 (1)	6	14 (5)	–	–	–	–		–		–		–		–		–	–	–	–	–
Verrucomicrobia			3	15 (8)	5	12 (1)	–	–	1	46		–		–		–		–		–	1	10	–	–
Eukarya												4												
Stramenopiles			5	16 (6)	–	–	–	–	1	15		–		–		–		–		–	–	–	–	–
Chlorophyta			5	17 (5)	5	51 (45)	4	55 (44) 40 (32)	1	13		–		–		–		–		–	2	16 (6)	2	11 (2)
Total			255		342		102		114			8		0		2		4		0	252		310	

Table 2. Restructuring of sponge microbiomes considered as losses of abundant OTUs (i.e., more than 10 sequences)

Bacteria			Dysidea avara						Agelas oroides						Chondrosia reniformis					
			ACID		CONTROL		ACID+CONTROL		ACID		CONTROL		ACID+CONTROL		ACID		CONTROL		ACID+ CONTROL	
Phylum	Class	Order	NOTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq	N OTUs	N Seq
Acidobacteria			–	–	–	–	3	48 (51)	–	–	–	–	1	11	–	–	–	–	–	–
Actinobacteria	Acidimicrobiia		–	–	–	–	1	122	–	–	–	–	–	–	–	–	–	–	–	–
	Actinobacteria		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bacteroidetes	Flavobacteriia		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sphingobacteriia			–		1	74	7	20 (21)	–	–	–	–	–	–	–	–	–	–	–	–
			–	–	–	–	3	20 (13)	–	–	–	–	–	–	–	–	–	–	–	–
Chloroflexi			–	–	–	–	1	34	–	–	–	–	5	15 (4)	–	–	–	–	–	–
Cyanobacteria			–	–	–	–	2	24 (8)	–	–	–	–	1	32	–	–	–	–	–	–
	Synechococcophycideae	Pseudanabaenales	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		Synechococcales	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Firmicutes	Bacilli		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Clostridia		–	–	–	–	1	16	–	–	–	–	–	–	–	–	–	–	–	–
Nitrospirae	Nitrospira	Nitrospirales	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Planctomycete: Phycisphaerae	Phycisphaerales		–	–	–	2	54 (59)	–	–	–	–	–	–	–	–	–	–	–	–	
	Planctomycetia	Pirellulales	–	–	–	–	1	11	–	–	–	–	–	–	–	–	–	–	–	–
		Planctomycetales	–	–	–	–	1	10	–	–	–	–	–	–	–	–	–	–	–	–
Proteobacteria	Alphaproteobacteria		–	–	1	31	13	25 (14)	–	–	–	–	–	–	–	–	–	–	–	–
		Rhizobiales	–	–	2	14 (1)	7	14 (6)	–	–	–	–	–	–	–	–	–	–	–	–
		Rhodobacterales	–	–	–	–	1	12		–	–	–	1	11	–	–	–	–	–	–
		Rhodospirillales	–	–	–	–	7	48 (58)	–	–	–	–	–	–	–	–	–	–	–	–
		Rickettsiales	–	–	–	–	2	23 (23)	–	–	–	–	–	–	–	–	–	–	–	–
	Deltaproteobacteria		–	–	–	–	7	24 (16)	–	–	6	15 (4)	9	14 (5)	–	–	–	–	–	–
	Gammaproteobacteria		–	–	–	–	17	518 (625)	–	–	–	–	–	–	–	–	–	–	–	–
		Alteromonadales	–	–	–	–	5	31 (16)	–	–	–	–	1	13	–	–	–	–	–	–
		Chromatiales	–	–	–	–	2	23 (13)	–	–	–	–	–	–	–	–	–	–	–	–
		HTCC2188	–	–	–	–	2	11 (0)	–	–	–	–	–	–	–	–	–	–	–	–
		Legionellales	–	–	–	–	7	22 (8)	–	–	–	–	–	–	–	–	–	–	–	–
		Oceanospirillales	–	–	1	17	9	90 (255)	–	–	–	–	–	–	–	–	–	–	–	–
		Xanthomonadales	–	–	–	–	1	23	–	–	–	–	–	–	–	–	–	–	–	–
SBR1093			–	–	–	–	–	–	–	–	–	–	4	73 (56)	–	–	–	–	–	–
TM6			–	–	–	–	1	26	–	–	–	–	–	–	–	–	–	–	–	–
Verrucomicrobia			–	–	–	–	–	–	–	2	11 (0)	16	16 (7)	–	–	–	–	–	–	
Eukarya																				
Stramenopiles			–	–	–	–	3	12 (2)	–	–	–	–	–	–	–	–	–	–	–	–
Chlorophyta			–	–	–	–	–	–	–	–	–	–	1	10	–	–	–	–	–	–
TOTAL			0		5		106		0		8		39		0		0		0	

Acquisition was interpreted as the number of OTUs that were not detected in the initial samples but were abundant at the end of the experiment. OTUs were considered lost when they were abundant in the initial samples but not detected (zero sequences) at the end of the experiment. Acid and Control columns refer to the OTUs that were exclusively acquired or lost in the indicated experimental condition. The Acid + Control column refers to the OTUs that were acquired or lost in both treatments. The last two columns refer to the ambient water OTUs at the end of the experiment. *N*: number, Seq: Sequences [mean (SD)].

control microbial growth and select for the presence of specific strains (Thacker and Freeman, 2012), the variation in the level of microbial restructuring observed among the different species indicates that horizontal transmission differs markedly among species. These differences are consistent with the proportion of the associated microbial community observed to exhibit temporal variation (i.e., microbes that are present during specific seasons or intermittently) in each species, as well as with the temporal scale over which this process occurs (i.e., months) (Björk *et al*., 2013). Thus, the reorganization capacity of a species may be related to the proportion of the microbiome exhibiting temporal variation in the field.

Certain studies have proposed that marine sponges in coral reef systems will benefit from climate change, primarily because they appear to be less affected than corals by warming and acidification (Kroeker *et al*., 2011; Fabricius *et al*., 2011; Bell *et al*., 2013). In general, acidification decreases the diversity, biomass and trophic complexity of the benthic community, which subsequently becomes dominated by non-calcified organisms (e.g., Kroeker *et al*., 2011), of which sponges are a major component. Measurements of sponge abundance across natural temperatures and pH ranges, such as those occurring in naturally acidified areas close to CO_2 seeps, demonstrate the resistance of certain sponges to low-pH conditions (Fabricius *et al*., 2011; Price *et al*., 2012; Goodwin *et al*., 2013). However, sponge cover and species abundance have also been observed to decrease in low-pH conditions (Fabricius *et al*., 2011, Goodwin *et al*., 2013). Based on a review of the carbonate mineralogy of sponges, calcareous sponges may be vulnerable to acidification. Both positive and neutral effects of acidification on sponge species have been shown in a small number of coral reef species (Durkworth *et al*., 2012, Morrow *et al*., 2015). Particular attention has recently been devoted to sponge bioeroders because they can increase reef vulnerability to storms (e.g., Wisshak *et al*., 2012). Our results have shown that the host-associated microbiomes of some sponge species can be dynamic and that the ability to alter the microbiome corresponded with the growth response to acidification exhibited by each of the three studied species. These findings indicate that the capacity to adapt to and survive acidic conditions appears to be species-specific, which is consistent with previous observations in CO_2 seeps (Goodwin *et al*., 2013, Morrow *et al*., 2015). These findings largely agree with those of Morrow and collaborators (2015) at a CO_2 seep, where they found distinct microbiomes in two sponge species that increased abundance at a low pH site in contrast to a control site; however, a third sponge species that did not alter its microbiome exhibited a decrease in abundance at the acidified site. The variability in the adaptation response among species may be related to the cost of adaptation (Guo and Gross, 2014). This adaptation response may be more common than previously thought because the results from a recent microbial metagenome study of 22 coral reefs support the hypothesis that the core microbial taxa adapt to local conditions by selecting for advantageous metabolic genes (Kelly *et al*., 2014).

We report differential effects on sponge holobiont growth under an OA scenario. The discrepancies in the responses of different sponge species could not be attributed to changes in microbial abundance, richness or major taxonomic group composition but were attributed instead to the sponges' abilities to restructure their microbiomes, mainly by acquiring new microbial components via horizontal transmission. Although the mechanisms linking microbiota composition and holobiont response to environmental change remain unknown, our results indicate that the ability to reorganize the microbiota may play a crucial role in the capacity of sponge species to withstand future environmental global changes such as OA.

Acknowledgements

The authors thank Paula Lopez-Sendino and Marc Catllá for the careful laboratory assistance provided. The study was funded by the Spanish Government through Project CGL2010-18466, CGL2013-43106-R and CTM2012-32017. This study constitutes a contribution from the Marine Biogeochemistry and Global Change Research Group funded by the Catalan Government through Grant 2014SGR1029.

References

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

Björk, J., Diez-Vives, C., Coma, R., Ribes, M., and Montoya, J. (2013) Specificity and temporal dynamics of complex bacteria – sponge symbiotic interactions. *Ecology* **94**: 2781–2791.

Bunce, C., Lundin, D., *et al*. (2016) Response of marine bacterioplankton pH homeostasis gene expression to elevated CO_2. *Nat Clim Chan* **6**: 483–487.

Cardenas, C.A., Bell, J.J., Davy, S.K., Hoggard, M, and Taylor, M.W. (2014) Influence of environmental variation on symbiotic bacterial communities of two temperate sponges. *FEMS Microb Ecol* **88**: 516–527.

de Caralt, S., Otjens, H., Uriz, M.J., and Wijffels, R.H. (2007) Cultivation of sponge larvae: Settlement, survival, and growth of juveniles. *Mar Biotech* **9**: 592–605.

Durkworth, A.R., West, L., Vansach, T., Stubler, A, and Hardt, M. (2012) Effects of water temperature and pH on

growth and metabolite biosynthesis of coral reef sponges. *Mar Ecol Prog Ser* **462**: 67–77.
Easson, C.G. and Thacker, R.W. (2014) Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. *Front Microbiol* **5**: 1–11.
Erwin, P.M., Pita, L., Lopez-Legentil, S, and Turon, X. (2012) Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Appl Environ Microbiol* **78**: 7358–7368.
Erwin, P.M., Coma, R., López-Sendino, P., Serrano, E., and Ribes, M. (2015) Stable symbionts across the HMA-LMA dichotomy: Low seasonal and inter-annual variation in sponge-associated bacteria from taxonomically divers hosts. *FEMS Microbiol Ecol* **91**: fiv115.
Eyre, B.D., Andersson, A.J., and Cyronak, T. (2014) Benthic coral reef calcium carbonate dissolution in an acidifying ocean. *Nat Clim Chan* **4**: 969–976.
Fabricius, K.E., Langdon, C. *et al*, (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nat Clim Chan* **1**: 165–169.
Fan, L., Liu, M., *et al*, (2013) Marine microbial symbiosis heats up: The phylogenetic and functional response of a sponge holobiont to thermal stress. *ISME J* **7**: 991–1002.
Gattuso, J.P., Hansson, L. (2011) Ocean acidification: Background and history. In Ocean Acidification. Hansson, L., Gattuso, J.P. (ed.). Oxford: Oxford University Press, pp. 1–20.
Goodwin, C., Rodolfo-Metalpa, R., Picton, B., and Hall-Spencer, J.M. (2013) Effects of ocean acidification on sponge communities. *Mar Ecol* **35**: 41–49.
Guo, M.S. and Gross, C.A. (2014) Stress-induced remodeling of the bacterial proteome. *Curr Biol* **24**: R424–R434.
Hooper, J.N.A., van Soest, R.W.M. (2002) *Systema Porifera: A Guide to the Classification of Sponges*. New York, USA, Kluwer Academic/Plenum Publishers.
IPCC (2013) Summary for Policymakers. *In Climate Change 2013: The Physical Science Basis. Contribution of Working Group I To the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. In Stocker, T.F, Qin, D., Plattner, G. K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (eds). Cambridge, United Kingdom: Cambridge University Press.
Kelly, L.W., Williams, G.J., Barott, K.L., *et al*. (2014) Local-genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *Proc Natl Acad Sci USA* **111**: 10227–10232.
Konopka, A. (2009) What is microbial community ecology? *ISME J* **3**: 1223–1230.
Konopka, A., Lindermann, S., *et al*. (2015) Dynamics in microbial communities: Unraveling mechanisms to identify principles. *ISME J* **9**: 1488–1495.
Kroeker, K.J., Micheli, F., Gambi, M.C., and Martz, T.R. (2011) Divergent ecosystem responses within a benthic marine community to ocean acidification. *Proc Natl Acad Sci USA* **108**: 14515–14520.
Lesser, M.P. Fiore *et al*. (2015) Climate change stressors destabilize the microbiome of the caribbean barrel sponge, *xentospongia muta*. *J Exp Mar Biol Ecol* **475**: 11–18.
Loreau, M. (2000) Biodiversity and ecosystem functioning: Recent theoretical advances. *Oikos* **91**: 3–17.
Maldonado, M., Ribes, M., and van Duyl, F.C. (2012) Nutrient fluxes through sponges: Biology, budgets, and ecological implications. *Adv Mar Biol* **62**: 113–182.
McFall-Ngai, M., Hadfield, M.G., and Bosch, T.C.G. (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci USA* **9**: 3229–3236.
Meron, D. and Atias, E., *et al*. (2011) The impact of reduced pH on the microbial community of the coral *acropora eurystoma*. *ISME J* **5**: 51–60.
Mohamed, N.M., Rao, V., Hamann, M.T., Kelly, M., and Hill, R.T. (2008) Monitoring bacterial diversity of the marine sponge *ircinia strobilina* upon transfer into aquaculture. *Appl Environ Microbiol* **74**: 4133–4143.
Morrow, K.M., Bourne, D.G., and Humphrey, C. (2015) Natural volcanic CO_2 seeps reveal future trajectories for host-microbial associations in corals and sponges. *ISME J* **9**: 894–908.
Olivier, A.E., Newbold, L.K., Whiteley, A.S., and van der Gast, C.J. (2014) Marine bacterial communities are resistant to elevated carbon dioxide levels. *Environ Microbiol Rep* **6**: 574–582.
Price, N.N., Martz, T.R., Brainard, R.E., and Smith, J.E. (2012) Diel variability in seawater pH relates to calcification and benthic community structure on coral Reefs. *PLoS ONE* **7**: e43843. doi:10.1371/journal.pone.0043843.
Ribes, M., Jimenez, E., Yahel, G., *et al*. (2012) Functional convergence of microbes associated with temperate marine sponges. *Environ Microbiol* **14**: 1224–1239.
Ribes, M., Dziallas, C., Coma, R., and Riemann, L. (2015) Microbiome diversity and putative diazotrophy in high- and low- microbial-abundance mediterranean sponges. *Appl Environ Microb* **17**: 5683–5693.
Schmitt, S., Tsai, P., Bell, J., and Fromont, J. (2011) Assessing the complex sponge microbiota: Core, variable and species-specific bacterial communities in marine sponges. *ISME J* **6**: 564–576.
Teixidó, N., Garrabou, J., and Harmelin, J.G. (2011) Low dynamics, high longevity and persistence of sessile structural species dwelling on mediterranean coralligenous outcrops. *PLoS ONE* **6**: e23744. doi:10.1371/journal.pone.0023744.
Thacker, R.W. and Freeman, C.J. (2012) Sponge–microbe symbioses: Recent advances and new directions. *Adv Mar Biol* **62**: 57–111.
Tilman, D. (1999) The ecological consequences of changes in biodiversity: A search for general principles. *Ecology* **80**: 1455–1474.
Webster, N.S., Negri, A.P., Flores, F., Humphrey, C., Soo, R., Botte, E.S., *et al*. (2013) Near-future ocean acidification casues differences in microbial associations within diverse coral reef taxa. *Environ Microb Rep* **5**: 243–251.
Wisshak, M., Schönberg, C.H.L., Form, A., and Freiwald, A. (2012) Ocean acidification accelerates reef bioerosion. *PLoS ONE* **7**: e45124.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Differences in sponge growth between low-pH and control conditions compared to the growth rate in the control condition. Values are given in percentages (%).

Fig. S2. Percentage (%) of sponge surface in Arc Tangent (ATan) covered by bacteria measured by CARD-FISH and confocal laser-scanning microscopy under the different conditions. *D. avara* (DA), *A. oroides* (AO) and *C. reniformis* (CR).

Fig. S3. Dendrogram of cluster analysis based on the Bray-Curtis dissimilarity index between OTUs defined at the 98% sequence identity in the sponge species *D. avara* (DA), *C. reniformis* (CR), *A. oroides* (AO) and seawater (SW). The y-axis represents within-cluster dissimilarity, with 0 indicating identical microbial communities and 1 indicating communities with no overlapping OTUs. Black, blue and red represent initial, control and acidified conditions, respectively. Cluster a include seawater (a1) and *D. avara* (a2) sequences. Cluster b includes *C. reniformis* (b1) and *A. oroides* (b2) sequences.

Fig. S4. Mean ± SE relative percentage of total 16S ribosomal DNA pyrosequencing reads from sponges under initial, control and acidified conditions.

Table S1. Parameters of the aquaria's seawater carbonate system for each treatment. pH_T = pH in the total scale at *in situ* temperature; TA = total alkalinity (μmol/kg−SW); S = salinity; T = temperature (°C); pCO_2 = partial pressure of CO_2 (μatm); DIC = dissolved inorganic carbon (μmol/kg−SW). All parameters are expressed as means ± SEs ($N = 3$).

Table S2. Summary output of an ANOVA evaluating the effects of two pH conditions (8.1 and 7.8 pH units) on the growth of *A. oroides, C. reniformis* and *D. avara*.

Table S3. Pyrosequencing metrics for the different sponge species and experimental conditions. Raw reads are the total number of rDNA gene reads generated. Clean reads are the total number of non-chimeric reads that passed the quality control criteria. OTUs: number of operational taxonomic units identified using a 97% similarity. DA, *D. avara*; AO, *A. oroides*; CR, *C. reniformis*; SW, seawater. NA, not available.